GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

GENETIC HETEROGENEITY AND PLEIOTROPY – THE ESSENCE OF MULTIDISCIPLINARITY IN MEDICAL GENETICS

LAVINIA CABA, MD, PhD

Associate Professor

2023

"To strive, to seek, to find, and not to yield"

Alfred Tennyson

TABLE OF CONTENTS

ABBREVIATION LIST iii

ABSTRACT 1

REZUMAT 3

I. OVERVIEW OF PROFESSIONAL, ACADEMIC AND SCIENTIFIC CONTRIBUTIONS 5

Chapter 1. CHROMOSOMAL ANOMALIES AND THEIR CONSEQUENCES . 13

1.1. Introduction 13

1.2. Congenital anomalies in patients with unbalanced translocation t(4;10) 14

1.3. Diagnostic and prenatal screening in chromosomal anomalies 27

1.3.1. Study of non-invasive prenatal testing in a Romanian Population 27

1.3.2. Comparison between paramagnetic and CD71 magnetic activated cell sorting of fetal nucleated red blood cells from the maternal blood 41

Chapter 2. GENETIC HETEROGENEITY AND PLEIOTROPY IN CANCER 49

2.1. Introduction 49

2.2. Familial adenomatous polyposis Gardner variant 49

2.3. Genomics, epigenomics and biomarkers in cancer 59

2.3.1. Circular RNA 59

2.3.2. Melanoma – genomics and epigenomics 69

Chapter 3. GENETIC HETEROGENEITY AND PLEIOTROPY IN BARDET-BIEDL SYNDROME – EXAMPLE OF THE MULTIDICIPLINARITY APPROACH IN CILIOPATHIES 82

3.1. Introduction 82

3.2. Pleiotropy and Variable Expressivity in Bardet–Biedl Syndrome 83

3.3. Study of Bardet-Biedl Syndrome 94

3.4. Multidisciplinary approach in BBS 97

Chapter 4. CHALLENGING DIAGNOSIS AND MULTIDISCIPLINARY APPROACH IN RARE DISEASES 104

4.1. Introduction 105

4.2. Study of Bartter Syndrome 105

4.3. Study of Epidermolysis Bullosa 111

4.4. Study of SHOX deletion 125
4.5. Study of Coffin Siris Syndrome 130
4.6. Study of Hereditary gingival fibromatosis 135
II. FUTURE DIRECTIONS OF CAREER DEVELOPMENT 138
III. REFERENCES 141

ABBREVIATION LIST

aCGH - Array Comparative Genomic Hybridization
ADH - antidiuretic hormone
AFAP - attenuated familial adenomatous polyposis
AFP - alpha-fetoprotein
ALT - alanine aminotransferase
AST - aspartate aminotransferase
ATP - adenosine triphosphate
AUC - Area Under the Curve
BBS - Bardet-Biedl Syndrome
BMI - body mass index
BS – Bartter Syndrome
CA199 - Carbohydrate antigen 19–9
CBC - complete blood cell count
CBCT - Cone-beam computed tomography
CD – collecting duct
CEA - Carcinoembryonic antigen
cfDNA - cell free DNA
CHRPE - congenital hypertrophy of the retinal pigment epithelium
circRNA - circular RNA
CKD - chronic kidney disease
CM - cutaneous melanoma
CNS - central nervous system
CNV - copy number variation
CRC - colorectal cancer
ctDNA - circulating tumor DNA
DEB - dystrophic epidermolysis bullosa
DNA - deoxyribonucleic acid
DT - desmoid tumors
EB - epidermolysis bullosa
EBS - epidermolysis bullosa simplex
ecircRNAs - exonic circRNAs
EDTA - ethylenediaminetetraacetic acid
EIciRNAs - exon-intron circRNAs
ENT - ears, nose and throat
ERG - electroretinography
ESCC - oesophageal squamous cell cancer
ESRD - end stage renal disease
FACS - fluorescence-activated cell sorting
FAP - familial adenomatous polyposis
PKD - polycystic kidney disease
circRNA - fusion circRNA
FF - fetal fraction
FISH - fluorescence in situ hybridization
FSH - follicle-stimulating hormone
GAPPS - gastric adenocarcinoma and proximal polyposis of the stomach
GFR - glomerular filtration rate
GGT - gamma- glutamyl transferase
GH - growth hormone
GnRH - gonadotropin-releasing hormone
HCC - Hepatocellular carcinoma
HDACs - Histone deacetylases
HGMD - Human Gene Mutation Database
IAM - immunofluorescence antigen mapping
ISS - idiopathic short stature
IFT - intra-flagella transport
IRES - Internal Ribosome Entry Site
ISCN - International System for Human Cytogenomic Nomenclature
IVF - in vitro fertilization
JEB - junctional epidermolysis bullosa
KATs - Histone Acetyltransferases
KEB - Kindler epidermolysis bullosa
LDH - Lactate dehydrogenase
LH - Luteinizing hormone
lncRNAs - long non-coding RNA
MACS - magnetic-activated cell sorting
mecciRNA - mitochondria-encoded circRNA
miRNA - MicroRNA
MKKS - McKusick–Kaufman syndrome
MKS - Meckel–Gruber Syndrome
NDI - nephrogenic diabetes insipidus
NGS - Next Generation Sequencing
NIFTY - Non-invasive Fetal Trisomy Test
NIPS - noninvasive prenatal screening
NIPT - non-invasive prenatal test
NPHP – nephronophthisis
NRCB - nucleated red blood cells
nt - nucleotide
OCT - optical coherence tomography
OGTT - oral glucose tolerance test
OMIM - Online Mendelian Inheritance in Man
OS – overall survival
PFS - progression-free survival
piRNA - piwi-interacting RNAs
SHBG - sex hormone binding globulin

PPV - positive predictive value
PT – prothrombin time
PTT – partial thromboplastin time
QF-PCR - quantitative fluorescent polymerase chain reaction
RBPs - RNA-binding proteins
RCD - rod-cone dystrophy
RFS - relapse-free survival
RNA - ribonucleic acid
rt-circRNAs - read-through circRNAs
SD - standard deviation
SGA - small for gestational age
SHOX - Short Stature Homeobox
snoRNA - small nucleolar RNA
SNP - Single nucleotide polymorphism
T2DM - Type 2 diabetes mellitus
TAL – thick ascending limb
TEM - transmission electron microscopy
TME - tumor microenvironment
V2R - vasopressin V2 receptor
WA - weeks of amenorrhea
WGS - whole genome sequencing
WHO - World Health Organization

ABSTRACT

The present habilitation thesis was made in accordance with the criteria recommended and approved by the National Council for Attestation of University Titles, Diplomas and Certificates (CNATDCU) and the methodology of the Doctoral School within the "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania.

The thesis entitled "Genetic heterogeneity and pleiotropy - the essence of multidisciplinarity in medical genetics" contains my academic, medical, and scientific achievements from the postdoctoral period (2014-2023) and the plans for evolution and career development in the three mentioned directions.

The thesis is structured in three sections as follows:

Section I comprises a synthesis of my academic, professional, and scientific achievements of the postdoctoral period, followed by four chapters presenting the major research themes as they have emerged over the last 10 years.

Chapter 1 entitled **"Chromosomal anomalies and their consequences"** includes two approaches to chromosomal anomalies, namely in the postnatal period and in the prenatal period. The first part presents a rare chromosomal abnormality, the sequential genetic testing strategy used in these cases, and genotype–phenotype correlations in partial trisomy 4q.

In the second part, two studies are presented that refer to non-invasive prenatal testing. The first of them analyzes the accuracy of fetal DNA testing in maternal blood, being a study that reports NIPT results on a group of 380 pregnant women. Various parameters were studied in correlation: detection rate, type of chromosomal abnormality, fetal fraction, fetal age and maternal age, indications for performing non-invasive testing.

The second study compares the effectiveness of two methods of isolating nucleated fetal erythroblasts that can be used later in prenatal screening: magnetic selection using the paramagnetic properties of hemoglobin and magnetic sorting using anti-CD71 antibodies.

Personal contributions in the field of "Chromosomal abnormalities and their consequences" were published in the following ISI journals: *Genes* (IF: 4.141), *Medicine* (IF: 2.948), and *Journal of Clinical Laboratory Analysis* (IF: 2.352).

Chapter 2 entitled **"Genetic heterogeneity and pleiotropy in cancer"** targets the second direction of research interest and has three subchapters. The first subchapter highlights the need for a multidisciplinary approach to cancer-predisposing syndromes and the usefulness of knowing the extra-organ manifestations affected by cancer in oncoprevention. In the last two subchapters, the value of using circular RNA as a biomarker in various types of cancer and genomic and epigenomic elements in melanoma are presented, elements that can constitute a premise for use as biomarkers.

Personal contributions in the field of oncogenetics have been published in the following ISI journals: *Diagnostics* (IF: 3.992), *Biomolecules* (IF: 6.064), and *International Journal of Molecular Sciences* (IF: 6.208).

Chapter 3 entitled **"Genetic heterogeneity and pleiotropy in Bardet-Biedl Syndrome - example of the multidisciplinary approach in ciliopathies"** brings together data on Bardet-Biedl Syndrome with reference to clinical, allelic, locus, and mutational heterogeneity and possible explanations of these phenomena. The chapter includes a study that quantified clinical aspects in 25 patients. At the end of this chapter, I summarized the main management directions as a result of collaboration with specialists from various medical specialties involved in the monitoring of these patients.

Personal contributions in the field of "Genetic heterogeneity and pleiotropy in Bardet-Biedl Syndrome - example of the multidisciplinary approach in ciliopathies" were published in the following ISI rated journals: *Genes* (IF: 4.141), *Journal of Multidisciplinary Healthcare* (IF: 2.919) and *American Journal of Medical Genetics Part A* (IF: 2.578).

Chapter 4 entitled **"Challenging diagnosis and multidisciplinary approach in rare diseases"** partially presents my concerns and those of the multidisciplinary teams I am part of in the vast field of rare diseases containing data on 5 rare diseases. Elements of genetic heterogeneity in two entities (Epidermolysis bullosa and Bartter Syndrome) and correlations with genetic testing and case management are presented at the beginning. This is followed by the presentation of a series of cases diagnosed with SHOX deletion highlighting the utility of genetic testing in the appropriate management of short stature. Coffin Siris syndrome and hereditary gingival fibromatosis are two rare diseases in which the differential diagnosis requires multidisciplinary clinical and paraclinical examinations.
Personal contributions in the field of rare diseases have been published in the following ISI rated journals: *Frontiers in Pediatrics* (IF: 3.569), *Diagnostics* (IF: 3.992), *Medicine* (IF: 2.948), *Experimental and Therapeutic Medicine* (IF: 2.751), *Genes* (IF: 3.688), *World journal of clinical cases* (IF: 1.534), and *International Journal of Molecular Sciences* (IF: 6.208).

Section II refers to future research directions in close connection with academic and medical activity. The strategies aim at the development of multidisciplinary research teams with already formed/new research cores from the university center where I carry out my activity, as well as from other centers according to the field of interest and competences of the partners. A particularly important aspect that I will promote is the involvement of young researchers (master's students, PhD students) but also students in these teams. I will also focus on identifying research resources and participating in research project competitions. The targeted research topics are based on the fields of interest from recent years or derived from them: chromosomal diseases, oncogenetics, rare diseases, epigenomics, nutrigenomics, genetic heterogeneity in genetic diseases.

Section III contains more than 800 bibliographic references that formed the basis of the thesis and the included articles.

REZUMAT

Prezenta teza de abilitare a fost realizată în conformitate cu criteriilor recomandate și aprobate de Consiliul Naţional de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU) și metodologia Școlii Doctorale din cadrul Universității de Medicină și Farmacie "Grigore T. Popa" Iași, România.

În teza intitulată **„Eterogenitatea genetică și pleiotropia – esența multidisciplinarității în genetica medicală"** sunt cuprinse realizările mele academice, medicale și științifice din perioada postdoctorală (2014-2023) și planurile de evoluție și de dezvoltare a carierei pe cele trei direcții amintite.

Teza este structurată în trei secțiuni astfel:

Secțiunea I cuprinde o sinteză a realizărilor academice, profesionale și științifice din perioada postdoctorală, urmată de patru capitole care prezintă temele majore de cercetare așa cum s-au conturat în ultimii 10 ani.

Capitolul 1 intitulat **"Anomaliile cromosomice și consecințele lor"** cuprinde două direcții de abordare a anomaliilor cromosomice și anume în perioada postnatală și în perioada prenatală. Prima parte prezintă o anomalie cromosomică rară, strategia secvențială de testare genetică utilizată în aceste cazuri și corelațiile genotip – fenotip din trisomia parțială 4q.
În a doua parte sunt prezentate două studii care fac referire la testarea prenatală neinvazivă. Primul dintre ele analizează acuratețea testării AND-ului fetal din sângele matern, fiind un studiu care raportează rezultatele NIPT pe un lot de 380 gravide. Au fost studiați în corelație diverși parametri: rata de detecție, tipul de anomalie cromosomică, fracția fetală, vârsta fetală și vârsta maternă, indicațiile de realizare a testării neinvazive.
Cel de-al doilea studiu compară eficacitatea a două metode de izolare a eritroblastelor fetale nucleate care pot fi utilizate ulterior in screeningul prenatal: selecția magnetică utilizând proprietățile paramagnetice ale hemoglobinei și sortarea magnetică utilizând anticorpi anti-CD71.
Contribuțiile personale în domeniul "Anomaliile cromosomice și consecințele lor" au fost publicate în următoarele reviste cotate ISI : *Genes* (IF: 4.141), *Medicina* (IF: 2.948), *Journal of Clinical Laboratory Analysis* (IF: 2.352).

Capitolul 2 intitulat **"Eterogenitate genetică și pleiotropie în cancer"** vizează a doua direcție de interes în cercetare și are trei subcapitole. Primul subcapitol evidențiază necesitatea abordării multidisciplinare a sindroamelor cu predispoziție la cancer și utilitatea cunoașterii manifestărilor extra organ afectat de cancer în oncoprevenție. În ultimele două subcapitole sunt prezentate valoarea utilizării ARN-ului circular ca biomarker în diverse tipuri de cancer și elemente genomice și epigenomice în melanom, elemente care se pot constitui ca și premisă pentru utilizarea ca biomarkeri.
Contribuțiile personale în domeniul oncogeneticii au fost publicate în următoarele reviste cotate ISI : *Diagnostics* (IF: 3.992), *Biomolecules* (IF: 6.064), *International Journal of Molecular Sciences* (IF: 6.208).

Capitolul 3 intitulat **"Eterogenitate genetică și pleiotropie în sindromul Bardet-Biedl – exemplu de abordare multidisciplinară în ciliopatii"** reunește date despre Sindromul Bardet-Biedl cu referire la eterogenitatea clinică, alelică, de locus și mutațională și posibile explicații ale acestor fenomene. În capitol este inclus un studiu care a cuantificat

aspectele clinice la 25 de pacienți. La finalul acestui capitol am sintetizat principalele direcții de management ca urmare a colaborării cu specialiști din diverse specialități medicale implicate în monitorizarea acestor pacienți.
Contribuțiile personale în domeniul "Eterogenitate genetică și pleiotropie în sindromul Bardet-Biedl – exemplu de abordare multidisciplinară în ciliopatii" au fost publicate în următoarele reviste cotate ISI: *Genes* (IF: 4.141), *Journal of Multidisciplinary Healthcare* (IF: 2.919) și *American Journal of Medical Genetics Part A* (IF: 2.578).

Capitolul 4 intitulat **"Provocări de diagnostic și abordarea multidisciplinară în bolile rare"** prezintă parțial preocupările mele și ale echipelor multidisciplinare din care fac parte în domeniul vast al bolilor rare conținînd date despre 5 boli rare. La început sunt prezentate elementele de eterogenitate genetică în două entități (Epidermoliza buloasă și Sindromul Bartter) și corelațiile cu testarea genetică și managementul cazurilor respective. Urmează apoi prezentarea unei serii de cazuri diagnosticate cu deleție de SHOX evidențiind utilitatea testării genetice în managementul adecvat al hipostaturii. Sindromul Coffin Siris și Fibromatoza gingivală ereditară sunt două boli rare la care diagnosticul diferențial necesită examene multidisciplinare clinice și paraclinice.
Contributiile personale in domeniul bolilor rare au fost publicate în următoarele reviste cotate ISI: *Frontiers in Pediatrics* (IF: 3.569), *Diagnostics* (IF: 3.992), *Medicina* (IF: 2.948), *Experimental and Therapeutic Medicine* (IF: 2.751), *Gene* (IF: 3.688), *World journal of clinical cases* (IF: 1.534) și *International Journal of Molecular Sciences* (IF: 6.208).

Secțiunea II face referire la direcțiile viitoare de cercetare în strânsă conexiune cu activitatea academică și medicală. Strategiile vizează dezvoltarea de echipe de cercetare multidisciplinare cu nuclee de cercetare deja formate/noi din centrul universitar în care îmi desfășor activitatea, precum și din alte centre în concordanță cu domeniul de interes și competențele partenerilor. Un aspect deosebit de important pe care îl voi promova îl constituie implicarea tinerilor cercetători (masteranzi, doctoranzi) dar și a studenților în aceste echipe. Un alt element de strategie pe care o voi aplica va fi identificarea resurselor de cercetare și participarea în competiții de proiecte de cercetare. Temele de cercetare vizate au la bază domeniile de interes din ultimii ani sau care derivă din acestea: bolile cromosomice, oncogenetica, bolile rare, epigenomica, nutrigenomica, eterogenitatea genetică în bolile genetice.

Secțiunea III conține peste 800 referințe bibliografice care au stat la baza realizării tezei de abilitare și a articolelor incluse.

I.OVERVIEW OF PROFESSIONAL, ACADEMIC AND SCIENTIFIC CONTRIBUTIONS

ACADEMIC ACTIVITY

I began my academic career in 2007 when I applied for the position of junior assistant professor in the Department of Medical Genetics of the Faculty of Medicine from the "Grigore T. Popa" University of Medicine and Pharmacy Iasi. I have completed all the steps of my academic career from assistant professor to associate professor as follows:

- 2007 – 2011, junior assistant professor of Medical Genetics;
- 2011 – 2016, assistant professor of Medical Genetics;
- 2016 – 2022, lecturer in Medical Genetics;
- 2022 – present, associate professor of Medical Genetics.

In 2008, I was admitted to the doctoral study program under the supervision of the distinguished Professor Mircea Covic, MD, PhD. The doctoral research topic was "Researches for improving the diagnosis, management and prevention of chromosomal diseases".

The main objectives of the doctoral thesis were:

- optimizing the clinical diagnosis in order to select the appropriate cytogenetic tests
- improving the cytogenetic diagnostic methods (through molecular techniques)
- improving the management of the families of the patients diagnosed with chromosomal diseases through genetic counseling and prenatal diagnosis
- improving prenatal diagnosis through invasive techniques or non-invasive prenatal diagnostic tests

The doctoral thesis's defense took place on the 1st of November, 2013. I obtained the academic degree of PhD in Medicine (Doctoral Diploma series I, no. 0006793) by Order of the Ministry of National Education no. 20 of January 21st, 2014.

In order to complete my teaching training, I graduated in 2010 from the Psychopedagogy Module for the training of the teaching personnel, organized by the Teacher's Training Department of the Faculty of Psychology and Educational Sciences from the "Alexandru Ioan Cuza" University Iasi. In the same period, in 2010, I took the "PRIME (Partnerships in International Medical Education) class for medical teachers getting the most out of teaching".

Since teaching to students also implied teaching in a foreign language, I also obtained two foreign language certifications (B2 level for French language - Certificate no. 618/25.05.2011; B2 level for English language – Certificate no. 974/7.10.2010) granted by the Center for Foreign Languages and Continuous Education, Faculty of Letters, "Alexandru Ioan Cuza" University Iași.

I conducted lectures and practical lessons in Medical Genetics with different categories of students from the Faculty of Medicine (Romanian and French Section); Dental Medicine (Romanian and French Section); General Medical Assistance Specialization in Iași, Botoșani and Bacău; Nutrition and Dietetics Specialization; Master programs' students.

I coordinated to completion 15 undergraduate theses of students from the Faculty of Medicine of "Grigore T. Popa" University of Medicine and Pharmacy Iasi, in the field of Human Genetics from 2012 to the present day. I am a member of the Doctoral Guidance Committee: one completed thesis and three ongoing doctoral theses.

I had practical activities and I gave lectures to resident doctors of several specialties: Medical Genetics, Paediatrics, Neonatology, Obstetrics-Gynaecology, Endocrinology, Pathology, Laboratory Medicine, Orthodontics, etc.

I coordinated 10 scientific papers at congresses for students and young doctors, one of them obtaining the first prize at the Internal Medicine Section at the 15th International Congress for Medical Students and Young Doctors 3rd-6th of May, 2018, Iasi, Romania:

- Oana Cornelia Gorduza, Andreea Florea, Mihnea Miron, Lavinia Caba Neurofibromatosis type I -Pleiotropic model.

I am a year tutor for the second-year students of the Faculty of Medicine, French program and in this position, I organized meetings to advise students on administrative or professional issues.

I was involved as a trainer in a project focused in professional development of different categories of medical personal (medical doctor, biologists):
"Improving the level of skills of professionals in the medical sector Professional training of medical personnel in medical genetics PROGEN". Project POCU/91/4/8/107623. Project Manager: Prof. Dr. Ileana Constantinescu, UMF Bucharest. Scientific Coordinator North East Region: Prof. Dr. Eusebiu Vlad Gorduza. Project value 11,702,201.54 lei. Implementation period 2 years (7.12.2017-7.12.2019) – project with completion date postponed to 1.12.2021.

I was actively involved in the administrative life of the university. I was a member of the Admissions Committee at the Faculty of Medicine (from 2008 – 2022) – supervision/scanning/envelopes committee; member of the Scanning/supervision Commission for the residency exam; secretary/member of the promotion committees (teaching assistant, lecturer, associated professor, professor) in the discipline of Medical Genetics; member of the commissions for obtaining the title of doctor/biologist specialist/principal or for filling the positions of medical genetics doctor in the medical network. Also, I was a member of the committee of Examination of Bachelor's Thesis in 2022.

The academic activity also included participation as a lecturer in postgraduate courses (medical genetics – 2018, 2022) and gastroenterology summer schools (2016, 2022 editions). At the invitation of the organizers, I held a workshop with the theme "How important is the family history in medical practice" at the 13th International Congress for Medical Students and Young Doctors 21st-24th of April, 2016, Iasi, Romania.

The teaching experience has materialized in writing as an author/co-author in 12 chapters of books/manuals/manual support and a book useful in the training of students, residents, and doctors.

1. **Lavinia Caba,** Eusebiu Vlad Gorduza. ***„Exerciții și teste de genetică medicală",*** Ed. Tehnopress Iaşi, 2022, ISBN 978-606-687-476-2
2. Maria-Christina Ungureanu, **Lavinia Caba**, Anamaria Hrișcă, Ștefana Bâlha. "Tratamentul cu rGH în tulburările de creștere - necesitatea unui diagnostic cât mai precis." – pp. 219-230 în Ingrith Miron, Laura Trandafir. ***"Actualități în Patologia Pediatrică",*** Ed. "Gr. T. Popa", U.M.F. Iasi 2022, ISBN: 978-606-544-674-8
3. Gorduza E. V., **Caba L**. *"Sfatul genetic",* pp. 464-481, în ***"Obstetrică",*** Socolov D., Grigore M., Nemescu D., Socolov R. (editori), 489 p, Ed. "Gr. T. Popa" Iași, 2020, ISBN 978-606-544-709-7
4. **Lavinia Caba**, Monica Cristina Pânzaru, E.V. Gorduza. Cap. "Sindromul congenital Zika" pp. 57-62. În **"Provocarea teoriei în practica medicală curentă"**. Ediția aV-a. Editura UMF "Gr. T. Popa" 2019, ISBN: 978-606-544-607-6
5. Monica-Cristina Pânzaru, Lăcrămioara Butnariu, **Lavinia Caba**, Roxana Popescu, Eva Gavril, Setalia Popa, Irina Resmeriţă, E.V. Gorduza, Cristina Rusu. Cap. "Aspecte imunologice în microdeleţia 22q11.2" pp. 282-287. În **"Provocarea teoriei în practica medicală curentă"**. Ediția aV-a. Editura UMF "Gr. T. Popa" 2019, ISBN: 978-606-544-607-6

6. Bembea M, Sandovici I, Pânzaru M, **Caba L**, Plăiașu V, Miclea D, Rusu C, Covic M. *Cap.15. "Genetica dezvoltării şi anomaliile congenitale"* – pp 463-502 în Covic M., Ștefănescu D., Sandovici I., Gorduza E.V. (editori), ***"Genetică Medicală"*** Ediţia a III-a revăzută integral şi actualizată, 678 p., Ed. Polirom, Iaşi 2017, ISBN 978-973-46-6526-6
7. **Lavinia Caba**, Eusebiu Vlad Gorduza. *Cap. IX. „Genetica în tulburările funcţionale digestive"*. – pp. 219-234 în Vasile Drug (editor), ***"Tulburări funcţionale digestive - actualităţi"***, 330p., Ed. Pro Universitaria Bucureşti, 2015, ISBN 978-606-26-0420-2
8. **Lavinia Caba**, Eusebiu Vlad Gorduza. *"La division cellulaire"* – pp. 35-56 în Eusebiu Vlad Gorduza (editor) „***Exercises et testes de génétique médicale***", 251 p., Ed. Tehnopress, Iaşi 2015, ISBN 978-606-687-234-8
9. **Lavinia Caba**, Eusebiu Vlad Gorduza. *„Les cas cliniques de syndromes chromosomiques"* – pp. 120-150 în Eusebiu Vlad Gorduza (editor) „***Exercises et testes de génétique médicale***", 251 p., Ed. Tehnopress, Iaşi 2015, ISBN 978-606-687-234-8
10. **Lavinia Caba**, Eusebiu Vlad Gorduza. *"Implicaţii practice ale progreselor geneticii în bolile inflamatorii intestinale"* – pp. 46-61 în Carol Stanciu, Anca Trifan, Ioan Sporea (editori), "***Bolile inflamatorii intestinale***", 400 p., Ed. "Gr. T. Popa", U.M.F. Iasi 2014, ISBN 978-606-554-220-7
11. Sandovici I, Covic M, Pânzaru M, **Caba L.** *Cap.15. "Genetica dezvoltării şi anomaliile congenitale"* – pp 495-538 în Covic M., Ștefănescu D., Sandovici I. (editori), ***"Genetică Medicală"*** Ediţia a II-a revăzută şi actualizată, 712 p., Ed. Polirom, Iaşi 2011, ISBN:978-973-46-1960-3
12. Lăcramioara Butnariu**,** Eusebiu Vlad Gorduza, **Lavinia Caba**, Monica Pânzaru, Elena Braha, Roxana Popescu, Cristina Rusu. Anomalii congenitale cardiace: Revizuirea descoperirilor recente legate de etiologia genetică, in Actualități în pediatrie, Nicolai Nistor, Alina-Costina Luca, Constantin C. Iordache, Ingrith Miron (editori), Editura Junimea, 2014, pag. 165- 170. ISBN 978-973-37-1798-0

MEDICAL ACTIVITY

I have graduated the Faculty of Medicine of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi in 1999. In 2000 I started my medical career as an intern at the Clinical Emergency Hospital "Sfantul Spiridon", Iasi. In the same year, I passed the national residency competition and I chose the specialty of family medicine. After one year, I once again passed the national residency competition. I changed the specialty and I chose the specialty of Medical Genetics.

In 2007 I completed the residency in the specialty of Medical Genetics and in 2008 I obtained the title of specialist physician in Medical Genetics. In 2013 I received clinical integration and I was employed as specialist physician in the Department of genetic explorations being part of the Immunology and Genetics Laboratory, "St. Spiridon" Emergency Clinical Hospital, Iasi. Six years later, in 2019, I sustained another exam and achieved the title of senior physician, being confirmed by the Ministry of Health.

During the residency, but also afterwards, I participated in various post-graduate training courses. Thus, I acquired skills and competences in the fields of interest, these being necessary for my teaching activity as well.

- 2012 **Oncogenetics** course Iasi, 24-26 October 2012, UMF "Grigore T. Popa" Iasi coordinator Prof. dr. Yves Jean Bignon
- 2013 Postgraduate course **"Les analises cytogenetiques dans la pathologie foetale**", Iasi, September 19-20, 2013

- 2014 Postgraduate course "**Management of chronic diseases**"; graduation certificate series CHX no 025 (Universitatea de Medicină și Farmacie „Grigore T. Popa" Iaşi, Universitatea de Medicină "Nicolae Testemitanu" Chisinau, Universitatea Nationala Medicala din Odessa) in the Project the East European Network of Excellence for Research and Development in Chronic Diseases CHRONEX-RD (2014-2015)
- 2015 „ **Oncogenetics – the current cancer prevention strategy**", UMF "Grigore T. Popa" Iasi
- 2017 **"Transfusion medicine – working protocols and practical clinical-therapeutic principles"** UMF "Grigore T. Popa" Iasi
- **The 2016 Course of Ichtiosys,** Iași, octombrie 2016, Coordinator prof dr. Cristina Haas, Universitatea Freiburg, Germany - Institutul de Chimie Macromoleculara Petru Poni Iași
- **Genodermatosis Network Training Session**, Iasi, June 2015 - Institutul de Chimie Macromoleculara Pentru Poni Iași
- Postgraduate course **"Management of genetic diseases - from theory to practice",** Bucuresti, 23 September 2014
- " **Evocative signs in Clinical Genetics " –** Gura Humorului, 25 September 2018
- "**Clinical Genetics**" - online, 25 February 2022

SCIENTIFIC ACTIVITY

Scientific activity is one of the three poles of a professor's activity in a medical university, along with teaching and medical activity. The three activities complement each other and influence each other.

The research activity started once I entered the residency when I started participating with poster-type scientific works at national and international medical genetics events. Later, I participated with scientific papers (oral/poster presentations) at national events organized by professional societies from other various specialties: internal medicine, pediatrics, gastroenterology and neurogastroenterology, surgery, cardiology, endocrinology, neonatology, laboratory medicine, infectious diseases, as well as demonstrations that focused on the topic of rare diseases.

The research carried out during my doctoral studies gave me the prerequisites to develop my research skills. The topic of the doctoral thesis related to chromosomal abnormalities represented a later concern, both postnatal and prenatal cytogenetic diagnosis being addressed. During my doctoral studies, I had the privilege of being a member of the team of an internal research project aimed at prenatal screening "Quantification of methylated fetal DNA - a new approach in noninvasive prenatal diagnosis of trisomy 21" - project director Prof. Eusebiu Vlad Gorduza, my mentor who supervised all my subsequent scientific activity. Worldwide, the year 2011 represents an important moment because NIPT was introduced into practice. During the postdoctoral period, I continued research related to the diagnosis and management of chromosomal abnormalities in the postnatal and prenatal periods. I participated as a member in a research grant "Implementation of an algorithm of diagnosis based on complex analyze of genomic profile of patients with congenital and developmental anomalies" (Project PN-II-PT-PCCA-2013-4-2240) took place during the period 2013-2017 - head of the team from "Grigore T. Popa" University of Medicine and Pharmacy from Iași in the national scientific project type "partnership" was Prof. Eusebiu Vlad Gorduza. In this project, different genetic analyzes (karyotype, FISH, MLPA, array-CGH) were applied with

the purpose of allowing a complete diagnosis of patients with multiple congenital anomalies selected in genetic centers from Iași, Bucharest, and Timișoara.

The results of this topics were published in the following papers:

- Popescu, R., Grămescu, M., **Caba, L.***, Pânzaru, M.C., Butnariu, L., Braha, E., Popa, S., Rusu, C., Cardos, G., Zeleniuc, M., Martiniuc, V., Gug, C., Păduraru, L., Stamatin, M., Diaconu, C.C., Gorduza, E.V. *A Case of Inherited t (4; 10)(q26; q26. 2) Chromosomal Translocation Elucidated by Multiple Chromosomal and Molecular Analyses. Case Report and Review of the Literature.* Genes (Basel) 2021, 12(12):1957
- Gug, C., Mozos, I., Ratiu, A., Tudor, A., Gorduza, E.V., **Caba, L.**, Gug, M., Cojocariu, C., Furau, C., Furau, G., Vaida, M.A., Stoicanescu, D. *Genetic Counseling and Management: The First Study to Report NIPT Findings in a Romanian Population*. Medicina (Kaunas) 2022, 58: 79.
- Nemescu, D., Constantinescu, D., Gorduza, V., Carauleanu, A., **Caba, L.** and Navolan, D.B. *Comparison between paramagnetic and CD71 magnetic activated cell sorting of fetal nucleated red blood cells from the maternal blood.* J Clin Lab Anal 2020, 34(9): e23420.
- Sireteanu, A., Popescu, R., Braha, E.E., Bujoran, C., Butnariu, L., **Caba, L**., Graur, E., Gorduza, E.V., Grămescu, M., Ivanov, I.C. and Pânzaru, M. *Detection of chromosomal imbalances using combined MLPA kits in patients with syndromic intellectual disability.* Rev Rom Med Lab 2014, 22(2): 157-164.
- Butnariu, L., Gramescu, M., **Caba, L.**, Panzaru, M., Braha, E., Popescu, R., Popa, S., Rusu, C., Cardos, G., Zeleniuc, M., Martiniuc, V., Plaiasu, V., Diaconu, C., Gorduza, E.V. *Using of multiple chromosomal and molecular analyses to elucidate the etiology of plurimalformative syndromes.* Rev Med Chir Soc Med Nat Iasi 2017, 121(3): 581-593.
- Pânzaru, M., **Caba, L.**, Rusu, C., Butnariu, L., Braha, E., Popescu, R., Grămescu, M., Popa, S., Resmerita, I., Bujoran, C., Martiniuc, V., Gorduza, E.V. *Phenotypic variability in Edwards syndrome: synopsis of 19 cases with trisomy 18.* Rev Med Chir Soc Med Nat Iasi 2017, 121(1): 172-177.

Another research topic addressed was oncogenetics. In this field, I gained skills by participating in the two postgraduate courses organized by UMF Iasi, the first of them in collaboration with Prof. Dr. Yves Jean Bignon. The topic of cancer-predisposing syndromes and genetic and epigenetic changes in familial or sporadic cancer is reflected in the following published works:

- Antohi, C., Haba, D., **Caba, L.***, Ciofu, M.L., Drug, V.L., Bărboi, O.B., Dobrovăț, B.I., Pânzaru, M.C., Gorduza, N.C., Lupu, V.V., Dimofte, D., Gug, C., Gorduza, E.V. *Novel Mutation in APC Gene Associated with Multiple Osteomas in a Family and Review of Genotype-Phenotype Correlations of Extracolonic Manifestations in Gardner Syndrome.* Diagnostics (Basel) 2021, 11(9) :1560.
- **Caba, L.**, Florea, L., Gug, C., Dimitriu, D.C., Gorduza, E.V. *Circular RNA—Is the Circle Perfect?* Biomolecules 2021, *11*(12), 1755.
- Zob, D. L., Augustin, I., **Caba, L.***, Panzaru, M. C., Popa, S., Popa, A. D., Florea, L., Gorduza, E. V. *Genomics and Epigenomics in the Molecular Biology of Melanoma—A Prerequisite for Biomarkers Studies*. Int J Mol Sci 2022, 24(1), 716.

Ciliopathies are rare genetic diseases, characterized by great heterogeneity and pleiotropy. The interest in such as entity - Bardet-Biedl syndrome - materialized through the publication of two synthesis articles regarding the phenomenon of heterogeneity (locus, allelic, mutational, and clinical) and the multidisciplinary management of this syndrome. I participated in a study that looked at the clinical aspects of a group of 25 patients with BBS

being the first in Romania as a result of the collaboration between several medical genetics centers.

- Florea, L., **Caba, L.*** and Gorduza, E.V. *Bardet–Biedl Syndrome—Multiple Kaleidoscope Images: Insight into Mechanisms of Genotype–Phenotype Correlations.* Genes (Basel) 2021, 12(9): 135.
- Focsa, I.O., Budisteanu, M., Stoica, C., Nedelea, F., Jurca, C., **Caba, L.**, Butnariu, L., Panzaru, M., Rusu, C., Balgradean, M. *Clinical Aspects of a Rare Disease: Bardet Biedl Syndrome.* Modern Medicine 2022, 29(1): 37.
- **Caba, L.**, Florea, L., Braha, E. E., Lupu, V. V., & Gorduza, E. V. *Monitoring and Management of Bardet-Biedl Syndrome: What the Multi-Disciplinary Team Can Do*. J Multidiscip Health 2022,15, 2153-2167.
- Khan, S., Focșa, I. O., Budișteanu, M., Stoica, C., Nedelea, F., Bohîlțea, L., **Caba, L**., Butnariu, L., Panzaru, M., Rusu, C., Jurca, C., Chirita-Emandi, A., Banescu, C., Abbas, W., Sadeghpour, A., Baig, S. M., Balgradean, M., Davis, E. E. *Exome sequencing in a Romanian Bardet-Biedl syndrome cohort revealed an overabundance of causal BBS12 variants*. Am J Med Genet 2023, 1–16.

Another area of interest is that of rare diseases. Rare diseases are a topic of interest worldwide, in Europe, but also in Romania because they are numerous, but the patients are few and sometimes the data is insufficient to establish management protocols. In this context, case presentation type articles are valuable through the information they bring to the characterization of diseases. The literature review type paper becomes valuable for the practicing doctor due to the synthesis achieved on a rare topic. The related works that target this topic of interest are the following:

- Florea, L., **Caba, L.*,** and Gorduza, E.V. *Genetic Heterogeneity in Bartter Syndrome: Clinical and Practical Importance*. Front Pediatr 2022, *June 3;10:908655. doi: 10.3389/fped.2022.908655. eCollection 2022.*
- Pânzaru, M.C., **Caba, L.***, Florea, L., Braha, E.E, Gorduza, E.V. *Epidermolysis Bullosa - a different genetic approach in correlation with genetic heterogeneity*. Diagnostics (Basel) 2022, 12(6): 1325.
- Ungureanu, M.-C., Hrisca, A., **Caba, L**., Teodoriu, L., Bilha, S., Preda, C., Leustean, L. *SHOX Deletion and Idiopathic Short Stature: What Does the Clinician Need to Know? Case Series Report*. Diagnostics (Basel) 2023, 13, 105.
- Momanu, A., **Caba, L.***, Gorduza, N.C., Arhire, O.E., Popa, A.D., Ianole, V. and Gorduza, E.V. *Gorham-Stout Disease with Multiple Bone Involvement—Challenging Diagnosis of a Rare Disease and Literature Review*. Medicina (Kaunas) 2021, 57(7):681.
- **Caba, L**., Gug, C., & Gorduza, E. V. *Heterogeneity in combined immunodeficiencies with associated or syndromic features*. Exp Ther Med 2021, 21(1): 1-1.
- Gug, C., **Caba, L**.*, Mozos, I., Stoian, D., Atasie, D., Gug, M., & Gorduza, E. V. *Rare splicing mutation in COL1A1 gene identified by whole exomes sequencing in a patient with osteogenesis imperfecta type I followed by prenatal diagnosis: A case report and review of the literature.* Gene 2020, 741: 144565
- Panzaru, M. C., Florea, A., **Caba, L.,** & Gorduza, E. V. *Classification of osteogenesis imperfecta: Importance for prophylaxis and genetic counseling*. World J Clin Cases 2023, 11(12), 2604-2620.
- Ciobanu, C. G., Nucă, I., Popescu, R., Antoci, L. M., **Caba, L**., Ivanov, A. V., ... & Pânzaru, M. C. *Narrative Review: Update on the Molecular Diagnosis of Fragile X Syndrome*. Int J Mol Sci 2023, 24(11), 9206.

- Bilha, S.C., Teodoriu, L., Velicescu, C. and **Caba, L**. *Pituitary hypoplasia and growth hormone deficiency in a patient with Coffin-Siris syndrome and severe short stature: case report and literature review*. Arch Clin Cases *2022*, 9(3): 121-125.
- Spiridon, M. R., Petris, A. O., Gorduza, E. V., Petras, A. S., Popescu, R., & **Caba, L.** *Holt-Oram syndrome with multiple cardiac abnormalities.* Cardiol Res 2018, 9(5): 324.
- **Caba, L**., Ungureanu, C., Grămescu, M., Pânzaru, M., Butnariu, L., Popescu, R., Resmeriţă, I., Saad, H. and Gorduza, E.V. *A new case of hereditary gingival fibromatosis.* Romanian Journal of Functional & Clinical, Macro-& Microscopical Anatomy & of Anthropology/Revista Româna de Anatomie Functionala si Clinica, Macro si Microscopica si de Antropologie 2017, 16(2): 143-147.
- Butnariu, L.I., Rusu, C., Pânzaru, M., **Caba, L.,** Popescu, R. and Gorduza, E.V. *Cleidocranial dysplasia: a case report*. Romanian Journal of Functional & Clinical, Macro-& Microscopical Anatomy & of Anthropology/Revista Româna de Anatomie Functionala si Clinica, Macro si Microscopica si de Antropologie 2017, *16*(2): 163-167.
- Pânzaru, M., Butnariu, L., Popescu, R., **Caba, L.**, Grămescu, M., Popa, S., Resmeriță, I., Gorduza, E.V. and Rusu, C. *Esophageal atresia and genetic conditions.* Romanian Journal of Functional & Clinical, Macro-& Microscopical Anatomy & of Anthropology/Revista Româna de Anatomie Functionala si Clinica, Macro si Microscopica si de Antropologie 2017, 16(1); 69-73.

In order to acquire useful skills in medical research, I participated in the "8th Meta-analysis Round", 27-28 February, 2019, Pécs, Hungary, and two Training Schools links to IBS within a project COST ACTION BM1106 - GENIEUR:

- 2014 - GENIEUR Training School (COST ACTION BM1106 – GENIEUR): „Assessment of IBS in Clinical Practice & Research: An Introduction for Scientists", Barcelona, Spain;
- 2016 - GENIEUR Training School (COST Action BM 1106 GENIEUR Training School Heidelberg "Molecular Methods in IBS Research: genetics, epigenetics and microbiota research"

The topic of genetics in IBS was capitalized through a book chapter (**Lavinia Caba**, Eusebiu Vlad Gorduza. Chapter IX. "Genetics in digestive functional disorders" in Vasile Drug (editor), "Digestive functional disorders - current affairs", Ed. Pro Universitaria Bucharest, 2015, ISBN 978-606-26-0420-2) and by a participation in Internal Research Projects 2017 at UMF Iasi with a grant proposal that was eligible, but not funded.

- Molecular Epidemiology Studies in Rome IV Irritable Bowel Syndrome Patients

  Project director: Lecturer Lavinia Caba

  Eligible proposal, 82 points - unfunded

I am a member of scientific national and international organizations:

- Romanian College of Physicians;
- Romanian Society of Medical Genetics;
- Romanian Society for Endocrinology;
- European Society of Human Genetics;
- European Cytogeneticists Association;
- European Society for Clinical Investigations.

As a result of the recognition of the scientific activity, our team has 5 articles awarded in PRECISI 2021 (found in the complete list of publications).

I am co-author of a chapter in the ***Tratat de Genetica Medicala*** (eds. Mircea Covic, Dragoș Ștefănescu, Ionel Sandovici and Vlad Gorduza) which was awarded in 2019 with the "C.I. Parhon" prize of the Romanian Academy.

International scientific visibility is reflected by:

- Hirsch index (Clarivate Analytics): **7**
- Number of publications in Clarivate Analytics database: **47**
- Cumulative impact factor (main author): **51.795**
- Total number of citations without self-citations (Clarivate Analytics): **114**
- Mean citation number/paper published = **2.79**
- h-index (Google Scholar) = **10**
- ORCID: 0000-0001-6327-4461

The scientific portfolio:
Book Author – 1
Book chapter author and co-author - 11

- **Papers published in extenso**:
  - 24 in ISI journals (15 - main author)
  - 16 in BDI journals (5 – main author)
  - 1 article - ISI indexed manifestation
  - Others papers in extenso – 38 (7 - main author)
- **100 papers published in abstract**:
  - 40 in ISI journals (11 - main author)
  - 60 in non-indexed journals/proceedings of different scientific meetings (16 - main author)

Oral presentations at national and international scientific meetings – 71 (26 - international)
Poster presentations at international and national scientific manifestations – 87 (46 international with 13 – main author; 41 national with 10 main author).

Chapter 1. CHROMOSOMAL ANOMALIES AND THEIR CONSEQUENCES

The results of researches about chromosomal anomalies and their consequences were published in the following papers
ISI ARTICLES
Popescu, R., Grămescu, M., **Caba, L.***, Pânzaru, M.C., Butnariu, L., Braha, E., Popa, S., Rusu, C., Cardos, G., Zeleniuc, M., Martiniuc, V., Gug, C., Păduraru, L., Stamatin, M., Diaconu, C.C., Gorduza, E.V. *A Case of Inherited t (4; 10)(q26; q26. 2) Chromosomal Translocation Elucidated by Multiple Chromosomal and Molecular Analyses. Case Report and Review of the Literature.* Genes (Basel) 2021, 12(12):1957 (**corresponding author**). **IF=4.141**
Gug, C., Mozos, I., Ratiu, A., Tudor, A., Gorduza, E.V., **Caba, L.**, Gug, M., Cojocariu, C., Furau, C., Furau, G., Vaida, M.A., Stoicanescu, D. *Genetic Counseling and Management: The First Study to Report NIPT Findings in a Romanian Population*. Medicina (Kaunas) 2022, 58: 79. **IF=2.948**
Nemescu, D., Constantinescu, D., Gorduza, V., Carauleanu, A., **Caba, L.** and Navolan, D.B. *Comparison between paramagnetic and CD71 magnetic activated cell sorting of fetal nucleated red blood cells from the maternal blood.* J Clin Lab Anal 2020, 34(9): e23420. **IF=2.352**
Sireteanu, A., Popescu, R., Braha, E.E., Bujoran, C., Butnariu, L., **Caba, L**., Graur, E., Gorduza, E.V., Grămescu, M., Ivanov, I.C. and Pânzaru, M. *Detection of chromosomal imbalances using combined MLPA kits in patients with syndromic intellectual disability.* Rev Rom Med Lab 2014, 22(2): 157-164. **IF=0.239.**
BDI ARTICLES
Butnariu, L., Gramescu, M., **Caba, L.**, Panzaru, M., Braha, E., Popescu, R., Popa, S., Rusu, C., Cardos, G., Zeleniuc, M., Martiniuc, V., Plaiasu, V., Diaconu, C., Gorduza, E.V. *Using of multiple chromosomal and molecular analyses to elucidate the etiology of plurimalformative syndromes.* Rev Med Chir Soc Med Nat Iasi 2017, 121(3): 581-593.
Pânzaru, M., **Caba, L.**, Rusu, C., Butnariu, L., Braha, E., Popescu, R., Grămescu, M., Popa, S., Resmerita, I., Bujoran, C., Martiniuc, V., Gorduza, E.V. *Phenotypic variability in Edwards syndrome: synopsis of 19 cases with trisomy 18.* Rev Med Chir Soc Med Nat Iasi 2017, 121(1): 172-177.

1.1. Introduction

Chromosomal anomalies are very important in pathology, because they produce major phenotypic and reproductive consequences. They produce over 100 chromosomal syndromes affecting 2% of the pregnancies of women older than 35 years, and 50% of miscarriages in the first trimester of pregnancy. There are two types of chromosomal anomalies regarding the phenotypic effect: unbalanced and balanced anomalies.

The identification of chromosomal abnormalities is important in genetic counseling and in the correct management of the respective family.

Unbalanced chromosomal abnormalities produces chromosomal diseases, identifiable by classic cytogenetic techniques combined with molecular technology (FISH, MLPA, array-CGH).

1.2. Congenital anomalies in patients with unbalanced translocation t(4;10)

1.2.1. Introduction

Congenital anomalies are structural defects produced during the prenatal period and are present at birth [WHO 2021]. Usually, the severe syndromes with congenital anomalies (SCAs) are generated by an unbalanced chromosomal rearrangement that disrupts large numbers of developmentally important genes, which results in specific and complex phenotypes. The confirmation of a chromosomal anomaly requires GTG-banded chromosomal analysis in association with supplementary analyses, such as FISH, MLPA, or array-CGH [Covic 2017; Shaffer, Theisen, 2010].

Partial trisomy 4q is a rare unbalanced structural chromosomal anomaly that was first described in the early 1970s [Francke 1972; Surana, Conen, 1972; Schrott et al., 1974]. Since then, less than 100 cases have been described. Some anomalies were *de novo*, but the majority are derived from the malsegregation of a parental balanced chromosomal anomaly. In such cases, the phenotype is induced by the association of 4q partial trisomy with partial monosomy of a different chromosome [Zhang et al., 2009]. The delineation of genotype–phenotype correlation in 4q trisomy is difficult because the described cases had different breakpoints and presented different associated monosomies and the ages of reported patients were variable [Battaglia et al., 2005; Cernakova et al., 2006].

Partial 10q monosomy was first described in 1978, and since then, about 110 cases have been reported in the literature [Lewandowski et al., 1978]. The chromosomal anomalies are: terminal 10q monosomy, terminal 10q monosomy associated with a partial trisomy of a different chromosome, and 10q26.1 microdeletion. The symptomatology is variable and depends on the type of anomaly, the fragment size, and coexistence with other anomalies. The phenotypic spectrum is heterogeneous, and it is characterized by some common clinical features, such as craniofacial anomalies, developmental delay, intellectual disability, urinary tract abnormalities, and heart defects [Irving et al., 2003; Scigliano et al., 2004; Kehrer-Sawatzki et al., 2005; Courtens et al., 2006; Yatsenko et al., 2009; Lin et al., 2016].

We present a case with a multiple congenital anomalies syndrome, identified during the neonatal period, that has an association between partial 4q trisomy and partial 10q monosomy. These anomalies were generated by the malsegregation of the maternal chromosomes 4 and 10, which are involved in a balanced reciprocal translocation. The 4q fragment located on the derivative chromosome 10 also presents a small 4q35.2 duplication, also inherited from the mother.

1.2.2. Materials and methods

1.2.2.1. Clinical Evaluation

The patient underwent a multidisciplinary evaluation, including the following specialties: genetics, pediatrics, cardiology, and neonatology. The patient's parents gave written informed consent (including for publication of images) considering the Declaration of Helsinki. This study was approved by the Ethics Committee for Scientific Research of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania.

1.2.2.2. Cytogenetic Analyses

Peripheral blood lymphocytes were cultured in a growth medium (RPMI 1640, Gibco®, Thermo Fisher Scientific Inc, Massachusetts, USA). Metaphase chromosomes were harvested, and slides were made for analysis. GTG banding was used for staining (at the 550-band level). Chromosomal analysis was performed using CytoVision software 2.81 (Applied

Imaging, USA), and the aberrations and karyotypes were classified according to the International System for Human Cytogenomic Nomenclature (ISCN 2016).

1.2.2.3. MLPA Technique

We used commercially available P036 and P070 SALSA® MLPA® kits (MRC-Holland, Amsterdam, The Netherlands). These kits screen for subtelomeric copy number variations and contain one MLPA probe for each subtelomeric region except for the short arms of autosomal acrocentric chromosomes (13p, 14p, 15p, 21p, and 22p), for which a probe on the q arm, close to the centromere, is included instead. To confirm the abnormalities detected, we applied kits P264 (for subtelomeric regions of chromosomes 1q, 2q, 3q, and 4q) and P286 (for subtelomeric regions of chromosomes 9q, 10q, 11q, and 12q) (MRC-Holland, Amsterdam, The Netherlands).
The DNA extraction from peripheral blood was performed using the Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA). The standard MLPA analysis was performed according to the manufacturer's instructions. Briefly, 300 nanograms of genomic DNA were denatured and hybridized with SALSA probes at 60 °C for 16 hours. After 15 minute ligation at 54 °C, PCR was performed in a Gradient Palm-Cycler (Corbett Research, Mortlake, NSW, Australia), available in a 96-well format, using Cy5 universally labeled primers. Fluorescent amplification products were subsequently separated through capillary electrophoresis in a CEQ 8000 GeXP Genetic Analysis System (Beckman Coulter, Brea, CA, USA) sequencer and were analyzed using the default software. The number of DNA copies was estimated using the Coffalyser.NET V9 software (MRC-Holland, Amsterdam, The Netherlands), which calculates the ratio of peak areas in test samples over those of normal controls for each target sequence. Reference DNA samples were collected from healthy individuals (without developmental delay, intellectual disabilities, or congenital abnormalities), prepared using the same DNA extraction method, and previously evaluated for copy number variation of target areas. There was a minimum of 3 reference samples per run, randomly distributed, with one more for every 7 test samples.

1.2.2.4. Array-CGH Technique

Array Comparative Genomic Hybridization (aCGH) analysis was performed using the Sure Print G3 ISCA V2 CGH 8x60K Array Kit (Agilent Technologies®, Santa Clara, CA, USA), the NimbleGen MS 200 Microarray Scanner (Roche Applied Science, Basel, Switzerland), and NimbleGen MS 200 Software v1.1.

1.2.3. Results

Clinical Presentation

We present a boy with a mild development delay and multiple congenital anomalies syndrome, resulted for a pregnancy supervised clinically and by prenatal screening. Prenatal ultrasound scan (second trimester) was normal and biochemical screening (maternal serum markers – alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol at 16th weeks of pregnancy) showed values without risk for chromosomal abnormalities. Parents are both healthies, youngs (father - 32 years, mother - 28 years), unrelated, with no "known" family history of genetic disorders or birth defects. The child is the first offspring of the couple, and he was born at 38 WA, with a weight of 2350 gr, height 50 cm, head circumference 35 cm, and an Apgar score of 8 at 1 minute and 8 at 5 minutes. The patient was first examined during the neonatal period and was re-evaluated at 6 months. He presented some facial dysmorphic features: flattened skull in the occipital region, with slight turricephaly; tall and broad forehead; hypertelorism—inner canthal distance 2.7 cm (+2SD), outer canthal distance

6.3 cm (+2SD); interpupillary distance 4.6 cm (+2SD); deep-set eyes, down slanting and short palpebral fissures; epicanthic folds; prominent nose, with wide root and bulbous tip; microstomia and micro-retrognathia; large, short philtrum with prominent reliefs; low set, prominent ears, with big scaphoid fossa and big cavum concha. At age of 6 months he developed the following skills: follows moving objects with eyes; listens to and follows simple directions; cries, babbles, and coos; reaches for objects and grasps; puts objects in mouth; lifts head and sits with back straight; smiles spontaneously. The ultrasound scan revealed a persistent ductus arteriosus (ΔP = 5 mm, velocity = 1 m/s, with left-to-right shunting) and atrial septal defect ostium secundum (3 mm, with left-to-right shunting), but without changes in kidneys. The cardiologist decided that no surgery was needed.

GTG banding showed a 46,XY,der(10)(10pter→10q26.2::4q26→4qter) chromosomal formula (Figure I.1.) that imposed parental karyotype. The father was normal, but the mother have a balanced reciprocal translocation between chromosomes 4 and 10: 46,XX,t(4;10)(q26;q26.2) (Figure I.2.).

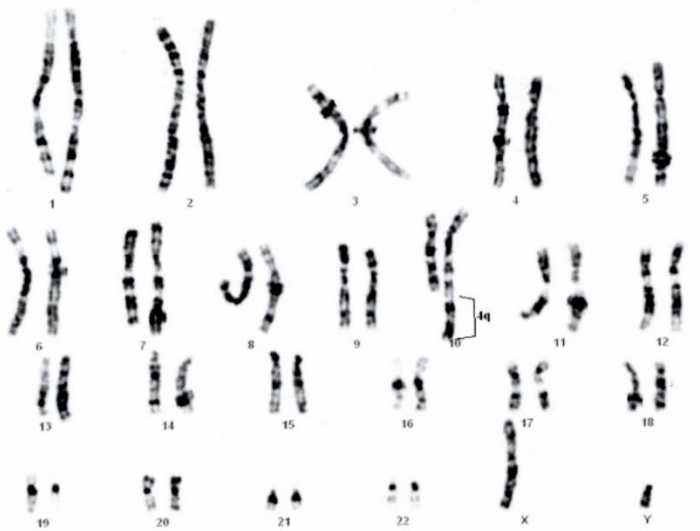


Figure I.1. Child's karyotype 46,XY,der(10)(10pter→10q26.2::4q26→4qter).

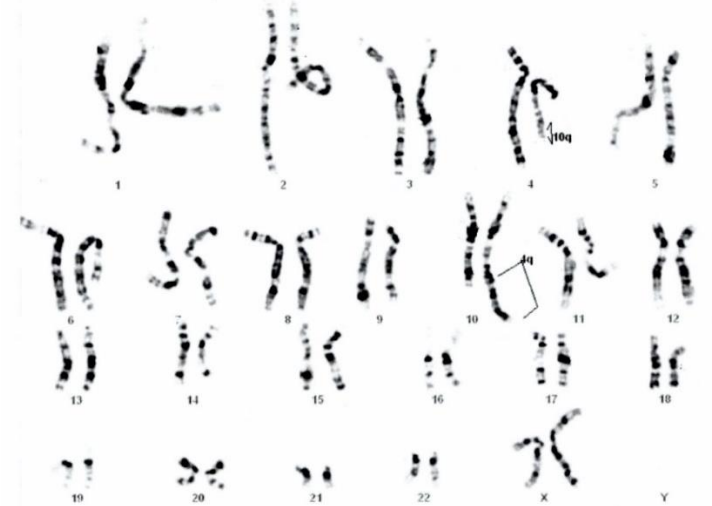


Figure I.2. Mother's karyotype 46,XX,t(4; 10)(q26; q26.2).

Using the MLPA technique with telomere probes (P036 and P070, MRC-Holland, Amsterdam, The Netherlands), we discovered a 1.5 fold amplification of the 4q35.2-4q35.1 segment (concordant with a 4q partial trisomy) and a 0.5 fold amplification of the 10qter segment (concordant with a partial 10q monosomy); we also discovered a 2-fold amplification of the 4q35.2 segment (concordant with a 4q partial tetrasomy—a segment that includes the

ZPF42 and *TRIML2* genes). To validate these changes, we used MLPA probe P264 (for subtelomeric regions of chromosomes 1q, 2q, 3q, and 4q) and probe P286 (for subtelomeric regions of chromosomes 9q, 10q, 11q, and 12q).

To complete the genetic investigation, we applied an array-CGH that confirmed a 71,057 kb triplication of the 4q26-q35.2 region (genomic coordinates on chromosome 4: 119839900—190896674) (Figure I.3.), a 562 kb microdeletion of the 10q26.3 region (genomic coordinates on chromosome 10: 134872533—135434178) (Figure I.4.), and a 795 kb quadruplication of the 4q35.2 region (Figure I.5.).

The triplication of 71,057 kb of the 4q26–q35.2 region was pathogenic. The microdeletion of 562 kb in the 10q26.3 region is probably not pathogenic. To verify if the 4q35.2 quadruplication is pathogenic, we performed array-CGH on both parents, and the mother presented a triplication of 795 kb in the 4q35.2 region (Figure I.6.).

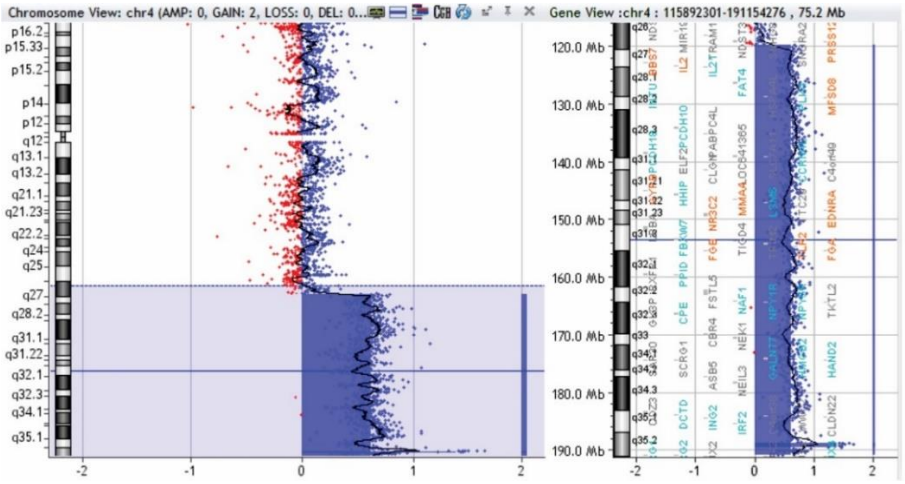


Figure I.3. Array-CGH test of patient—triplication of 71,057 kb in the 4q26-q35.2 region (shaded in blue). Red and blue dots represent the log2 ratio of fluorescence (Cy5/Cy3), calculated by analytical software; log2 ratio at 1 indicates a duplication of the DNA of the region (3 copies) in the test sample versus the control (in blue color), and a log2 ratio at -1 indicates a deletion of the DNA of that region in the test sample versus the control (red deviation).

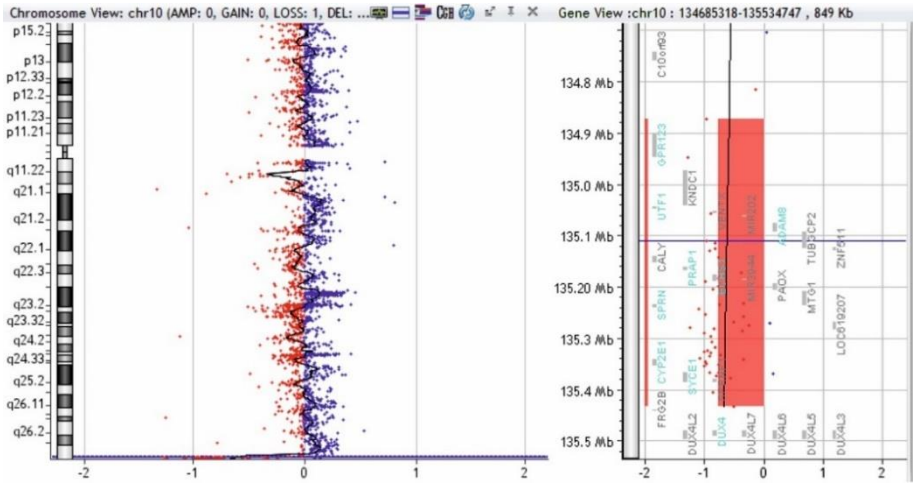


Figure I.4. Array-CGH test of patient—microdeletion of 562 kb in the 10q26.3 region (shaded in red). Red and blue dots represent the log2 ratio of fluorescence (Cy5/Cy3), calculated by analytical software; log2 ratio at 1 indicates a duplication of the DNA of the region (3 copies) in the test sample versus the control (in blue color) and a log2 ratio at -1 indicates a deletion of the DNA of that region in the test sample versus the control (red deviation).

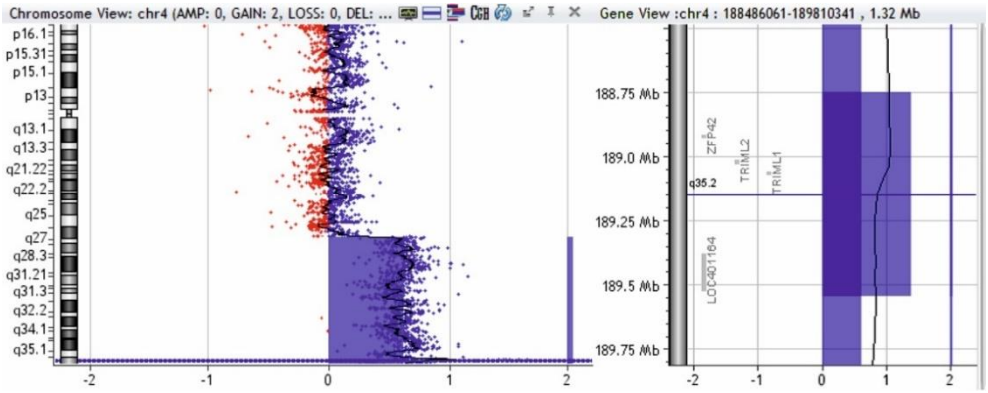


Figure I.5. Array-CGH test of patient—quadruplication of 795 kb in the 4q35.2 region (shaded in blue). Red and blue dots represent the log2 ratio of fluorescence (Cy5/Cy3), calculated by analytical software; log2 ratio at 1 indicates a duplication of the DNA of the region (3 copies) in the test sample versus the control (in blue color), and a log2 ratio at -1 indicates a deletion of the DNA of that region in the test sample versus the control (red deviation).

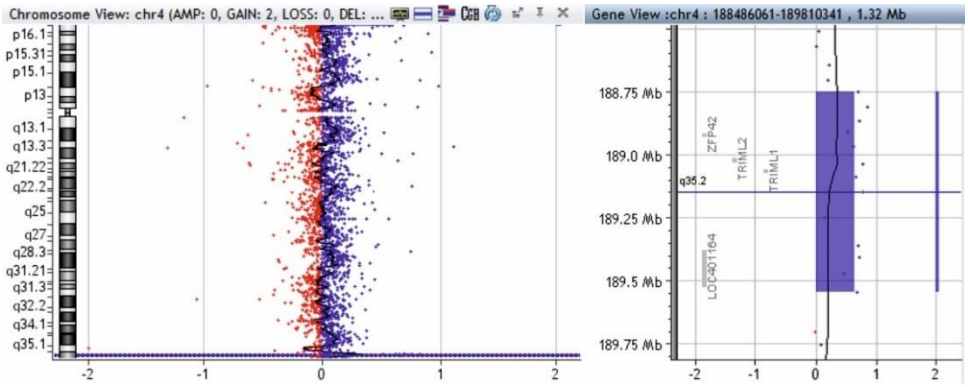


Figure I.6. Array-CGH test of patient's mother—triplication of 795 kb in the 4q35.2 region (shaded in blue). Red and blue dots represent the log2 ratio of fluorescence (Cy5/Cy3), calculated by analytical software; log2 ratio at 1 indicates a duplication of the DNA of the region (3 copies) in the test sample versus the control (in blue color) and a log2 ratio at -1 indicates a deletion of the DNA of that region in the test sample versus the control (red deviation).

1.2.4. Discussion

We present a case that associates an important 4q partial trisomy (4q26–q35.2) and a small 10q partial monosomy (10q26.3). These chromosomal anomalies originate from the mother, who carries a balanced reciprocal translocation t(4q;10q)(q26;q26.3). Moreover, the son presents a 795 kb quadruplication in the 4q35.2 region, inherited on maternal line. Due to the small size of the 10q partial monosomy, we could presume that the phenotype of our patient is determined mainly by the 4q partial trisomy.

Our case is the second cited in the literature. Zhang et al. presented a patient that associated a 4q partial trisomy (4q26–q35.2) and a 10q microdeletion (10q26.3) [Zhang et al., 2009]. The breakpoints on chromosomes 4 and 10 are similar in these two cases. The breakpoints on chromosome 4q are located at 119,839,900 bp from the 4p telomere in our case and at 118,785,802 bp from the 4p telomere in the case presented by Zhang et al. The breakpoints on chromosome 10q are located at 134,872,533 bp from the 10p telomere in our case and at 134,750,859 bp from the 10p telomere in the case presented by Zhang et al. Thus, in both cases, the size of the 4q duplication is approximately 71 Mb, while the 10q microdeletion's size is approximately 0.55 Mb.

Trisomy 4q syndrome is a heterogeneous disorder because the reported patients have different breakpoints located on the long arm of chromosome 4; some patients associate different monosomies, and the age of reported patients vary between newborn and adulthood [Celle et al., 2000]. The breakpoints in trisomy 4q syndrome are distributed throughout the long arm of chromosome 4, with an only one exception: 4q11 band. The distribution of different types of 4q partial trisomy is presented in Figure I.7.

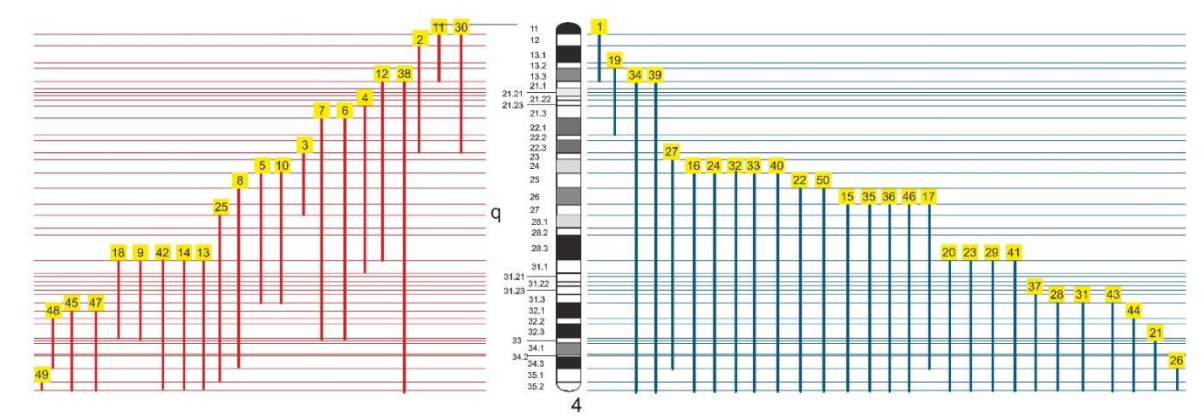


Figure I.7. Ideogram of chromosome 4 and distribution of breakpoints in 4q partial trisomy. Red lines represent different 4q duplications identified by different authors; blue lines represent the cases with a translocation between chromosome 4 and other chromosomes; the numbers 1–50 represent the references presented in Table I.1.

Table I.1. Different types of 4q partial trisomy.

No	Reference	Chromosome 4 region	Type of anomaly
1	Mattei et al. 1979	q12–q13	IH, t(2;4) mat
2	Zollino et al. 1995	q13.3–q22.2	DN, dup(4)(q13.3q22.2)
3	Halal et al. 1991	q23–q27	DN, dup(4)(q23q27)
4	Jeziorowska et al. 1993	q21.3–q31.3	DN, dup(4)(q21.3q31.3)
5	Fryns 1980	q25–q31	DN, dup(4)(q25q31)
6	Vogel et al. 1975	q22–q34	DN, dup(4)(q22q34)
7	Dutrillaux et al. 1975	q22–q34	DN, dup(4)(q22q34)
8	Taylor et al. 1977	q26–q35	DN, dup(4)(q26q35)
9	Goodman et al. 1997	q31.1–q32.3	IH, dup(4)(q31.1q32.3)
10	Muraki et al. 1997	q25–q31.3	DN, dup(4)(q25q31.3)
11	Shashi et al. 1999	q12–q13	DN, dup(4)(q12q13)
12	Guillen Navarro et al. 1996	q21–q28	DN, dup(4)(q21q28)
13	Otsuka et al. Case 1, 2005	q31.22–q35.2	IH, dup(4)(q31.22q35.2)
14	Otsuka et al. Case 2, 2005	q31.22–q35.2	IH, dup(4)(q31.22q35.2)
15	Lundin et al. 2002	q27–q35	DN, t(4;7)(q27;p22)
16	Mikelsaar et al. 1996	q25–qter	DN, t(4;22)(q25;p11)
17	Cui et al. 2006	q27–q35	DN, t(4;5)(q27;q35)
18	Maltby et al. 1999	q31–q33	IH, dup(4)(q31q33)
19	Assawamakin et al. 2012	q13.2–q22.1	IH, der(8)(20qter–>20q12::4q22.1–>q21.21::4q13.3–>4q13.2::8q22.1–>8p11.12: :8q22.3–>qter),der(20)(20pter –>20q12::4q13.3–>q21.21: :8q22.3–>q22.1::8p11.12–>pter)
20	Angulo et al. 1984	q31–qter	IH, t(4;12)(q31;q24)
21	Watanabe et al. 1977	q33–qter	IH, t(4;13)(q33;q33)
22	Schrott et al. 1974	q26–qter	IH, t(4;13)(q26;q34)
23	Jenkins et al. 1975	q31–qter	DN, t(4;13)(q31;q14)
24	Zhang et al. 2009	q25–qter	IH, t(4;10)(q26;q26.3)
25	Cernakova et al. 2006	q28–q35.2	DN, dup(4)(q28q35.2)
26	Horbinski et al. 2008	q35–qter	DN, t(4;18)(q35;q23)
27	Rinaldi et al. 2003	q24–q35	DN, t(4;14)(q24;p12)
28	Kadotani et al. 1981	q32–qter	IH, t(4;9)(q23;p24)

Table I.1. (continued)

No	Reference	Chromosome 4 region	Type of anomaly
29	Issa et al. 1976	q31–qter	IH, t(4;9)(q31;q34)
30	Collia et al. 2012	q12–q22	DN, dup(4)(q11q22)
31	Baccichetti et al. 1975	q32–qter	IH, t(4;21)(q32;q22)
32	Patel et al. 2006	q25–qter	IH, t(4;18)(q25;q22)
33	Cervenka et al. 1976	q25–qter	IH, t(X;4)(q27;q25)
34	Biederman and Bowen 1976	q21–qter	IH, t(2;4)(p25;q21)
35	Bonfante et al. 1979, case A	q27–qter	IH, t(4;18)(q27;p11)
36	Bonfante et al. 1979, case B	q27–qter	IH, t(4;18)(q27;p11)
37	Škrlec et al. 2014	q31.3–qter	IH, t(2;4)(p25.1;q31.3)
38	Gorukmez et al. 2014	q21–q35	DN, dup(4)(q21q35)
39	Vargas Machuca et al. 2016	q21–qter	IH, t(4;20)(q21;q13.1)
40	El-Ruby et al. 2007	q25–qter	IH, t(4;21)(q25;q22)
41	Anneren et al. 1984	q31–qter	IH, t(4;8)(q31;p23)
42	Carrascosa Romero et al., 2008	q31–q35	DN, dup(4)(q31q35)
43	Bellucco et al., 2018	q32.1–q35.2	47,XX,+der(21)t(4; 21)(q32.1;q21.2)mat. arr[GRCh37/hg19] 4q32.1q35.2(158907036_190957460)x3,21q11.2q21.2(15016486_ 25605895)x3
44	Lin et al. 2018	q32.3–qter	ND, t(4;5)(q32.3;p14.2)
45	Mohamed et al. 2018	q32.1–q35.2	DN 46,XX,add1(q44)
46	Shenoy et al. 2018	q27q35.2	IH, t(4;21)(q27;q22)
47	Thapa et al. 2014, case A	q32.1–q35.2	ND, dup(4)(q32.1q35.2)
48	Thapa et al. 2014, case B	q32.2–q34.3	ND,dup(4)(q32.2q34.3)
49	Zaki et al. 2019	q35.2	DN, dup(4)(q35.2)
50	This case	q26–qter	IH, t(4;10)(q26;q26.3)

IH –inherited; DN – de novo; ND – not discussed.

Trisomy 4q syndrome shows a complex phenotype that includes growth retardation, intellectual disability, a specific craniofacial dysmorphism (microcephaly, epicanthic folds, high nasal bridge, short philtrum, microretrognathia, and low set and malformed ears), congenital heart defects (atrial or ventricular septal defect and patent ductus arteriosus), renal defects, and/or thumb anomalies (Table I.2.).

Table I.2. Phenotype changes in different types of 4q partial trisomy.

No	Reference	Chromosome 4 region	a	b	c	d	e	f	g	h	i	j	k
1	Mattei et al. 1979	q12–q13	+	+	+	+	–	+	–	–	–	–	–
2	Zollino et al. 1995	q13.3–q22.2	–	+	–	–	–	–	+	–	–	–	–
3	Halal et al. 1991	q23–q27	+	+	+	+	–	–	–	–	–	+	–
4	Jeziorowska et al. 1993	q21.3–q31.3	+	+	+	+	+	+	+	+	+	–	+
5	Fryns 1980	q25–q31	+	+	–	+	+	+	–	+	–	–	–
6	Vogel et al. 1975	q22–q34	+	+	–	+	+	+	+	+	+	–	+
7	Dutrillaux et al. 1975	q22–q34	+	+	+	+	+	+	+	+	+	–	+
8	Taylor et al. 1977	q26–q35	+	+	+	+	+	+	+	+	–	+	ND
9	Goodman et al. 1997	q31.1–q32.3	+	+	–	+	–	ND	ND	–	–	+	–
10	Muraki et al. 1997	q25–q31.3	+	+	–	–	–	–	–	+	–	–	–
11	Shashi et al. 1999	q12–q13	+	+	+	+	+	–	–	+	–	–	–
12	Guillen Navarro et al. 1996	q21–q28	+	+	–	+	+	ND	+	+	+	–	–
13	Otsuka et al. 2005, Case 1	q31.22–q35.2	+	+	+	+	+	+	+	+	+	–	+
14	Otsuka et al. 2005, Case 2	q31.22–q35.2	+	+	+	+	+	+	+	+	+	–	+
15	Lundin et al. 2002	q27–q35	+	+	+	+	+	ND	–	–	+	–	–
16	Mikelsaar et al. 1996	q25–qter	+	+	+	+	+	+	–	+	–	–	–
17	Cui et al. 2006	q27–q35	+	+	–	+	+	ND	–	–	+	–	–
18	Maltby et al. 1999	q31–q33	–	–	–	–	–	ND	–	–	–	–	–
19	Assawamakin et al. 2012	q13.2–q22.1	–	+	+	–	–	ND	–	–	–	+	–
20	Angulo et al. 1984	q31–qter	+	+	–	+	+	+	ND	+	–	–	+
21	Watanabe et al. 1977	q33–qter	+	+	+	–	+	ND	–	+	ND	–	+
22	Schrott et al. 1974	q26–qter	+	+	+	–	–	ND	+	–	ND	–	+
23	Jenkins et al. 1975	q31–qter	+	+	–	+	+	ND	–	+	ND	+	–
24	Zhang et al. 2009	q25–qter	+	+	+	+	+	+	+	+	–	–	–
25	Cernakova et al. 2006	q28–q35.2	+	+	+	+	+	–	+	+	–	+	–
26	Horbinski et al. 2008	q35–qter	+	+	+	+	+	–	–	+	–	+	–
27	Rinaldi et al. 2003	q24–q35	+	+	+	ND	+	ND	+	+	+	+	+
28	Kadotani et al. 1981	q32–qter	+	ND	+	ND	+	ND	+	+	+	ND	ND
29	Issa et al. 1976	q31–qter	+	+	–	ND	+	ND	–	+	–	–	ND

Table I.2. (continued)

30	Collia et al. 2012	q12–q22	−	−	−	+	+	ND	−	+	−	−	−
31	Baccichetti et al. 1975	q32–qter	+	+	+	−	+	−	+	+	−	ND	−
32	Patel et al. 2006	q25–qter	+	+	+	ND	+	ND	+	+	+	−	+
33	Cervenka et al. 1976	q25–qter	+	+	+	+	+	−	−	+	+	−	+
34	Biederman and Bowen 1976	q21–qter	+	+	+	ND	+	+	+	+	+	−	ND
35	Bonfante et al. 1979, case A	q27–qter	+	+	−	−	−	−	+	+	−	+	−
36	Bonfante et al. 1979, case B	q27–qter	+	+	−	+	+	−	−	+	−	−	−
37	Škrlec et al. 2014	q31.3–qter	−	+	−	+	−	ND	ND	+	−	−	−
38	Gorukmez et al. 2014	q21–q35	+	+	+	−	+	+	+	+	+	+	+
39	Vargas Machuca et al. 2016	q21–qter	+	ND	+	ND	+	ND	+	+	−	+	−
40	El-Ruby et al. 2007	q25–qter	+	+	+	−	+	−	−	+	+	−	−
41	Anneren et al. 1984	q31–qter	+	+	+	+	+	ND	−	+	−	−	−
42	Carrascosa Romero et al. 2008	q31–q35	−	+	+	ND	+	+	+	+	−	−	+
43	Bellucco et al. 2018	q32.1–q35.2	+	+	+	−	+	−	−	−	−	−	−
44	Lin et al., 2018	q32.3–qter	ND	+	ND	ND	ND	ND	ND	ND	+	+	+
45	Mohamed et al. 2018	q32.1–q35.2	+	+	+	−	−	ND	+	+	−	−	−
46	Shenoy et al. 2018	q27q35.2	+	ND	+	ND	ND	ND	+	+	ND	+	+
47	Thapa et al. 2014, case A	q32.1–q35.2	+	+	ND	−	+	+	+	+	−	ND	−
48	Thapa et al. 2014, case B	q32.2–q34.3	+	+	ND	−	ND	ND	−	+	+	ND	−
49	Zaki et al. 2019	q35.2	−	+	+	ND	ND	ND	ND	+	−	−	−
50	This case	q26–qter	+	+	+	+	+	+	+	+	−	+	−

ND—not discussed; a—growth retardation; b—psychomotor retardation; c—microcephaly; d—epicanthic folds; e—high nasal bridge; f—short philtrum; g—micrognathia; h—low set and malformed ears; i—thumb anomalies; j—congenital heart diseases; k—renal anomalies.

Celle et al. analyzed the duplications of the long arm of chromosome 4 and concluded that 4q partial trisomy could be generated by multiple genetic mechanisms, such as unbalanced translocation (the result of malsegregation of parental derivative chromosomes involved in a balanced reciprocal translocation) de novo duplication, de novo unbalanced translocation, and, more rarely, a chromosomal insertion [Celle et al., 2000]. Table I.1. illustrates the heterogeneity of chromosomal anomalies in 4q partial trisomy. Thus, in 22 cases, the anomaly was de novo (16 cases with de novo duplication and 6 cases with de novo translocation). The other cases were inherited anomalies/unknown status of inheritance, and most of them (28 cases) are represented by different translocations between chromosome 4 and other chromosomes. Out of the translocations (de novo or inherited), the most frequent are: t(4;18) and t(4;21)—4 cases; t(4;13), t (2;4) and t(4;10)—3 cases; and t(4;8), t(4;9), and t(4;20), each of them with two cases.

Figure I.7. shows the heterogeneity of the breakpoints' position on the long arm of chromosome 4. Thus, we can divide 4q partial trisomy into four categories: proximal (between 4q12 and 4q23), intermediate (between 4q22–23 and 4q31), terminal (between 4q31 and 4qter), and large 4q partial trisomy (between 4q21–25 and 4qter). Phenotypic changes generated by these anomalies are different, and we will analyze them separately.

The proximal 4q partial trisomies are represented by the cases 1 [Mattei et al., 1979], 2 [Zollino et al., 1995], 11 [Shashi et al. 1999], 19 [Assawamakin et al., 2012], and 30 [Colla, 2012] from Table I.1 and Figure I.7. In all these cases, the phenotype is milder and the main characteristics are growth and psychomotor retardation, microcephaly, and low set malformed ears. For example, Shashi et al. reported a boy with a 4q12–q13 duplication that presented microcephaly and intellectual disability as main features [Shashi et al., 1999]. Additionally, minimal phenotypic changes were reported by Bonnet et al., who presented a child with a 4q11–4q13.2 partial trisomy that had only a mild psychomotor delay [Bonnet et al., 2006]. There were similar findings by Matoso et al., who presented two siblings that have a 4q13.1–q13.3 partial trisomy that presented only delayed developmental milestones and attention-deficit hyperactivity disorder [Matoso et al., 2013].

Additionally, a mild phenotype seems to be associated with a 4q partial trisomy of the 4q23–q27 region. Thus, Case 3 from Table I.2 and Figure I.7, reported by Halal et al., presented growth and psychomotor delay and nonspecific dysmorphic features such as epicanthic folds and a high nasal bridge [Halal, Vekemans, Chitayat, 1991].

Cases 4 [Jeziorowska et al., 1991], 5 [Fryns, Berghe, 1980], 6 [Vogel et al., 1975], 7 [Dutrillaux et al., 1975], 8 [Taylor et al., 1977], 12 [Navarro et al., 1996], and 17 [Cui et al., 1996] from Table I.2 and Figure I.7 represent the intermediate 4q partial trisomy of the 4q23–q31 region. All these cases presented a severe phenotype characterized by: growth retardation (7/7), intellectual disability (7/7), high nasal bridge (7/7), epicanthic folds (6/7), low set malformed ears (6/7), micrognathia (5/7), thumb anomalies (5/7), and short philtrum (5/7). However, the genes located in this region seem to have a lesser impact on the development of the heart because only one of these patients presented a congenital heart defect. Lundin et al. presumed that the 4q27.4–q31 partial trisomy induced the most severe clinical effects, including growth retardation, intellectual disability, microcephaly, facial asymmetry, thumb anomalies, hearing impairment, epilepsy, and congenital heart defects [Lundin et al., 2002].

Terminal 4q partial trisomies are represented by Cases 13 [Otsuka et al., 2005], 14 [Otsuka et al., 2005], 20 [Angulo et al., 1986], 21 [Watanabe et al., 1977], 23 [Jenkins et al., 1975], 26 [Horbinski et al., 2008], 28 [Kadotani et al., 1981], 29 [Issa, Potter, Blank, 1976], 31 [Baccichetti et al., 1975], 37 [Skrlec et al., 2014], 41 [Annerén, Lubeck 2009], 42 [Romero et al., 2008], 43 [Bellucco et al., 2018], 44 [Lin et al., 2017], 45 [Mohamed et al., 2018], 47 [Thapa et al., 2014], 48 [Thapa et al., 2014], and 49 [Zaki et al., 2019] (Table I.2, Figure I.7). The majority of these encompassed the 4q31-q35(qter) region. The main phenotypic changes

were represented by low set malformed ears (16/18), intellectual disability (17/18), high nasal bridge (13/18), growth retardation (14/18), microcephaly (11/18) epicanthic folds (7/18), and micrognathia (8/18). Thumb anomalies (6/18) and short philtrum (5/18) are rare, while cardiac heart defects were found in only 4 cases. Renal anomalies could also be considered less frequent, being found in 6/18 cases.

The majority of the cases presented in Table I.2 and Figure I.7 are represented by large 4q partial trisomies that encompassed at least the 4q27–qter region (Cases 15 [Lundin et al., 2002], 16 [Mikelsaar, Lurie, Ellus, 1996], 22 [Schrott et al., 1974], 24 [Zhang et al., 2009], 25 [Cernakova et al., 2006], 27 [Rinaldi et al., 2003], 32 [Patel et al., 2006], 33 [Cervenka, Djavadi, Gorlin, 1976], 34 [Biederman, Bowen, 1976], 35 [Bonfante, Stella, Rossi, 1979 case A], 36 [Bonfante, Stella, Rossi, 1979 - case B], 38 [Sag et al., 2014], 39 [Machuca 2016], 40 [El-Ruby, Hemly, Zaki, 2007], 46 [Shenoy R D, Shenoy V, Shetty, 2018], and our case). The phenotype of these patients is severe, being correlated with the large amount of genetic material present in the three copies. The major anomalies reported are: growth retardation (15/16), low set malformed ears (13/16), intellectual disability (13/16), microcephaly (14/16), high nasal bridge (13/16), and micrognathia (12/16). Epicanthic folds (7/16) and short philtrum (6/16) are not very frequent. Visceral anomalies are relatively frequent, congenital heart disease having been documented in 7 cases and renal anomalies in 6 cases.

Rinaldi et al. discussed the correlation between 4q partial trisomy and the phenotype and observed an important clinical heterogeneity correlated with the variability of the duplicated region. They considered that psychomotor and growth retardation, retromicrognathia, and low set and/or malformed ears are non-specific clinical findings associated with chromosomal unbalances [Rinaldi et al., 2003].

The relationship between 4q partial trisomy and renal anomalies has been discussed by different authors but still lacks certitude. Zollino et al. suggested that the 4q22–q23 region is involved in the development of the acrorenal field [Zollino et al., 1995]. On the other hand, Battaglia et al. considered that the development of the acrorenal field is more likely associated with genes from the 4q25–q28 region [Battaglia et al., 2005]. Otsuka et al. suggested that renal hypoplasia may have a close relationship with the duplication of 4q33–q34 [Otsuka et al., 2005]. This fact is also suggested by the elements presented in Table I.2. Thus, all the cases with renal anomalies presented a minimum of a 4q33–qter partial trisomy.

Congenital cardiac defects were present in only 14 of 50 patients with 4q partial trisomy (see Table I.2). In seven of those cases, the presence of congenital heart disease could be considered a consequence of supplementary genetic material from chromosome 4 because they had either a 4q duplication [Cernakova et al., 2006; Halal, Vekemans, Chitayat, 1991; Dutrillaux et al., 1975; Goodman et al., 1997; Sag et al., 2014] or a translocation, but the monosomy of the other chromosome did not alter the phenotype [Assawamakin et al., 2012; Rinaldi et al., 2003]. In the rest of the seven cases, an association between 4q partial trisomy and monosomy of chromosomes 13 [Jenkins et al., 1975], 18 [Horbinski et al., 2008; Bonfante, Stella, Rossi, 1979], 20 [Machuca, 2016], 21 [Shenoy R D, Shenoy V, Shetty, 2018], 5 [Lin et al., 2017], and 10 (our case) was present, and thus, the delineation of a chromosomal region involved in the development of congenital heart disease is difficult. However, Rinaldi et al. presumed that the 4q26-q27 region could be the critical region that contains genes for congenital development; this segment is duplicated in 9 of the 12 cases discussed above [Rinaldi et al., 2003]. The *HAND2* gene (4q34.1) is implicated in cardiac morphogenesis. The effects on the phenotype are different for haploinsufficiency and for duplication of the *HAND2* gene because only haploinsufficiency seems to be associated with congenital heart defects [Mohamed et al., 2018].

Thumb anomalies are considered a particularity of 4q partial trisomy, but the data from Table I.2 indicate such an anomaly in only 17 out of 50 cases. The analysis of these data

enables us to presume that the 4q27–q28 segment could be the region that contains genes involved in thumb development because all cases (except for the siblings reported by Otsuka et al. 2005 and Lin 2018, Thapa case B) presented 4q duplication that encompasses this segment [Otsuka et al., 2005; Lin et al., 2017; Thapa et al., 2014]. A similar hypothesis formulated by Rinaldi et al. indicates that the 4q25-q28 region is a minimal commonly duplicated segment [Rinaldi et al., 2003].

The 10q subtelomeric deletion is a rare anomaly. The symptomatology of this syndrome includes: variable dysmorphic facial features (microcephaly, dolichocephaly, triangular asymmetric face, prominent nasal root with beaked nose and flared nostrils, long philtrum, thin upper lip, small pointed jaw, malformed and low set ears, low posterior hairline), delayed psychomotor development, and poor speech and language development [Irving et al., 2003; Courtens et al., 2006; Mehta, Duckett, Young, 1987; Tanabe et al., 1999; Miller et al., 2009]. In all these cases, the chromosomal deletion was of minimum 5 Mb and encompassed a region located between the 10q26 band and the 10q telomere. Yatsenko et al. considered that the minimum critical region is approximately 600 kb, and it is located in the 10q26.2 region [Yatsenko et al., 2009]. In this segment are present genes *DOCK1* and *C10ORF90*. The *DOCK1* gene influences phagocytosis, cell migration, apoptosis and tumorigenesis, and cardiovascular development. Lin et al. suggested that the distal 10q26 terminal deletion with a breakpoint at ~130.0 Mb from the 10p telomere may be involved in the phenotype of the 10q26 deletion syndrome, but this could be influenced by other pathogenic genes: *FGFR2*, *CTBP2*, *CALY*, *WDR11*, *HMX2*, and *HMX3* [Lin et al., 2016]. A similar hypothesis was proposed by Sangu et al., who presented a patient with a de novo microdeletion of 10q26.11-q26.13, where genes *FGFR2*, *HMX2*, and *HMX3* are located [Sangu et al., 2016].

However, in our case, the 10q26.3 microdeletion is located much closer to the 10q telomere, and the dimension is only 562 kb. The 10q26.3 region contains 27 genes, 18 of which encode proteins (Figure I.8). Three of these, the genes *ECHS1* (enoyl-CoA hydratase, short chain 1), *SYCE1* (synaptonemal complex central element protein 1), and *TUBGCP2* (tubulin gamma complex associated protein 2) have been associated with autosomal recessive disorders [OMIM]. Therefore, the absence of a gene copy in our patient is not involved in phenotypic changes. This is concordant with other reports. Martin et al. presented two relatives (mother and child) that had a 10q telomere deletion with minimum phenotypic changes (mother healthy; child with some unspecified neurological features) [Martin et al., 2002]. A similar case was discussed by Ravnan et al., who identified a mother–child transmission of a 10q telomeric deletion and considered this anomaly to be a chromosomal variant [Ravnan et al., 2006]. Zhang et al., who presented an anomaly similar to that reported by us, considered that the 10q26.3 microdeletion was without phenotypic consequences and all phenotypic changes in their patient resulted from the 4q26–q35.2 partial trisomy [Zhang et al., 2009]. Riegel et al. reported two siblings with a der(10)t(4;10)(q35.2;q26.3) anomaly that presented moderate intellectual disability, dysmorphic features (triangular face; hypertelorism; downward slanting palpebral fissures; short, upturned nose with broad and prominent root and hypoplastic alae; thin upper lip; downturned corners of the mouth; bilateral preauricular tags), smooth palmar creases, and short fingers [Riegel et al., 2001]. Yan et al. presented a 10q26.3 microdeletion (arr10q26.3(131,585,685–134,832,720)x1) identified prenatally in a child with a ventricular septal defect, overriding aorta, and right ventricular hypertrophy [Yan et al., 2013]. The absent segment, in this case, does not encompass the 10q chromosomal region identified by us. Considering all this information, we presume that the 10q26.3 microdeletion had minimal influence on the phenotype of our patient. This is concordant with the theory postulated by Kowalczyk et al., who explained the absence of phenotypic changes in microdeletion by several facts: the lost material is non-essential; the

genes located in the deleted region are haplosufficient; some deleted genes have additional copies, which can compensate for the loss; the chromosomal region is imprinted and the remaining allele on the other chromosome of the pair is active [Kowalczyk, Srebniak, Tomaszewska, 2007].

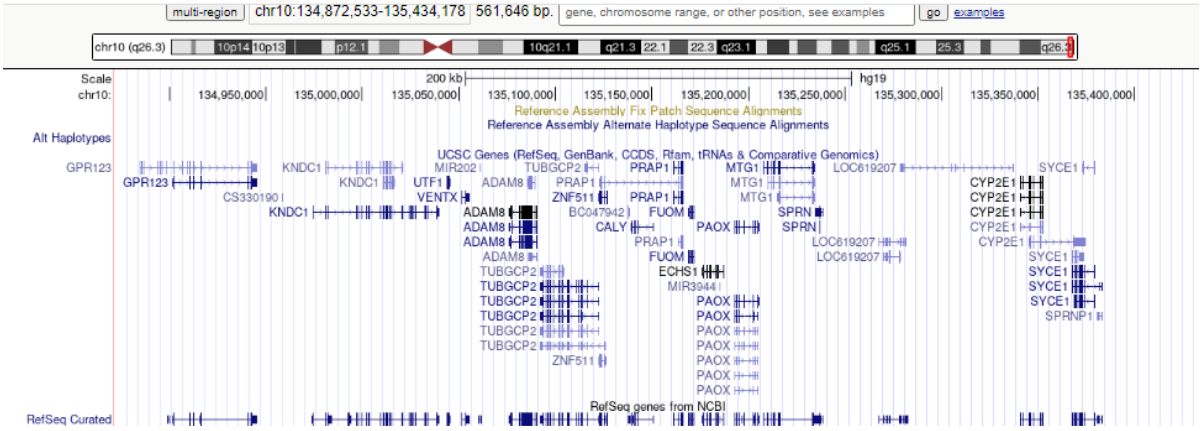


Figure I.8. UCSC Genome Browser image of genes in the 10q26.3 region (accessed on 29 November 2021).

The fragment of 795 kb located in the 4q35.2 region, present in three copies in the mother and in four copies in the child, can be considered non-pathogenic, although it contains the genes *ZFP42*, *TRIML1*, and *TRIML2*. All these genes encode proteins that are members of the superfamily of zinc-finger. The *ZFP42* gene is not involved in human pathology, while mice homologous gene *Zfp42* intervenes in the reprogramming of X chromosome inactivation [Navarro et al., 2010]. Genes *TRIML1* and *TRIML2* are involved in early embryogenesis [Tian et al., 2009].

Human duplication is favored by the existence of low copy repeat sequences involved in homologous recombination events mediated by high sequence identity between the low copy repeats [Ji et al., 2009]. Abnormal recombination induces chromosomal rearrangements involved in genomic disorders such as Williams-Beuren syndrome [Peoples et al., 2000], Smith-Magenis syndrome and the corresponding 17p11.2 duplication [Potocki et al., 2000], and the DiGeorge/velo-cardio-facial syndrome and cat-eye syndrome produced by rearrangements in the 22q11 region [Yobb et al., 2005].

In other cases, such chromosomal changes do not modify the phenotype and generate only a copy number variation (CNV) [Weckselblatt, Rudd, 2015]. Some duplications (or even triplication/quadruplication) without phenotype changes were identified on the long arm of chromosome 4. For example, Chen et al. reported a 4.5 Mb benign 4q12–q13.1 triplication transmitted from father to son, and Chen et al. presented a 4.4 Mb benign 4q12-q13.1 quadruplication transmitted from parent to child [Chen et al., 2014; Chen et al., 2011].

Bertelsen et al. reported a patient with Tourette syndrome that associated a 1.7 Mb benign duplication in the 4q35.2 region (chr4.hg19:g.(?_188104036)_(189795272_?)), transmitted from father to child [Bertelsen et al., 2014]. The duplicated segment includes *ZFP42*, *TRIML1*, and *TRIML2* genes. This duplication is similar to the anomaly discovered by us, and, thus, we can conclude that the duplication in the 4q35.2 region is without phenotypic changes. The 4q35.2 duplication could be favored by the genomic architecture of this chromosomal region. Close to the region that contains the *ZFP42*, *TRIML1*, and *TRIML2* genes is located an array of 3.3 kb repeats (D4Z4). The contraction of these repeats from 11–100 copies to ≤10 generates facio-scapulo-humeral muscular dystrophy and can modify the organization of chromatin in this segment of chromosome 4. In addition, the D4Z4 repeats of chromosome 4 have a similar macrosatellite homologous segment in the 10q26.3 region [Pope et al., 2011].

Matsumura et al. described a high rate of translocation between the 4q35.2 region and the 10q26.3 region in both Asiatic and European populations, and these rearrangements are favored by genomic homology between these chromosomal segments [Matsumura et al., 2002]. Starting from this, we can presume that this homology could favor the chromosomal rearrangements between chromosomes 4 and 10, including the complex rearrangements described by us.

In these conditions, prenatal diagnosis of the future pregnancies of the mother becomes mandatory due to the theoretical 2/3 risk of forming fetuses with unbalanced chromosomal abnormalities. However, the practical risk is only around 5% because viable unbalanced embryos can result from an adjacent-1 segregation of maternal derivative chromosomes and the embryos carrying the association between 4q trisomy and 10q monosomy. The embryos with an association between 10q trisomy and 4q monosomy (generated by an adiacent-1 segregation) and those that result from an adjacent-2 segregation of maternal derivative chromosomes are unviable [Gug et al., 2019; Gug et al., 2020; Gug et al., 2018; Stene, Stengel-Rutkowski, 1988].

1.2.5. Conclusions

We present a rare complex chromosomal anomaly identified in one patient with multiple congenital anomalies syndrome. The boy has a chromosomal formula of 46,XY,der(10)(10pter→10q26.2::4q26→4qter) and his mother presents an apparently balanced reciprocal translocation - 46,XX,t(4;10)(q26;q26.2). Using molecular analyses (MLPA and array-CGH), we confirmed the presence in our patient of an association between a large triplication of 71,057 kb in the 4q26–q35.2 region, a 562 kb microdeletion in the 10q26.3 region, and a 795 kb quadruplication of the 4q35.2 region. In these conditions, we consider that phenotype was modified mainly by the 4q partial trisomy, while the 10q26.3 microdeletion allowed a minimal influence on the phenotype; thus, we consider our case as a "pure" 4q partial trisomy. Because of the mother–son transmission of the 4q35.2 triplication, we consider that this genomic change is only a CNV without pathogenic importance. This report highlights the importance of the use of different cytogenetic and molecular methods to identify specific chromosomal anomalies present in some multiple congenital anomalies syndromes.

1.3. Diagnostic and prenatal screening in chromosomal anomalies

1.3.1. Study of non-invasive prenatal testing in a Romanian Population

1.3.1.1. Introduction

Non-invasive prenatal testing (NIPT) has been increasingly used in the last decade, initially for the screening of common fetal aneuploidies and then for several structural chromosomal abnormalities [Yu et al., 2019 Benn P, Cuckle H, Pergament E, 2013; Minear et al., 2015]. Conventional aneuploidy screening technology by NIPT was introduced in 2011. The discovery of cell-free fetal DNA in the plasma of pregnant women by Lo et al. in 1997 [Lo et al., 1997] provided the opportunity for NIPT. The introduction and rapid progress of next generation sequencing (NGS) technologies have increased the accuracy of the method [Wang et al., 2020]. Several studies reported a combined specificity for aneuploidies of 99.9%, much more accurate than biochemical screening [Russo, Blakemore, 2014; Health Quality Ontario, 2019; Nicolaides, 2011; Gorduza, Socolov, Socolov, 2018; Dey, Sharma, Aggarwal, 2013]. Sonography and maternal biochemistry prenatal screening of fetal aneuploidies have a

combined detection rate within 50–95% [Wang et al., 2020].

Sex chromosome aneuploidies that can also be identified using NIPT have a lower detection rate, especially monosomy X, with a higher false positive rate compared to autosomal trisomies. The average positive predictive value (PPV) for sex chromosome anomalies screening was estimated at 40.56% [Wang et al., 2020; Lu et al., 2021]. NIPT has several limitations regarding the detection of more rare fetal chromosomal abnormalities, other than aneuploidy for 3 (13, 18, 21) or 6 pairs (13, 18, 21, 9, 16, 22) of autosomal chromosomes, or the identification of cases with mosaicism.

Segmental duplications or deletions, also called copy number variations (CNVs), are extensively distributed in the human genome and many are associated with diseases [Zarrei et al., 2015; Popescu et al., 2021; Gug et al., 2020; LaLonde et al., 2020; Gug et al., 2018; Goldenberg, 2018]. The incidence of microdeletions/microduplications is independent of maternal age [Wapner et al., 2015]. Structural chromosome abnormalities also remain difficult to detect. An algorithm known as Fetal Copy Number Analysis through Maternal Plasma Sequencing was developed to detect, close to 100%, deletions/duplications ≥10 Mb [Liu et al., 2016].

NIPT widely used today, it is especially applied for the screening of autosomal trisomies, with the advantage of not requiring invasive sampling and having high accuracy, sensitivity and specificity [Sun, Lu, Ma, 2019]. Based on multiple meta-analyses, false positive rates range between 0.04% and 0.06% for the autosomal trisomies 21, 18 and 13. The detection rate of sex chromosome aneuploidies varies, being 90.3% for monosomy X with a 0.23% false positive rate. The detection rate for sex chromosome trisomies was 93.0%, and the false positive rate was 0.14% [Samur, 2020]. However, invasive diagnostic tests such as amniocentesis or chorionic villus sampling remain the gold standard for the accurate diagnosis of chromosomal abnormalities, but the number of such procedure was reduced by the use of NIPT.

In the present study, we aimed to assess the accuracy of cell free DNA (cfDNA) testing based on low-level whole-genome sequencing to screen for common aneuploidies and some microdeletions and evaluated the clinical performance of NIPT using maternal plasma cfDNA.

1.3.1.2. Materials and methods

Patients and Sample Collection

A retrospective study was conducted including data obtained from a single private genetic center that collects cases from the Western part of Romania. Our study analyse the results of NIPT from 380 Caucasian pregnant women, including 4 twin pregnancies and 7 pregnancies obtained by in vitro fertilization (IVF). Exclusion criteria were as follows: more than two fetuses, pregnancy after stem cell therapy or organ transplantation, invasive cancers. All pregnancies were assessed by the obstetrician– gynecologists before maternal blood sampling. All subjects had a pretest ultrasound scan to determine the gestational age and number of fetuses.

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was provided by the Human Research Ethics Committee from the Emergency Clinical Municipal Hospital Timisoara, Romania (No. 6-2846 from 8 July 2020). Written informed consent was obtained from all investigated women to perform NIPT and to publish the results in the future.

NIFTY (Non-Invasive Fetal Trisomy) in 225 cases and NIPS (noninvasive prenatal screening) in 155 cases were performed. The samples were processed in Hong Kong and the USA, respectively. The NIFTY test was the first NIPT test available in Romania.

Genetic counseling pre-test and post-test was provided to each couple. In all high-risk cases, we recommended and performed a genetic diagnosis using an invasive method, chorionic villus sampling (CVS), or amniocentesis, followed by a combination of tests: Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR), array Comparative Genomic Hybridization (aCGH) and karyotyping.

NIPT: NIFTY and NIPS Tests

The NIFTY test (BGI Laboratory) enables the isolation of cfDNA (including both maternal and fetal DNA) from a maternal blood sample and performs low coverage whole genome sequencing using NGS technology. The unique reads for each chromosome are calculated and compared to an optimal reference control sample. Data is analyzed using a BGI's bioinformatics algorithm, and a risk score is generated for the tested conditions. For gender identification, cfDNA isolation is necessary (including both maternal and fetal DNA) from a maternal blood sample, followed by molecular genetic testing.

The NIFTY test enables the identification of 6 autosomal aneuploidies (chromosomes 13, 18, 21, 9, 16, and 22), sex chromosomes aneuploidies and 84 microdeletion syndromes. The test was validated for deletion/duplication (del/dup) syndromes larger than 10 Mb. The detection rate is over 90% if the size of the deletion/duplication is 3 Mb, according to the specifications of the BGI Laboratory. The NIFTY test was used from 2014 to 2020 in 225 cases.

NIPS was performed in 155 cases, which allows the identification of pregnancies at risk for aneuploidies of chromosomes 13, 18, 21, and for 6 microdeletion syndromes: 1p36 deletion syndrome, 4p16.3 deletion (Wolf - Hirschhorn syndrome), 5p15.2 deletion (*Cri du chat* syndrome), 15q11.2 deletion (Prader-Willi and Angelman syndromes) and 22q11.2 deletion (velocardiofacial syndrome) in singleton pregnancies. Invitae laboratory uses cfDNA extraction from maternal blood, DNA sequencing and analysis of sequenced regions to determine the risk of the above mentioned fetal chromosomal abnormalities. Other mechanisms that cause Prader-Willi and Angelman syndromes, such as uniparental disomy, have not been examined. A percentage of Y chromosome ≥ 0.0048 and <0.0048 is considered as a male and a female fetus, respectively. The accuracy is at least 97%.

The NIPT is validated to detect fetal chromosome trisomy 21, 18, and 13 and the presence of the Y chromosome for twin pregnancies at a minimum gestational age of 10 weeks 0 days. Both tests can reveal fetal sex and sex chromosomes aneuploidies.

Cell Culture

CVS or amniocentesis was performed in every case with positive NIPT results. All cytogenetic analyses were performed in Romania. Amniotic or chorionic villus cell cultures were initiated in 2 different flasks with AmnioMAX™-II complete medium (Gibco-Thermo Fisher, Waltham, MA, USA) containing fetal bovine serum, gentamicin and L-glutamine. On the fifth day of cell culture, the growth medium was changed, and subsequently, cell growth and number of mitoses were monitored daily. Long-term cultures were performed (12 days), and the cells were then harvested by routine techniques.

Cytogenetics

At least 20 metaphases per each sample were analyzed using GTG banding (at the 550-band level). Results were regularly available within 14–18 days. Analysis of the chromosomes was performed using LUCIA Karyo G software, and the aberrations and karyotypes were classified according to the International System for Human Cytogenomic Nomenclature 2016 [ISCN, 2016].

QF-PCR Assay

If a high-risk of aneuploidy for chromosomes 13, 18, 21, X, and Y was detected by NIPT/NIPS, the samples were verified by the rapid QF-PCR assay. QF-PCR tests were performed by a partner laboratory (Cytogenomic Medical Laboratory, Bucharest, Romania).

Array CGH

Array CGH was performed using Cyto Chip Focus Constitutional BAC array (Illumina Inc., San Diego, CA, USA), and the used reference was the normal human male genomic DNA (Promega Corporation, Madison, WI, USA). Array CGH analyses were also performed by Cytogenomic Medical Laboratory.

Data Analysis

For a statistical presentation, the results were reconsidered after classifying pregnancies according to maternal age (<34 years and ≥35 years).
Statistical processing was performed using SPSSv17 (version 17, SPSS Inc., Chicago, IL, USA) and Microsoft Excel (version 2013, MS Corp., Redmond, WA, USA).

1.3.1.3. Results

NIPT was performed in 380 pregnant women in a private genetic center from Timisoara, Romania. There were 380 cfDNA maternal plasma samples and 378 of them were successful (99.47%). In two cases, the analysis could not be performed even after a second blood sample was taken. Seven pregnancies resulting from IVF were included. A low risk was identified in 95.76% (362/378) of cases that were considered normal pregnancies and were further monitored. A high risk of aneuploidy has been identified in 2.64% (10/378) of cases. Increased risk for microdeletion syndromes has been identified in 1.05% (4/378) of cases. Combined anomalies risk (one aneuploidy and one microdeletion) has been identified in 0.52% (2/378) of cases. The number of investigations increased from 2014 to 2020, which shows the growing interest in this type of testing for pregnant women in Romania. Figure I.9 shows the distribution of cases and anomalies identified throughout the studied period.

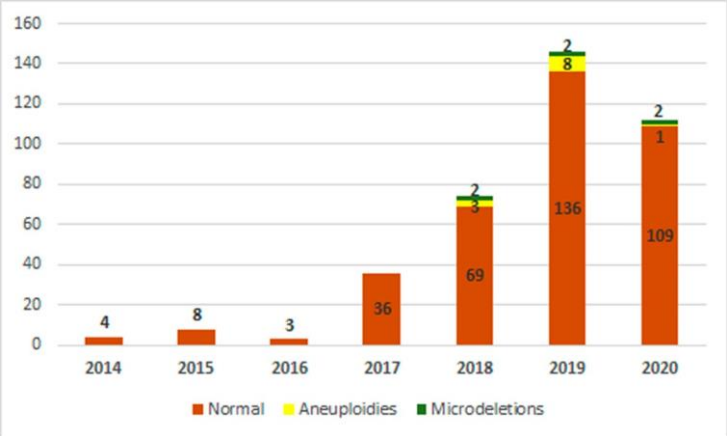


Figure I.9. Distribution of cases over time, specifying the type and number of detected abnormalities (aneuploidies and microdeletions).

The mean gestational age was 12.62 ± 2.47 weeks (range: 10–22 weeks)
Most cases (55 cases) had a gestational age of 10 weeks.

Fetal Fraction

NIPT is based on the percentage (%) of cell-free DNA in the mother's blood, also called fetal fraction (FF). In our study, these percentages were highly variable, with a mean of 10.96% ± 4.14, ranging between 3.5% and 28.12% (Figure I.10).
In 4 cases the analysis failed initially, but it was successful in 2 of them after a second blood sample was taken, hence finally, 378 cases received a NIPT risk result. FFs in these 2 cases were 8.58% and 10.96%, respectively. As FF values were within such a wide range, we evaluated the relationships between FF and different parameters to find significant associations (Table I.3.).

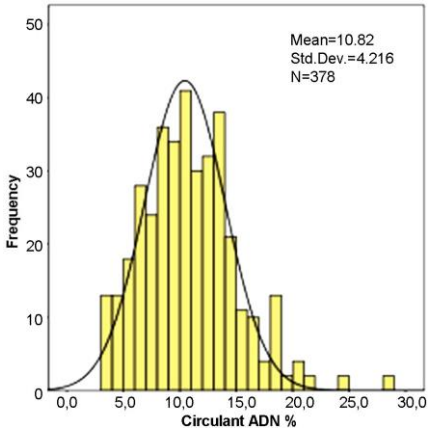


Figure I.10. Distribution of cell-free DNA percentage in maternal blood.

Table I.3. Fetal fraction descriptive statistics for different parameters.

Parameters	Result	Number of Cases	Mean FF[1]	SD[2]	Standard Error of the Mean
Maternal age	Up to 34 years	215	11.23	4.28	0.37
	Over 35 years	163	10.61	3.93	0.39
NIPT[3] risk of aneuploidy	No risk	362	11.03	4.14	0.28
	Risk for aneuploidy as a unique abnormality	10	10.40	4.18	0.82
NIPT risk of aneuploidy	Risk for aneuploidy and microdeletion	2	10.21	4.07	0.54
	No risk	372	10.93	4.16	0.27
NIPT risk of microdeletion	Risk for microdeletion as a unique abnormality	4	12.57	2.76	1.23
NIPT risk of microdeletion	Risk for microdeletion and aneuploidy	2	11.03	3.07	0.89
Risk confirmation	Not necessary	362	11.00	4.22	0.28
	Not confirmed	7	9.17	2.40	0.98
Risk confirmation	Confirmed	9	11.24	2.61	0.87
	Total	378	10.96	4.14	0.27

FF[1], Fetal fraction (%); SD[2], standard deviation; NIPT[3], non-invasive prenatal test.

A gestational age of up to 22 weeks had no influence on fetal fraction. We did not find significant differences in fetal fraction across the high and low-risk groups.
The results of the associations between FF and different parameters were not statistically significant (Table I.4)

Table I.4. Associations between fetal fraction and different parameters.

Parameters Associated with FF[1]	Statistical Significance	Statistical Analysis/ p Values
Gestational age	no significant correlation	Spearman Correlation $\rho = 0.053/p = 0.418$
Pregnant women in the age risk category, over 35 years	insignificantly lower	Mann-Whitney U test/p = 0.400
Maternal age	no significant correlation	Spearman Correlation $\rho = -0.022/p = 0.740$
Pregnant women with NIPT normal result	insignificantly increased	Mann-Whitney U test/p = 0.728
Pregnant women with no risk of aneuploidy	insignificantly increased	Kruskal-Wallis test/p = 0.315
Pregnant women at risk of microdeletion as a single abnormality	insignificantly increased	Kruskal-Wallis test/p = 0.176
Cases confirmed by invasive methods	insignificant association	Kruskal-Wallis test/p = 0.490

FF[1], Fetal Fraction (%)

The mean maternal age in the entire group was 33.4 ± 4.96 years, ranging between 21 and 47 years. The patients were divided into two groups: under 34 years old and 35 years or older. We also calculated the mean age of pregnant women who were at risk for aneuploidy depending on the type of aneuploidy (Table I.5).

Table I.5. Mean maternal age in different groups.

Maternal Age (Years)	Mean ± SD[1]	Minimum–Maximum	Number of Cases
Group up to 34 years	29.8 ± 2.87	21–34	216
Group over 35 years	38.2 ± 2.61	35–47	164
Cases with NIPT risk for trisomy 21	34.8 ± 6.38	25–42	5
Cases with NIPT risk for trisomy 18	36.3 ± 7.10	30–44	3
Cases with NIPT risk for trisomy 13	39	39	1
Cases with NIPT risk for trisomy 14	41	41	1
Cases with NIPT risk for X monosomy	33.5 ± 4.95	30–37	2

Table I.5. (continued)

Group with NIPT risk for all types of tested aneuploidy	35.83 ± 5.57	25–44	12
Group with confirmed aneuploidy	34.5 ± 5.21	25-42	8
Group with NIPT risk of aneuploidy and microdeletion	35.5 ± 6.09	26–41	6
Group with confirmed microdeletion	41	41	1
Entire group	33.4 ± 4.96	21–47	380

SD[1], Standard deviation

The association between advanced maternal age risk (age > 35 years) and the aneuploidy risk was not statistically significant (chi-square test, $p = 0.824$). Age over 35 years was not a significant risk factor for the occurrence of aneuploidy (OR = 1.18, cu 95% CI = [0.5, 2.78]), $p = 0.824$ (Chi2 Test). Most cases were in the 30–34 age group, followed by the 35–39 age group (Figure I.11).

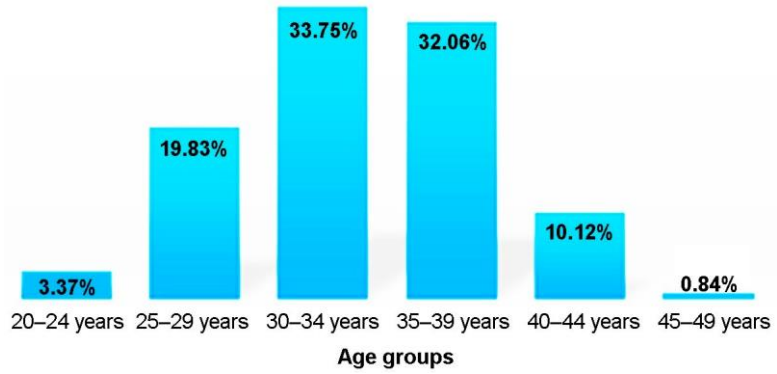


Figure I.11. Distribution of cases considering maternal age.

The Main Indications for the NIPT

The indications for non-invasive prenatal testing were established for all 380 patients during the pre-testing genetic counseling. Most of the tests were performed at the gynecologist's indication for supervision of normal pregnancy (43.9%), followed by the cases with advanced maternal age (43%) and positive obstetric history (13.9%). Of the 8 cases with aneuploidy confirmed by invasive tests, 2 cases were in the age group under 34 years: maternal age was 25 years and trisomy 21 was confirmed in one case; another 30-year-old patient with twin pregnancy had a female twin with trisomy 18. There were also cases with biochemical risk at the double test (DT), prenatal ultrasound abnormalities or with a balanced chromosomal abnormality. A total of 3.8% of cases had a positive family history for single-gene diseases; in these latter cases, the test was performed to identify the sex of the fetus.

Ultrasound abnormalities were identified in 19 fetuses (5%), including 5 cases with increased fetal nuchal translucency (NT) (≥3 mm). The following abnormalities were recorded: intrauterine growth retardation, cardiac anomalies (tricuspid valve regurgitation, tetralogy of Fallot; aortic override, pulmonary artery stenosis, atrial septal defect), renal abnormalities (renal pyelectasis, bilateral hyperechogenic kidney), hyperechogenic fetal bowel, abnormal anterior abdominal wall, central nervous system abnormalities (banana-shaped cerebellum with risk of neural tube defect, choroid plexus cysts of 5 mm), abnormal venous duct with absent, bilateral hydrothorax, ascites, cystic hygroma, sexual ambiguity, polydactyly, clinodactyly. Combinations of indications for testing were registered in 12 cases (3.15%), one of them having 3 and another one having 4 indications.
Four pregnant women (1.05%), carriers of an X-linked recessive gene, were offered the analysis for fetal sex determination. In all cases, there was a female fetus and the NIPT result was normal.
In 10 cases (2.63%), there was an indication related to the existence of a chromosomal disease in the family. In 6 cases there was a relative with trisomy 21. In 3 cases other chromosomal abnormalities were identified: maternal translocation, paternal inversion and a de novo duplication of a previous child.

The NIPT Results

Of the 378 cases, 362 (95.76%) had a low-risk, and 16 (4.24%) had a high-risk. Of these, 8 cases (2.12%) were confirmed by invasive methods, and 8 (2.12%) were false-positive.
Correlations between the risk assessed by NIPT and the results of invasive prenatal diagnosis have also been performed (Table I.6).

Table I.6. The correlation between the risk assessed by NIPT and the final result.

NIPT Risk Assessment	Number of Cases	(%)	Invasive Prenatal Diagnosis			PPV[1] (%)
			Confirmed	False Positive	False Negative	
Total number of cases with NIPT result	378	100				
NIPT low risk	362	95.76	-	-	-	100
NIPT increased risk	16	4.23	8			
NIPT increased risk for isolated aneuploidies	10	2.64	7	3	-	70
Trisomy 21	5	1.32	5	0	0	100
Trisomy 18	3	0.79	2	1	0	66.66
Trisomy 13		0	0	0	1	0
Monosomy X	2	0.53	0	2	0	0
NIPT increased risk for aneuploidy and microdeletion	2	0.53	1	1	0	16.66
Trisomy 13 and 1p36 microdeletion syndrome	1	0.26	0 0	1 1	0	0

Table I.6. (continued)

Trisomy 14 and del(X)(p22.33-11.21)	1	0.26	0 1	1 0	0	0
NIPT increased risk for isolated microdeletions	4	1.04	0	4	0	0
del(10q25.2-q26.3)	1	0.26	0	1	0	0
del(15)(q11.2-13.1)	1	0.26	0	1	0	0
del(20q11.21-q13.13)	1	0.26	0	1	0	0
del(22q11.2) microdeletion syndrome	1	0.26	0	1	0	0

PPV[1], Positive predictive value

In one case, the NIPT result suggested a large chromosomal deletion that was confirmed by aCGH, but with increased size, 61.88 Mb, compared to the size of 54.27 Mb determined by the NIPT. In this case, FF was 15.64%, the highest in the group at risk of microdeletions. The rest of the cases found to be at risk of microdeletions were not confirmed by direct methods (aCGH) (Table I.7).

Invasive Methods for Verifying (Confirmation/Exclusion) NIPT Results

Direct analysis of fetal cells obtained by invasive methods was performed to verify the 16 cases identified to be at risk. Six CVS and ten amniocenteses were performed, followed by combinations of QF-PCR, karyotyping and aCGH. Thus, 29 analyzes were performed, of which 12 were QF-PCR, 11 karyotypes and 6 microarrays. One of the twin pregnancies benefited from 2 karyotypes and the other from 2 QF-PCR analyzes and 2 karyotypes.

Table I.7. Description of cases at risk for microdeletions

NIPT High Risk for Microdeletions	Size (Mb[1]) Determined by NIPT	FF[2] (%)	Gestational Age (Weeks)	aCGH[3] and Size (Mb)
del(X)(p22.33-11.21)	54.27	15.64	13	arr[GRCh37] Xp22.33p11.1 (168551_62051248)x1 61.88 Mb
del(10q25.2-q26.3)	22.57	5.3	11	False-positive
del(20q11.21-q13.13)	18.40	8.32	12	False-positive
del (15)(q11.2-13.1)	5.6	10.96	13	False-positive
del(22q11.2) microdeletion syndrome	2.54	9	12	False-positive
1p36 microdeletion syndrome	2.30	12.3	16	False-positive

Mb[1], Megabase; FF[2], fetal fraction; aCGH[3], Array CGH

In all cases at risk of aneuploidies, 12 fetal karyotypes and 10 QF-PCR analyzes were performed, the latter in order to obtain rapid results. The cases at risk of X monosomy and trisomy 14 were not confirmed.

Two cases at risk of aneuploidy, one with trisomy 21 and one with trisomy 18, were from twin pregnancies. Two amniocenteses were performed separately for each fetus, confirming trisomy 21 in the male fetus from one twin pregnancy and trisomy 18 in the female fetus from the other twin pregnancy.
Two cases were at risk for both numerical and structural abnormalities, and amniocentesis was required, followed by fetal karyotyping and microarray. One case (0.26%) was at risk for trisomy 14, which has not been confirmed, in association with Xp microdeletion, which was confirmed, and the pregnancy was stopped for medical reasons. Another case had a high-risk for trisomy 13 and for microdeletion 1p36, but they were both ruled out and the pregnancy continued.
A high-risk of microdeletion syndromes as unique abnormalities was identified in 4 cases (1.04%): del (10q25), del(15q), del(20q), and del(22q11.2). As mentioned, a high-risk for a microdeletion and an aneuploidy was identified in 2 cases: del(1p36) and trisomy 13 in one case and del(Xp) and trisomy 14 in the other one (Tables I.6 and I.7). In all these 6 cases aCGH was performed. The case at risk for a large deletion, of 61.88Mb, del(X)(p22.33–11.21) was confirmed, but the other 5 cases proved to be a false-positive: del(10q25.2–q26.3), del(15)(q11.2–13.1), del(20q11.21–q13.13), and two microdeletion syndromes: 1p36 and del(22q11.2).

Positive Predictive Value (PPV)

Regarding the high-risk for aneuploidies, out of the 12 cases, 7 were confirmed; therefore, PPV was 58.33%, while regarding the high-risk for microdeletions, out of the 6 cases, only one was confirmed, and PPV was 16.66. Of the 12 aneuploidies, 5 cases (including one from a twin pregnancy) had an increased risk for trisomy 21, and all were confirmed; therefore, we report 100% PPV for trisomy 21. The other 3 cases (including one from a twin pregnancy) had an increased risk for trisomy 18, and two were confirmed; therefore, we report 66.66% PPV for trisomy 18. One case had an increased risk for trisomy 13, but it was ruled out, and we reported 0% PPV for trisomy 13. A special case was one obtained by IVF with an increased risk for X monosomy. CVS with QF-PCR ruled out this diagnosis, but identified trisomy 13, and post-test ultrasound at week 12 showed specific congenital anomalies (polydactyly). This case was a false-negative for trisomy 13 and false-positive for monosomy X. As it was the only case at high-risk for trisomy 13 in the entire group, the NPV was 100%. The second case with increased risk only for X monosomy has not been confirmed. Both cases at risk of X monosomy were false positive.

A comparison of specificity, sensitivity, and PPV scores for aneuploidies is presented in Table I.8.

Table I.8. Comparison of specificity, sensitivity and PPV scores for aneuploidies between NIFTY and NIPS tests

Chromosomal Abnormality	Sensitivity		Specificity		PPV[1]		
	NIFTY BGI	**NIPS INVITAE**	**NIFTY BGI**	**NIPS INVITAE**	**NIFTY BGI**	**NIPS INVITAE**	**Present study**
Trisomy 21	99.17%	99.99%	99.95%	99.89%	92.19%	92.89%	100%
Trisomy 18	98.24%	99.99%	99.95%	99.99%	76.61%	89.11%	66.66%
Trisomy 13	>99.9%	99.99%	99.96%	99.69%	32.84%	73.54%	0%
Monosomy X	>99.9%	99.99%	-	99.89%	40%	69.15%	0%

PPV[1], Positive predictive value.

1.3.1.4. Discussion

Until recently, prenatal screening for fetal aneuploidies relied on the measurement of maternal serum biochemical markers combined with fetal ultrasound markers [Koumbaris et al., 2019].

Non-invasive prenatal testing analyzing FF of DNA in maternal blood has rapidly applied in the last few years. It is used as a screening test for the most common chromosomal disorders. NIPT relies on the presence of fragments of cell-free placental and maternal DNA in maternal blood to assess the risk of an affected pregnancy. The test compares the total maternal and fetal DNA in a maternal blood sample to a control sample using advanced bioinformatics analysis. It is provided for screening only. False-negative and false-positive results may occur. This test assesses the risk for the following chromosomal abnormalities: trisomy 21, 18, 13, sex chromosome aneuploidies, and several microdeletions [Liu et al., 2016; Yao et al., 2014]. NIPT can be performed at any stage of the pregnancy, generally from 10 weeks' gestation onwards, to ensure adequate cfDNA in the maternal plasma sample [Bianchi et al., 2012; Bianchi, Chiu, 2018; Taneja et al., 2017]. In our study group, NIPT was performed between 10 and 22 weeks of pregnancy. However, most cases came immediately after 10 weeks of gestation, with a mean of 12.62 ± 2.47 weeks.

Fetal Fraction

The proportion of cfDNA molecules is expressed as the fetal DNA fraction in the plasma of pregnant women [Samur, 2020]. We analyzed the association between FF and the following parameters: gestational age, maternal age, and NIPT risk result for aneuploidies and for microdeletions, but no significant correlations were found.

In our study, a gestational age of up to 22 weeks had no influence on FF, consistent with the results of Hestand et al. [Hestand et al., 2018]. Other studies found that the FF positively correlated with gestational age [Guo et al., 2019] and significantly increased especially beyond 23 weeks of gestation, but waiting for a late gestational age is not a reliable approach [Taneja et al., 2017; Hou et al., 2019]. We found lower FF values in pregnant women over 35 years compared to those under 35 years, but the difference was not statistically significant. Hou et al. [Hou et al., 2019] found that the percentage of FF significantly decreased with an increase in maternal age. A negative correlation between FF of cfDNA and maternal age was reported by Guo et al. [Guo et al., 2019], while Hestand et al. found no influence of maternal age on FF [Hestand et al., 2018].

We did not find significantly increased FF values in pregnant women with low-risk NIPT results. In a study regarding FF in pregnancies with a low and high-risks for fetal chromosomal aneuploidies, Hudecova et al. reported no statistically significant difference in FF across the high, intermediate, and low-risk groups [Hudecova et al., 2014]. In our study, the FF values were not significantly increased in pregnant women at risk of a microdeletion. Using multivariate regression analysis to determine several maternal and fetal factors that might be significant predictors of the FF, Ashoor et al. found that fetal karyotype did not provide a significant independent prediction of FF [Ashoor et al., 2012]. Another study compared FF in euploid versus aneuploid pregnancies and found that the median FF was significantly higher in Down syndrome pregnancies and significantly lower in trisomy 18 and triploid pregnancies. Some results were not informative, and this was due to low FF, but the authors considered that the high-risk for trisomy 18 and/or triploidy warranted offering additional assessments [Palomaki et al., 2015]. NIPT could establish a risk in our study in 378 cases and the FF range was found between 3.5% and 28.12%, with a mean of 10.96 ± 4.14%. It was confirmed that the important prerequisites for NIPT are FF > 3.5% and gestational age over 10 weeks of pregnancy. An FF-based risk model was built for pregnancies with a FF too low to receive a result on standard NIPT. This algorithm can identify a subset of cases at

increased risk for trisomy 13, trisomy 18, or triploidy [McKanna et al., 2019].

Invasive assays used in our cases were CVS and amniocentesis. The main advantage of CVS is that it can be performed immediately after obtaining the NIPT result; hence a result can be obtained in the shortest possible time. Patients who underwent amniocentesis could not have CVS because the pregnancies were advanced, close to week 16.

The mean maternal age in the group detected to be at high-risk for aneuploidies was 35.83 years (range between 25–44 years), but if we exclude the false-positive cases and consider only the confirmed aneuploidies, mean maternal age was 34.5 years (range between 25–42), very close to the mean age of the entire study group, which was 33.4 years (ranging between 21–47 years). The mean values were somewhat close, therefore not statistically significant, and considering the very wide ranges found in the study group, it did not help us in assessing the risk before NIPT was performed. This is one of the reasons why we advocate for performing the NIPT in all pregnancies, even in the absence of classical indications.

If a fetus has a trisomy, the fetal-derived DNA molecules from the extra chromosome should be increased in maternal plasma, when compared to a pregnancy with a euploid fetus [Chen et al., 2011; Lo et al., 2010]. Trisomy 21 was the only abnormality for which the NIPT risk result was confirmed in full, PPV being 100%. The mean maternal age in these cases was 34.8 years. According to BGI versus Invitae specifications for trisomy 21, sensitivity is 99.17% versus 99.99%, specificity is 99.95% versus 99.89%, and PPV is 92.19% versus 92.89% (Table I.8.). Only 20% of our cases had ultrasound abnormalities, 40% had abnormal prenatal biochemical screening results-double test, and another 40% had maternal age over 35 years as the only indication for NIPT. Yamada et al. reported a lower maternal age-specific risk for trisomy 21 based on the clinical performance of NIPT compared to the risk predicted in several other studies [Yamada et al., 2018].

NIPT was positive for trisomy 18 in 3, cases but only 2 have been confirmed, PPV being 66.66%. Interestingly, the case with the false-positive result that was not confirmed by amniocentesis followed by QF-PCR was 44 years old. Much higher PPV values were available from both partner laboratories: 76.61% (BGI) and 89.11% (Invitae) (Table I.8) [Samur, 2020; Zhang et al., 2015; Jiang et al., 2012].

One of our cases had a high-risk for trisomy 13 and for 1p36 microdeletion, both abnormalities being excluded after amniocentesis followed by QF-PCR and aCGH. The pregnant woman was 39 years old and gave birth to a healthy baby. In contrast, another 37-year-old woman with the pregnancy achieved by IVF, with cystic hygroma and heart abnormality on a prenatal ultrasound, and NIPT high-risk for monosomy X was found to have a fetus with trisomy 13 after CVS with QF-PCR was performed [Vaida et al., 2021]. Preimplantation genetic testing was not performed in this case. Post-NIPT ultrasound identified polydactyly, and the pregnancy was stopped for medical reasons. In a report on NIPT using fetal DNA from maternal plasma for trisomy 13 detection, Yu et al. found the sensitivity and specificity of 95.2% and 99%, respectively [Yu et al., 2014].

NIPT identified an increased risk for monosomy X in two cases, but both were false-positive, PPV being 0% in our study group. Monosomy X has the lowest PPV, 40% according to BGI and 69.15% according to Invitae Laboratories [Bianchi et al., 2012; Jiang et al., 2012]. Other trisomies can be detected by chance with the low genome coverage version. Of the aneuploidies found in our study group, only trisomy 14 falls into this group, noting that this abnormality is not part of the standard aneuploidies expected to be identified, but it is an abnormality that can be additionally detected by NIFTY [Kleinfinger et al., 2020]. In addition to the standard aneuploidies, in one case, a high-risk for trisomy 14 and for Xp deletion has been found. The 41-year-old pregnant woman had a positive medical history: a trisomy 21 pregnancy resulted in a stillbirth. The case was special because, after amniocentesis followed by QF-PCR, aCGH, and fetal karyotyping, trisomy 14 was ruled out; this was a false-positive

result. The deletion of the short arm of the X chromosome was confirmed, and a translocation between chromosome 14 and the Xq arm was identified. The fetus had numerous ultrasound abnormalities, and termination of pregnancy for medical reasons was decided. Rare autosomal trisomies are important but not so rare. Scott et al. found that they are often associated with poor obstetric outcomes [Scott et al., 2018]. Preimplantation testing was performed in two pregnancies (2/7) obtained by IVF (outside Romania). NIPT was also carried out (NIFTY in one pregnancy and NIPS in the other one), confirming the sex of the fetus and the absence of aneuploidies.

Twin cases are particular cases. First, NIPT analysis can only evaluate the risk of aneuploidy and not of microdeletions. Our study included 2 pregnant women (30 and 36 years old) without other indications besides NIPT increased risk for trisomy 21 and trisomy 18, respectively. In both cases the twins were of different genders. In each case, two amniocenteses were performed followed by QF-PCR and fetal karyotyping and in both cases, one of the twins was confirmed with a trisomy (one male fetus with trisomy 21 and in the other pregnancy one female fetus with trisomy 18). In both cases, the parents decided to stop the pregnancy. Another peculiarity of twin pregnancies is related to the vanishing twin, whose sex can cause false-positive results for fetal gender. As the pregnancy progresses, the ultrasound sex is observed, and if there are discordances, a disorder of sex development must be considered. In these cases, genetic counseling and detailed anamnesis are especially useful and can further guide pregnancy management. BGI recommends waiting 8 weeks after the disappearance of a twin before taking the NIPT test; it is considered that the FF of the vanished twin has decreased considerably during these weeks and no longer influences the result. However, this is impossible to achieve when the pregnant woman presents to the first obstetric consultation too late for confirmation of pregnancy, a few weeks after the signs of a twin pregnancy disappears. Therefore, we encourage pregnant women to have their first obstetric consultation as soon as possible. A recent study has found that trisomy 21, 18, and 13, as well as X chromosome aneuploidies, were accurately detected by NIPT in twin pregnancies. As in our study, the aneuploidies mostly occurred in only one twin. The authors agreed that NIPT could be used as routine prenatal screening in twin pregnancies [Yu et al., 2019]. If there are more than two fetuses, the risk of a false-positive and negative results increases. In their study, Hartwig et al. reported four false negative aneuploidy cases, of which two were explained by a vanishing twin. The sensitivity and specificity, when no-calls and vanished twins were excluded, were 100% and 99.5% for trisomy 21, 91% and 99.2% for trisomy 18, and 100% and 99.6% for trisomy 13 [Hartwig et al., 2018].

Many factors, both maternal (such as maternal blood transfusion, surgery, immunotherapy, neoplasm, or mosaicism) and pregnancy-related (vanishing twin, fetal demise, confined placental mosaicism, true fetal mosaicism, uniparental disomy, and polyploidy), can affect the results. In our study, the pregnant women denied such medical conditions. Regarding pregnancy-related factors, fetal ultrasonography did not reveal the presence of a vanishing twin. The other above-mentioned chromosomal abnormalities were not identified with invasive techniques. Fetal demise during weeks 20–30 of gestation was registered in 2 cases, both having NIPT low-risk results, and the causes were not established. We also gathered information about births and offspring, revealing that a female newborn from a twin pregnancy was diagnosed with Treacher Collins syndrome after birth. All other newborns were considered healthy at birth without any suspicion of chromosomal syndromes.

Microdeletion and microduplication syndromes are caused by CNVs and are relatively rare disorders [Qi et al., 2020]. It is estimated that they account for 1–2% of all newborn congenital anomalies and the most common is 22q11.2 deletion syndrome [Wapner et al., 2012]. NIPT test can detect specific loci relevant to microdeletion/microduplication syndromes according to OMIM and Decipher databases. The risk of false-positive/negative

results in these cases can be increased compared to common trisomies. Due to the low prevalence of these microdeletion syndromes and the limited performance data for these disorders, it is not possible to calculate a precise and accurate PPV.

Our study included cases analyzed during 2014 using the NIFTY test. At that time, three aneuploidies (for chromosomes 13, 18, 21) and three microdeletions (1p36 deletion syndrome, 5p Cri du Chat syndrome, 2q33.1 deletion syndrome) could be tested. During 2015, the test was improved, enabling identification of six aneuploidies (chromosomes 9, 16, 22 were added) and eight microdeletion syndromes (5p, 1p36, Van der Woude 1q32.2, 2q33.1, 16p12.2, DiGeorge 2, Jacobsen 11q23, Prader-Willi/Angelman). In 2017 the number of microdeletions that could be tested increased to 63, and in 2018 the test changed its name to Nifty-pro, and the number of microdeletions increased to 84.

In our study, 5 out of 6 cases identified as having a high-risk of microdeletions, were ruled out; therefore, our PPV was only 16.66%. One of our cases had a high-risk of Xp microdeletion, of 54.27 Mb; after aCGH was performed, it has been confirmed, but having a larger size, 61.88 Mb. FF, in this case, was 15.64%, the highest in the group at risk of microdeletions.

The other microdeletions, which were not confirmed, were smaller, their sizes ranging from 5.06 Mb to 22.57 Mb. The high risk for deletions was identified in these 5 cases: del (10q25), del (15q), del (20q), del 1p36 and del (22q11.2) microdeletion syndrome. All five were ruled out following direct analysis of fetal cells by aCGH. The gestational age was variable, ranging between 11–16 weeks.

Detection of microdeletions can be performed by the BGI laboratory through low coverage whole genome sequencing using NGS technology and a bioinformatics algorithm, which allows the identification of 84 deletions and duplications. Internal analysis of BGI showed a sensitivity exceeding 90% (cfDNA9.5%) in selected del/dup syndromes with abnormal size over 3 Mb.

The maternal presence of CNVs, as recently reported in two cases, could explain why the results yielded by NIPT are not confirmed by invasive methods [Qi et al., 2020]. Microdeletions can occur de novo in most cases but can also be inherited if a parent has a balanced translocation. Results should always be reviewed with a qualified healthcare professional. High-risk results must be followed by confirmatory diagnostic testing.

The PPV and NPV reported scores do not consider additional clinical information such as previous screening results, positive family history, or abnormal ultrasound findings. It is not possible to calculate a precise and accurate PPV for sex chromosome aneuploidies due to their uncertain prevalence and the limited performance data for these disorders. Moreover, due to the low prevalence of microdeletion syndromes and the limited performance data for these disorders, it is not possible to calculate a precise and accurate PPV as well. The Netherlands launched a study on NIPT as a first-tier test offered to all pregnant women. This study confirmed that genome-wide NIPT is a reliable and robust screening test. PPVs found by the authors were 96% for trisomy 21, 98% for trisomy 18, and 53% for trisomy 13 and were higher than expected [van der Meij et al., 2019].

Another study that examined over 8000 single pregnancies with NIPT reported that PPVs for trisomy 13, 18, 21 and sex chromosome aneuploidy were 14.28%, 60%, 80%, and 45.83%, respectively. At the same time, they have found 0.63% positive cases for chromosomal microdeletions or microduplications, but only 36.11% of them were true-positive cases. They concluded that NIPT had the highest accuracy for trisomy 21 detection, while accuracy was low for chromosomal microdeletion and microduplications [Hu et al., 2019].

NIPT is an advanced screening test, incorporated into clinical practice in the context of genetic counseling. The genetic counselor should highlight that NIPT is a screening test, but

the detection rate is superior to that of maternal serum screening, which has a misdiagnosis rate of 5 to 50% [Nicolaides, 2011]. NIPT results may indicate an increased risk for specific conditions. The healthcare provider will further interpret the NIPT results in the context of the patient's clinical data and family history and will recommend genetic counseling and additional testing when appropriate. Diagnostic testing should be performed to confirm a positive result [Buchanan et al., 2014].

In our study, four pregnant women (1.05%) were carriers of an X-linked recessive gene, and the analysis was performed for result-oriented care, focusing on the fetal sex. In all cases, the fetal gender was identified as female and had a low-risk NIPT. Pan et al. developed a fetal sex determination method based on maternal plasma sequencing and assessed its potential use in X-linked disorders counseling, concluding that high accuracy of non-invasive fetal sex determination can be achieved [Pan et al., 2014].

Several other cases had a history of single-gene diseases in the family, and even if NIPT could not detect a risk for these conditions, further explanations were needed, and amniocentesis was recommended. In two cases with advanced maternal age, after a low- risk NIPT result, the management of the pregnancy was oriented towards amniocentesis to test the fetus for known mutations that could have been inherited from the carrier's parents [Belengeanu et al., 2004; Gug, Mihaescu, Mozos,2018; Gug, Gorduza et al., 2020; Gug, Caba et al., 2020].

NIPT newer technologies have already expanded prenatal screening beyond common autosomal aneuploidies to smaller chromosomal abnormalities. Other tests for single-gene disorders are currently developed, but their validation is important. Parents should be provided with appropriate genetic counseling by a qualified professional [Shaw et al., 2020; Belengeanu et al., 2009].

1.3.1.5. Conclusions

This is the first study in our country reporting the NIPT results from a series of 380 cases (Caucasians from Eastern Europe). The confirmation rate was higher for autosomal aneuploidies compared to sex chromosome aneuploidies and microdeletions. All cases at risk for trisomy 21 were confirmed. A trisomy 13 with a false-negative NIPT result was identified via invasive prenatal diagnosis. Only one large fetal microdeletion detected by NIPT has been confirmed. False-positive NIPT results, which were not confirmed by invasive methods, led to the decision to continue the pregnancy, bringing relief to pregnant women. A gestational age of up to 22 weeks had no influence on fetal fraction. We did not find significant differences in fetal fraction across the high and low-risk groups. The main limitation of the study is the small number of patients included. NIPT can be used as a screening method for all pregnancies, but in high-risk cases, an invasive confirmation test is strongly recommended, and no definitive measure of pregnancy termination should be taken without a result obtained by an invasive method.

1.3.2. Comparison between paramagnetic and CD71 magnetic activated cell sorting of fetal nucleated red blood cells from the maternal blood

1.3.2.1.Introduction

Prenatal diagnosis has become critical for the management of high-risk patients and fetal ultrasound defects. Currently, a definitive prenatal diagnosis needs an invasive procedure, most frequent a chorionic villous biopsy or an amniocentesis. These procedures are associated with a low, measurable risk for the fetus and the mother [Salomon et al., 2019]. Therefore, significant efforts have been made to develop non-invasive diagnosis methods with similar performance.

Currently, non-invasive prenatal testing (NIPT) techniques are based mainly on cell-free fetal DNA analysis in the maternal blood. However, these methods can accurately identify common aneuploidies (trisomy 21, 18, and 13), but they are, at this moment, partial ineffective in detecting copy-number variations, which are another important group of prenatal abnormalities [Gil et al., 2017].

Many studies proved the presence of fetal cells in maternal blood [Sekizawa et al., 2007]. Intact fetal cells from the maternal circulation can overcome the cell-free NIPT's limitations because they could be a source of pure fetal genomes. They are a rare event, with a frequency of one in 104 to 109, which represents a challenge for actual technologies [Hamada et al., 1993]. However, these cells can be enriched from the maternal circulation and used in different tests to assess the fetus genetics. That leads to interesting opportunities for prenatal evaluation.

After years of oblivion, the interest in the analysis of the fetal cells non-invasively isolated from maternal circulation reborn [Pin-Jung et al., 2019]. That is due to recent developments in single-cell analysis technologies, which opens opportunities for prenatal screening [Vossaert et al., 2018; Hua et al., 2015].

Fetal nucleated red blood cells (NRBC) are a reliable candidate target because they have a limited lifespan [Pearson, 1967], can be differentiated morphologically from maternal cells [Huang et al., 2011] contain a representation of whole fetal genome [Liou et al., 1993] have specific markers (eg, embryonic and fetal globin) and allow analysis with chromosomal fluorescence in situ hybridization (FISH) [Nemescu, Martiniuc, Gorduza, Onofriescu, 2011].

Because the number of fetal NRBC in maternal blood is small, many attempts have been made to enrich these cells: density gradient centrifugation, magnetic-activated cell sorting (MACS), fluorescence-activated cell sorting (FACS), lectin-binding method, micro-beads sedimentation [Wei et al., 2018] and micromanipulation. However, the procedures are complex that limits extensive studies. In recent years, many approaches based on microfluidics chips have been developed to capture circulating fetal cells [Easley et al., 2006; Huang et al. 2008; Ma et al., 2019; Feng et al., 2018; Huang et al., 2017].

Despite considerable progress, reproducibility, and reliability of isolation and detection of fetal cells from maternal blood remain poor [Bianchi et al., 2002]. That was attributed to the rarity and variability of fetal cells among pregnancies [Choolani, 2012]. Also, current methods require a great deal of work and specific cellular localization or manipulation technologies that loose or affect the cells. Thus, the selection methods should be relatively simple, efficient, and reproducible.

This study aimed to compare two simplified selection methods of the fetal cells in the blood of normal pregnant women. First was a magnetic cell selection based on the paramagnetic proprieties of the hemoglobin, converted to methemoglobin. Second was a positive MACS enrichment, using anti-CD71 monoclonal antibodies. The fetal cells were recognized through the visualization of the Y chromosome by FISH.

1.3.2.2. Materials and methods

Patients

We included women with singleton pregnancies with a male fetus, attending for routine antenatal care or prenatal diagnosis. After an informed consent, approved by the University Ethics Committee, we collected 10-16 mL of maternal peripheral venous blood into K2 EDTA vacutainers and processed within 1-4 hours. In all cases, there was a normal fetal development without structural or chromosomal abnormalities. The fetal sex determined by ultrasound, was confirmed by amniocentesis or at birth. We harvest the maternal blood samples before any invasive fetal procedure.

Separation and enrichment of nucleated cells from the maternal blood

In the laboratory, maternal samples were mixed for 15 minutes on a roller, diluted 1:1 with phosphate-buffered saline (PBS) Dulbecco without Ca and Mg (Biochrom AG) and carefully layered on a double- density gradient prepared with 10 mL of 1.119 g/mL and 10 mL of 1.077 g/mL Percoll (Fluka). Then, at room temperature (20°C), samples were centrifuged at 500 *g* for 30 minutes (2 acceleration, no brake). After that, the cell ring at the interface of the two density gradients was collected with a Pasteur pipette and washed twice with PBS Dulbecco's solution (1:1 dilution). Then, another by centrifugation at 500 *g* for 10 minutes followed. The cell layer of interest was localized with density marker beads (green 1.102 g/mL and orange 1.035 g/mL, Sigma). After removal of the supernatant, the washed cells were re-suspended in 3 mL buffer prepared by a 1:10 dilution of the MACS BSA Stock Solution with autoMACS™ Rinsing Solution (named Buffer B). The viable cells thus selected were counted using a Bürker-Türk chamber, after which the sample was divided into two aliquots. These were processed as paired samples using the hemoglobin enrichment and magnetic-activated cell sorting (MACS) techniques.

Magnetic cell selection with hemoglobin was based on the paramagnetic properties of the nucleated cells when the contained hemoglobin is converted to methemoglobin. For this, one aliquot of the suspension was incubated with 1.5 mL of 50 mmol/L $NaNO_2$ solution for 10 minutes at room temperature. The probe was then passed through a MiniMACS MS+ magnetic column under a magnetic field of 1.4 T provided by a VarioMACS™ magnet (Miltenyi Biotech). We used a low flow rate of 270 s/mL controlled with a NE-500 programmable syringe pump (World Precision Instruments Inc) [Huang et al, 2008]. The column was then washed with 500 µL buffer B at the same flow rate. After rinsing, the column, removed from the magnetic field, was eluted with 2.5 mL Hanks' balanced salt solution (HBSS) at high flow, according to the manufacturer's protocol. Cells from the other aliquot of the suspension were enriched by CD71 magnetic positive sorting technique, following a slightly modified manufacturer protocol (Miltenyi Biotech), previously described [Nemescu, Constantinescu, 2011].

Viable cells from the paramagnetic and CD71 positive fractions were counted using a Burker-Turk chamber. The suspension was gently mixed with a fresh Carnoy solution (1:1 dilution) and centrifuged at 300 *g* for 10 minutes. The pellet was re-suspended in 500 µL Carnoy fixative for a minimum of 2 hours. We placed the enriched NRBCs on increased adherence slides (Superfrost®Plus Gold, Menzel-Glaser) by cytocentrifugation at 270 *g* for 5 minutes (Rotofix 32, Hettich) at medium acceleration and brake, using a mean size cyto chamber (60 mm^2 area, Hettich Cyto System 2). We obtained, for each case, a single-cell spot, with 0.3-3 × 105 total cells/slide. Then, the slides were cytocentrifuged at 1100 *g* for 1 minute and air-dried. Finally, the slides were successively fixed with 100% methanol for 5-7 minutes and formaldehyde (2% in PBS) for 10 minutes at room temperature.

To assess the protocol results and to check the presence of hemoglobin-rich cells, slides from some patients were stained with benzidine and May-Grunwald-Giemsa. In practice, the slides were exposed to a solution of 0.25% benzidine (Sigma-Aldrich, #3503-1G) in methanol for 3 minutes, then developed in a solution of hydrogen peroxide and methanol for 1.5 minutes in the dark (1.25 mL 30% hydrogen peroxide in 50 mL 50% ethanol). After two rapid rinses in dH_2O, the slides were fixed for 10-15 minutes in absolute methanol. We performed the May Grunwald Giemsa staining according to the standard methods, the slides being finally washed with dH_2O, and then with tap water for color enhancement [Huang Huang, 2008] However, we found that these treatments affected the quality of FISH, so this step was removed from the study protocol.

Fluorescence in situ hybridization analysis (FISH)

The FISH analysis was performed using specific satellite enumeration probes for chromosomes X and Y (Kreatech Poseidon™, Leica Biosystems), and the rapid protocol was presented elsewhere [Nemescu, Martiniuc, Gorduza, Onofriescu, 2011]. The fluorescent signals were analyzed using an Axioscop 1.0 microscope with ×100 objective and triple band-pass filters (Zeiss). The images were captured and processed using a digital camera and TissueFAXS software (TissueGnostics). The system automatically recorded the cells from the whole cytocentrifugation spot, followed by manual analysis of images. We choose for analysis only intact cells that were not overlapping. When a Y chromosome was suspected, the field was re- checked manually. Generally, all identified spots were counterchecked for non-specificity in all other filters at a magnification of ×1000.

The enrichment steps and analysis of fetal cells were performed without knowledge of clinical details or fetal sex.

Statistical analysis

Data were analyzed using the SPSS program, version 23.0 (SPSS). The Gaussian distribution of data was evaluated with the Shapiro-Wilk test. Non-parametric tests (Wilcoxon Signed Ranks Test) were used for comparison between paramagnetic and anti-CD71 sorting of nu- cleated cells as well as for differentiation of XY cell numbers in the two groups, as all these data had a non-gaussian distribution. The purity of cell isolation was expressed as 1-NRBC/total cells for each enrichment method. The efficiency of the two methods in detecting fetal sex (at least one XY cell) was compared using the McNemar test. A *P*-value of<.05 was considered significant.

1.3.2.3. Results

We tested peripheral blood samples from 27 normal pregnancies with unique, healthy fetuses. Seventy-seven percent of them were at their first pregnancy, and the others have had no history of a male fetus. The gestational age had a mean of 21 weeks (range 12-30). Maternal age has a median of 26 years (range 16-38). From 10 mL of maternal blood, we isolated a mean of 9.6 ± 1.2 × 106 mononuclear cells (Figure I.12.) (mean ± SE). The median number of isolated NRBC, corrected for 10 mL blood, was 29.7 × 104 cells (range: 0.9-76.8 × 104) after hemoglobin enrichment and 10.1 × 104 cells (range: 1-28.8 × 104) after anti-CD71 magnetic-activated sorting. There is a significant statistical difference between the numbers of total NRBC isolated by the two techniques (Wilcoxon Signed Ranks Test; $P < .001$) (Figure I.13.). The median depletion rate (1-NRBC/total cells) was significantly lower for hemoglobin selection (96.7%) than for the anti-CD71 sorting (99.1%) (range: 89.2%-99.8% vs 95.7%-99.8%; Wilcoxon Signed Ranks Test; $P < .001$).

The total number of NRBC harvested from double-density gradient centrifugation, or the total number of cells from hemoglobin or MACS enrichment were not influenced by parity or gestational age.

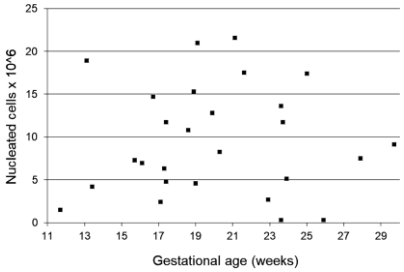


Figure I.12. Number of NRBC isolated by centrifugation in the density gradient, depending on gestational age

In the case of paramagnetic selection, the FISH analysis found at least one male cell in 22 of the 27 (81.5%) cases. The average number of XY cells viewed was 5.09 ± 2.5 (mean ± SE) cells, with a range between 2 and 10. For anti-CD71 selection, the FISH analysis found at least one male cell 16 from the 27 women (61.5%). The average number of XY cells, thus visualized, was 3.38 ± 1.7 (average ± SE), with a range between 1 and 6. That represents about one cell per 1 mL of maternal blood. There was no statistically significant difference between the percentage of nuclei with an XY signal in hemoglobin and anti-CD71 sorted samples (McNemar test, N = 26, $P = 0.109$). However, the number of NRBC was significantly higher in hemoglobin selected samples ($P = 0.03$, Wilcoxon Signed Ranks Test) (Figure I.14). All control slides, with male cord blood samples, achieved easily identifiable spots for all fluorochromes. Our FISH protocol achieves an efficiency over 90% for the X and Y chromosome samples. All probes were processed by female assistants only, to limit the contamination.

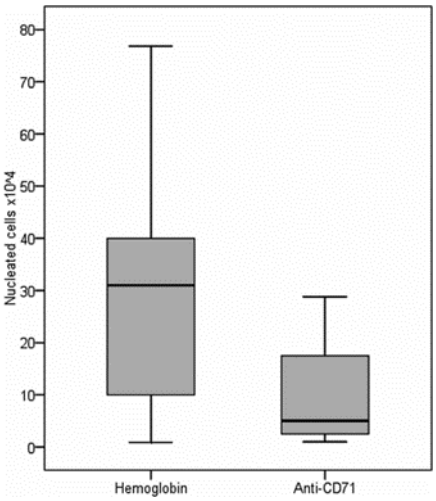


Figure I.13. NRBC isolated by paramagnetic hemoglobin and anti-CD71 techniques. The horizontal line represents the median. The bottom and top of each box show 25% and 75% of all values, respectively. The upper and lower bars represent the 90% and 10% limits, respectively

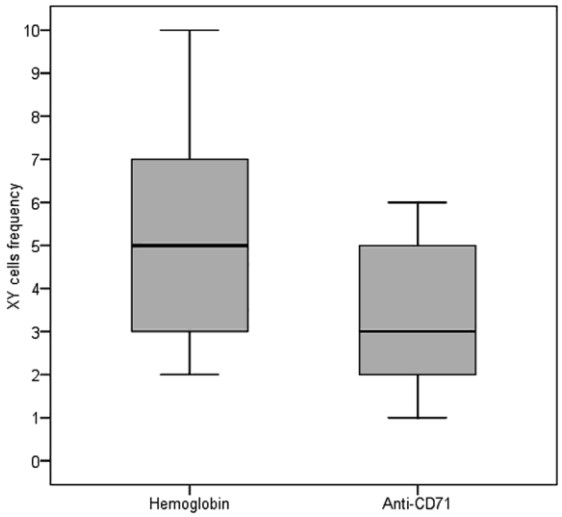


Figure I.14. The number of XY nuclei identified by FISH for hemoglobin enriched and anti-CD71 sorted samples. The horizontal line represents the median, the inferior and superior bars represent the limits of 10%, respectively, 90%. The bottom and top of each box show 25% and 75% of all values, respectively

The final enriched fractions were assessed using benzidine/May- Grunwald-Giemsa staining. That found the isolation of several cell types, including erythrocytes, white blood cells, and NRBCs. The NRBCs were recognized as cells with a low nucleus to cytoplasm ratio, a small dense nucleus, and an orthochromatic nongranular cytoplasm [Troeger, 1999]. The hemoglobin in the erythroid cells was specifically stained using a pseudo-peroxidase reaction with benzidine. Thus, the cytoplasm of the hemoglobin containing NRBCs appears golden-brown, improving their detection (Figure I.15).

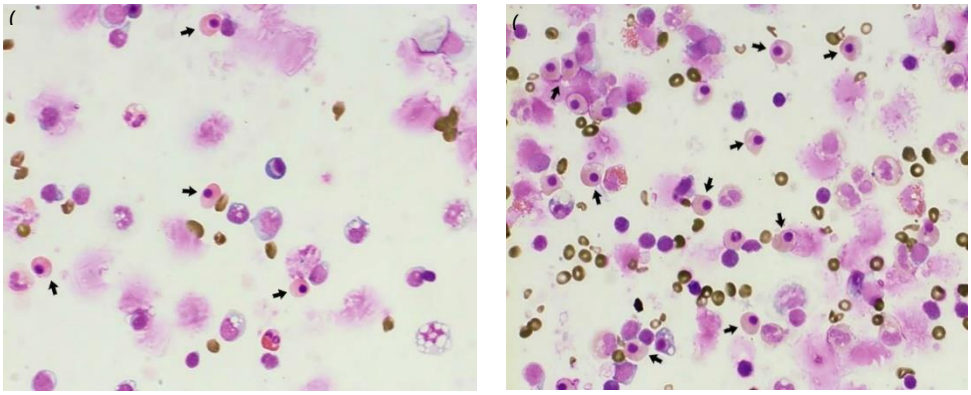


Figure I.15. Cytospin preparation of enriched cells from anti-CD71 positive magnetic sorting (A) and paramagnetic selection (B), stained with benzidine/May-Grunwald-Giemsa (×400). The NRBCs (arrows) were recognized as cells with a low nucleus to cytoplasm ratio, a small dense nucleus, and an orthochromatic nongranular cytoplasm. Also, by benzidine staining, the cells with a golden- brown cytoplasm contain hemoglobin (NRBC, RBC), and cells without hemoglobin (WBC) have a blue cytoplasm

1.3.2.4. Discussion

Our study assessed the ability to isolate the fetal nucleated erythroblasts by a simplified method. After a common double-density gradient enrichment, we compared the efficacy of the magnetic selection using the paramagnetic properties of hemoglobin with the magnetic sorting using anti-CD71 antibodies. In the first case, the method selects erythroblasts containing magnetic active hemoglobin, converted to methemoglobin, while in the second, the fetal erythroblasts are screened according to the presence of CD71 antigens. The assessment of the two methods was performed by highlighting the Y chromosome using FISH method.

Double-density gradient centrifugation provided mononuclear concentrations with a wide range of variations. Similar values acquired Lim et al. [Lim, Tan, Goh, 1999] (mean of 11.8 × 106 cells/ 10 mL blood) and Reading et al. [Reading et al., 1995] (mean of 17.47 × 106 cells/ 10 mL whole blood), using a 1.077 g/mL density gradient centrifugation with Percoll or Histopaque, respectively. Kwon et al, using the same double-density gradient as in our study, but with other osmolarities, found similar results: a mean of 7, respective 4.9 × 106 NRBC cells per 10 mL blood, for gestational ages of 17 and 28 weeks [Kwon et al., 2007]. Our value (950 NRBC/µL) also fits in the range described in neonates [Christensen et al., 2011]. The number of cells recovered by the double-density gradient had a considerable variation. That may directly influence the number of cells from magnetic sorting and probably the number of identified fetal cells.

The stationary capture of fetal nucleated erythroblasts in the magnetic column through a single separation cycle allowed the elimination of non-specific cells with a 96% efficiency (median 96.7%, percentiles 20 and 80, 94.9% and 98.7%, respectively). However, these values are lower than those obtained by Huang et al. in a similar experiment (median separation efficiency 98.6%, percentiles 20 and 80, 98.1% and 99%, respectively). This difference could appear by the higher number of cells initially introduced in the experiment because the separation was carried out by a microfluidic device [Huang et al., 2008].

The second method was a positive magnetic sorting using CD71, the transferrin receptor present on erythroid cells such as nucleated erythrocytes. We achieved a mean depletion of 98.6%, similar to that obtained by others [Reading et al., 1995]. However, other groups, using a similar method, have obtained significantly more CD71 positive cells (mean of 34 to 149 × 104 cells/10 mL blood), with a lower depletion rate (94%-97%) [Kwon et al.,

2007; Prieto et al., 2002]. Variations in the initial cell number and the different measurement methods (automatic vs manual) can explain these differences.

We did not find the variation of isolated total NRBC with gestational age like others, using a microfluidic or microbead selection [Wei et al., 2018; He Z et al., 2017]. In this study, we found through FISH at least one XY cell in 81.5% of the pregnant women bearing a male fetus, in the case of the paramagnetic selection and in 59% for that with anti-CD71 antibodies, respectively.

The rate at which the CD71 positive selection followed by FISH found a male sex fetus varies widely in the literature from 24% to 100% [Kwon et al., 2007; Jansen et al., 1997]. Most studies report values ranging from 50% to 60% [Fernandez et al., 2005; Zhao et al., 2004]. This phenomenon could appear because: (a) only a small part of the fetal cells is nucleate [Seppo et al., 2008] (b) we can select only a reduced proportion of the fetal erythroblasts, (c) the fetal erythroblasts have a large number variation in the maternal circulation, (d) the fetal erythroblasts do not survive or are strongly affected through the enrichment process, with numerous cell treatments. From this point of view, some studies have found that most fetal erythroblasts are not suitable for FISH analysis, about 43% of them being apoptotic [Sekizawa et al., 2000]. Erythroblasts could find in the maternal blood a higher oxygen concentration, which promotes apoptosis [Kondo et al., 2002] and reduces nucleus size [Babochkina et al., 2005]. Thus, probably most fetal erythroblasts are not suitable for the FISH analysis. Other authors suggest contamination, especially with persistent fetal cells from previous pregnancies [Kwon et al., 2007]. In our study, the percentage of women at their first pregnancy was high (80%), and the others did not have male children. These could partially explain the reduced frequency of identification of XY fetal cells.

Finally, we found a low number of confirmed XY cells in all samples, an average of five cells in 10 mL blood for paramagnetic selection, and three cells in 10 mL blood for anti-CD71 selection. New technologies based on microfluidic devices achieved a slightly improved number, in normal pregnancies: 2.38-7.25 fetal cells per mL of maternal blood in Huang et al [Huang et al., 2017] study, or at least 1-11 fetal cells per mL in Ma et al [Ma et al., 2019] study.

However, there are some limitations to the study. First, the CD71 and hemoglobin are not specific to fetal cells, being expressed as well in maternal erythroid precursors. Therefore, we expect many maternal cells selected by the two enrichment methods, which must be differentiated by FISH. Second, the number of available fetal cells is inevitably reduced by the numerous procedures like centrifugation, resuspension. Third, the cytospin itself could be a limiting factor, leading to an uneven cell distribution, not fixing all cells, and even degrading some of them. At this moment we cannot estimate the weight of these procedures in the result. Fourth, the automatic microscopy has allowed the study of hundreds of thousands of cells on a single slide, the time required to scan a single centrifugation spot requiring about 12 hours. The scan is limited by the fact that the fluorescent signals are on different levels in the same nucleus. We needed a special algorithm to focus and combine multiple acquired planes. Fluorescence visualization signaled a lot of candidate cells, some with red, as well as green signals. We do not know the cause of these false-positive red signals. They may appear by non-specific binding of the sample or maybe small particles present in the wash solution [Kolvraa et al., 2005].

Our methods were based on a single phase of detection and confirmation of the fetal cells, applying the FISH detection to all cells resulting from the positive selection process. Thus, we excluded many centrifugations, various cell staining and discoloration treatments, which could make the fetal cells unsuitable for FISH analysis. This approach, however, required automatic slide assessment on the fluorescence microscope.

From a technical point of view, the combination of density gradient centrifugation with paramagnetic selection represents a unique, original approach. It has the advantage of simplicity and achieves a minimal manipulation and treatment of cells. The technology is much easier to be applied compared to most approaches commonly used to enrich and screen fetal nucleated erythroblasts.

1.3.2.5. Conclusions

The isolation and identification of fetal cells in the maternal blood is a considerable challenge due to the low number of these cells. To be used in prenatal screening, new methods of cell enrichment or other specific antibodies are required.

Chapter 2. GENETIC HETEROGENEITY AND PLEIOTROPY IN CANCER

The results of researches about genetic heterogeneity and pleiotropy in cancer were published in the following papers
ISI ARTICLES
Antohi, C., Haba, D., **Caba, L.***, Ciofu, M.L., Drug, V.L., Bărboi, O.B., Dobrovăț, B.I., Pânzaru, M.C., Gorduza, N.C., Lupu, V.V., Dimofte, D., Gug, C., Gorduza, E.V. *Novel Mutation in APC Gene Associated with Multiple Osteomas in a Family and Review of Genotype-Phenotype Correlations of Extracolonic Manifestations in Gardner Syndrome.* Diagnostics (Basel), 2021, 11(9) :1560. (**corresponding author**). **IF=3.992** • awarded in the **UEFISCDI** competition "Premierea Rezultatelor cercetarii-Articole PRECISI 2021"
Caba, L., Florea, L., Gug, C., Dimitriu, D.C., Gorduza, E.V. *Circular RNA—Is the Circle Perfect?* Biomolecules 2021, *11*(12), 1755. **IF=6.064**
Zob, D. L., Augustin, I., **Caba, L.***, Panzaru, M. C., Popa, S., Popa, A. D., Florea, L., Gorduza, E. V. *Genomics and Epigenomics in the Molecular Biology of Melanoma—A Prerequisite for Biomarkers Studies*. Int J Mol Sci 2022, 24(1), 716. (**corresponding author**) **IF=6.208**

2.1. Introduction

Cancer is a genetic disease caused by an accumulation of mutations with a final effect of uncontrolled cell growth and proliferation. Genetic changes in cancer can be either inherited through the germ line, resulting in cancer susceptibility, or acquired as somatic mutations. Genetic aberrations in cancer include point mutations and extended genome rearrangements. Cancers with a genetic predisposition represent about 30% of all cancers, while hereditary cancers represent 1-2% of all cancers. Thus, the special attention directed towards these categories of cancers is justified, with the objective of their primary prophylaxis. The following elements are very important: family history that can highlight family aggregation, the age of appearance of tumors, the presence of multiple congenital anomalies or other non-oncological symptoms or the presence of precursor lesions or benign lesions.

2.2. Familial adenomatous polyposis Gardner variant

2.2.1. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide (approximately 1.8 million new cancer diagnoses yearly) and the second most common cause of cancer death (over 880,000 deaths in 2018) [Bray et al., 2018]. A special entity is represented by early onset CRC that has a different clinical, pathological and molecular profile compared to late onset CRC. Thus, distinction between the two forms is important for effective prevention, detection and the therapeutic plan [Akimoto et al., 2020].

Usually, colorectal cancer in young people is discovered at an advanced stage due to the lack of screening programs in this age group. Exceptions are cases of hereditary syndromes predisposed to CRC (10% of all colorectal cancers), where, after the discovery of an index case, a large screening of the family follows [Silla et al., 2014; Pearlman et al., 2017].

Most early-onset cancers are monogenic (16–35% of total) [Akimoto et al., 2020; Pearlman et al., 2017; Patel, Ahnen, 2018; Stoffel et al., 2018; Mork et al., 2015]. The most common forms are Lynch Syndrome and familial adenomatous polyposis (FAP). The high

percentage of monogenic cancers with early onset indicates and justifies genetic counselling and multigene panel testing [Pearlman et al., 2017]. However, nearly half of patients with a genetic form of CRC do not have a positive family history [Stoffel et al., 2018]. Thus, genetic diagnosis is important for patients, but also for the identification and management of relatives at risk of inheriting the mutation.

Familial adenomatous polyposis accounts for approximately 1% of all colorectal cancers [Yalcin, Philip, 2019]. FAP (OMIM #175100) is caused by germline mutations in a tumor suppressor gene—*APC* regulator of WNT signaling pathway (*APC*). Mutations of the *APC* gene have an autosomal dominant inheritance pattern. FAP can be divided in several forms: classic FAP (characterized by the presence of a large number of colorectal adenomatous polyps −>100), attenuated FAP (AFAP—characterized by a lower number of polyps, usually under 30), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), Gardner syndrome (associates FAP with osteomas and soft tissue tumors) and Turcot syndrome (association of FAP with central nervous system tumors) [Mao et al., 2021]. The presence of extracolonic non-malignant manifestations in Gardner syndrome (including osteomas, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium, benign cutaneous lesions, desmoid tumors and adrenal masses) and their early appearance, even before the development of polyps/cancer, is an important factor for diagnosis and prevention.

We present two related cases, diagnosed with a new pathogenic variant in the *APC* gene to highlight the importance of extracolonic manifestations and family history in the early diagnosis of FAP. We also reviewed the extracolonic manifestations in FAP and their correlations with the location of mutations in the *APC* gene.

2.2.2. Materials and methods

Patients

The two patients are represented by the proband and her brother. The proband is a young Caucasian female (16 years old at the time of diagnosis). She was referred to an oral surgeon because of a mandibular osteoma discovered during a routine consultation. The proband's brother (12 years old at the time) had supernumerary teeth and a mandibular tumor on palpation. Family history showed that the proband's mother was diagnosed with mandibular osteoma, supernumerary teeth and died at 34 years old because of intra-abdominal and abdominal wall tumors (with unspecified origin). The maternal grandfather had supernumerary teeth and soft tissue tumors and died around the age of 35 (Figure I.16.).

The association of multiple osteomas, dental abnormalities, and family history of colon cancer with dominant transmission has led to clinical diagnosis of familial adenomatous polyposis Gardner variant.

Methods

Patients were evaluated by a multidisciplinary team consisting of an oral surgeon, radiologist, geneticist, gastroenterologist, endocrinologist and an ophthalmologist.

A complete evaluation was performed by anterior-posterior cephalometric and panoramic X-rays, Cone-beam computed tomography (CBCT), digestive endoscopic evaluation, genetic molecular testing, ophthalmological and endocrinological evaluation. At the age of 18, the proband underwent surgical removal of the most prominent osteoma of the left angle of the mandible and a pathological examination was performed.

The patients underwent Invitae Multi-Cancer panel (Invitae, San Francisco, CA, USA). Full-gene sequencing, deletion/duplication analysis and variant interpretation were performed at Invitae.

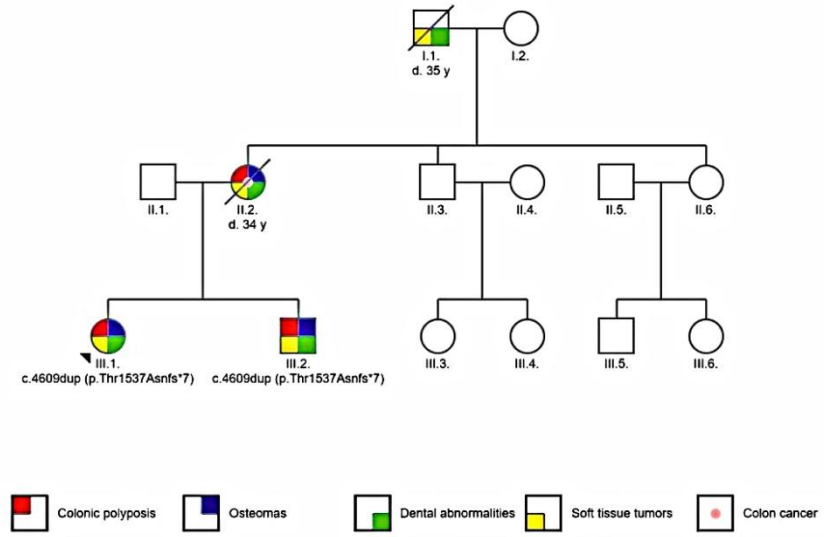


Figure I.16. Pedigree of the analyzed family (arrow indicates the proband).

2.2.3. Results

Radiographic Assessment

Initial radiographic assessment of the female proband (III.1.) included: anterior-posterior cephalometric and panoramic X-rays which showed multiple osteomas affecting the skull bones and mandible, slight facial asymmetry and several impacted teeth (Figure I.17a–c.). CBCT revealed diffuse sclerosis of the jaw bones (Figure I.17d–g.), expansion of the body of the mandible, multiple peripheral osteomas including a large osteoma at the left angle of the mandible associated with facial asymmetry (Figure I.17d–j.). Small osteomas were registered within the anterior ethmoidal cells, frontal sinus, several skull bones (frontal, temporal, parietal, occipital), zygomatic arch, nasal bones (Figure I.17g–j.) and the left external auditory canal with a discrete decrease of its diameter (Figure I.17i.).

Cephalometric and panoramic radiographs of the male patient (III.2.) showing multiple radiopaque masses with relatively well-defined borders affecting the jaw bones, diffuse sclerosis of the body of the mandible and large masses near the gonions (Figure I.18a,b.). CBCT axial, cross-section, panoramic, sagittal, coronal, 3D lateral-oblique and 3D sagittal reconstructions showing multiple enostoses located in the medullary bone of the body of the maxilla and mandible, delayed dental eruptions and hypercementosis (Figure I.18c–i.). Bilateral mushroom-shaped osteomas affect the angle of the mandible, temporal and frontal bones (Figure I.18h,i.).

In comparison, the male patient presented overall larger osteomas affecting the left angle of the mandible and both temporal bones, left coronoid process, impacted teeth, and increased bone sclerosis of the jaw bones. Additionally, he had a breast lipoma. Specific for this case is the presence of a pedunculated osteoma attached to the anterior wall of the right sphenoidal sinus and mucositis of the posterior ethmoidal cells.

Pathological Examination

The pathological examination showed compact bone tissue, consisting of mature bone lamellae with Haversian canals and paucicellular fibrous stroma (Figure I.19.). These morphological aspects confirm the presence of osteoma.

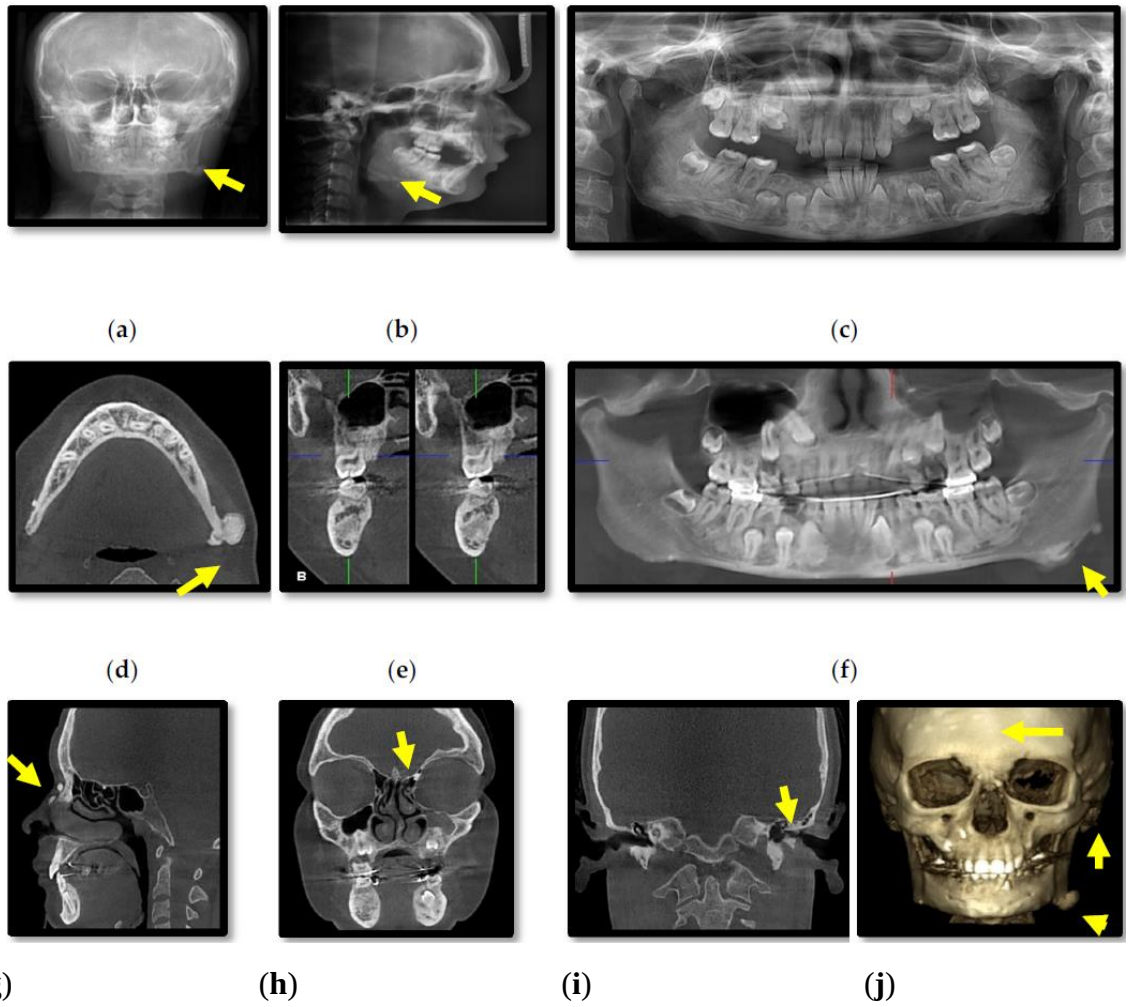


Figure I.17. Patient III.1. Cephalometric (**a**,**b**) and panoramic (**c**) radiographs; Cone-beam computed tomography (CBCT) with axial (**d**), cross-section (**e**), panoramic (**f**), sagittal (**g**), coronal (**h**,**i**) views and 3D reconstructions of bone tissue (**j**).

Digestive Endoscopic Evaluation

Upper digestive endoscopy in the proband showed a normal aspect of esophageal and gastric mucosa. A pseudopolipoid appearance (<2 mm) and hypertrophic papilla were observed in the duodenum. Lower digestive endoscopy revealed multiple polyps (approximately 50–70), the largest located in the sigmoid colon with a diameter of 5–6 mm. Polypoid lesions were observed in cecum (a 2 mm polyp) and at 5 cm from the ileocecal valve (also a 2 mm polyp).

Pathological examination of the biopsied fragments shows the structure of tubular adenomatous polyp with low-grade epithelial dysplasia. The colonic mucosa fragment has glandular architecture and preserved mucosecretion, with moderate lymphoplasmacytic inflammatory infiltrate and chorion edema.

Similar aspects of colon were discovered by endoscopy in the male patient.

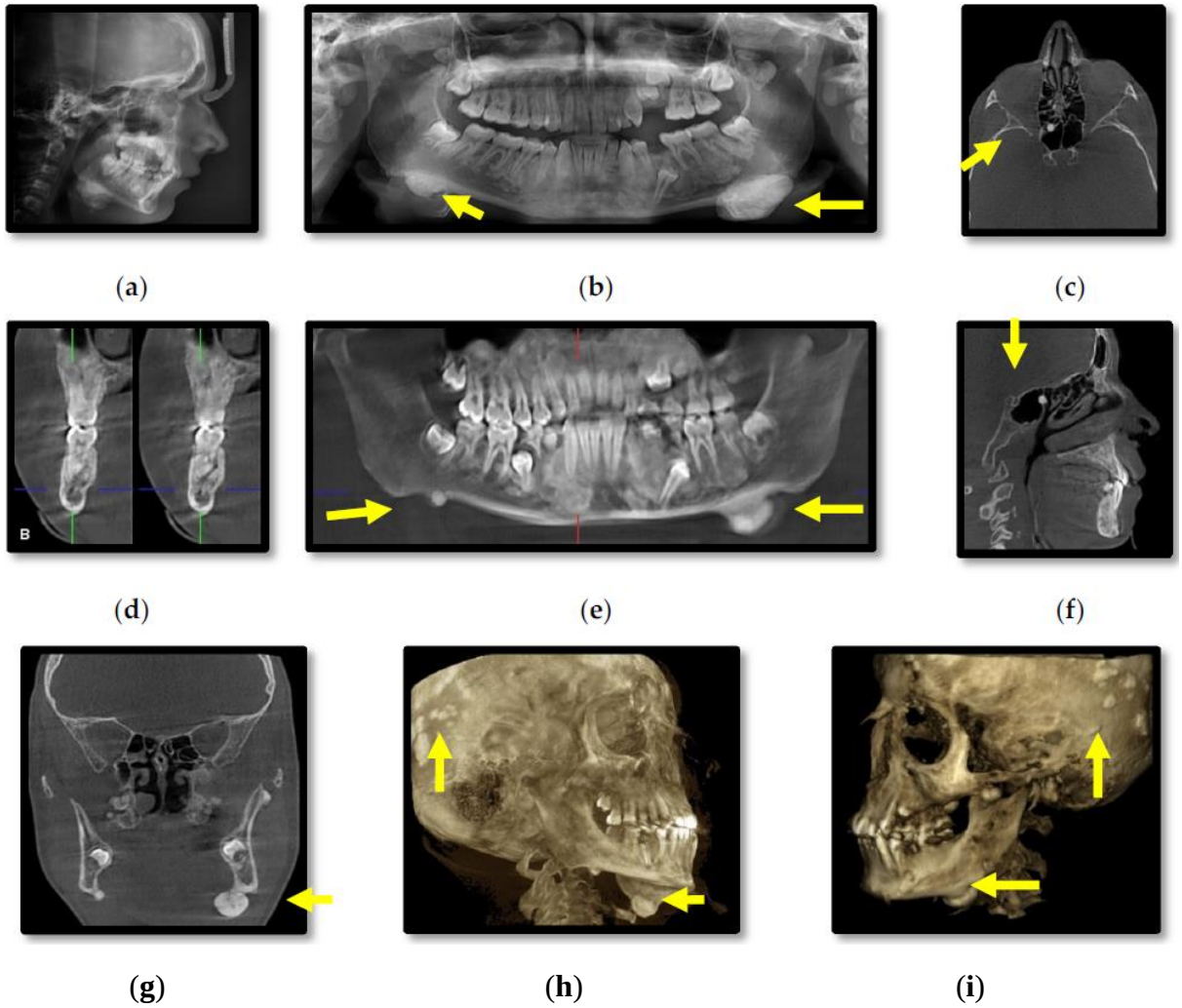


Figure I.18. Patient III.2. Cephalometric (**a**) and panoramic (**b**) radiographs; CBCT axial (**c**), cross-section (**d**), panoramic (**e**), sagittal (**f**), coronal (**g**), 3D lateral-oblique (**h**) and 3D sagittal (**i**).

Genetic Testing

Gene sequencing (by NGS) showed a novel pathogenic variant— **c.4609dup (p.Thr1537Asnfs*7)** —in heterozygous status in the *APC* gene in the siblings. The reference sequence is: *APC*: NM_000038.5. The novel sequence has a premature translational stop signal in the *APC* gene (p.Thr1537Asnfs*7). While this is not anticipated to result in nonsense mediated decay, it is expected to disrupt the last 1307 amino acids of the APC protein. This variant is not present in population databases (ExAC no frequency) [National Center for Biotechnology Information. ClinVar; 2021]. It is expected to disrupt the domains of the protein, which mediate interactions with the cytoskeleton. A different truncation (p.Tyr2645Lysfs*14) that lies downstream of this variant has been determined to be pathogenic. This suggests that deletion of this region of the APC protein is causative of disease. For these reasons, this variant has been classified as pathogenic [National Center for Biotechnology Information. ClinVar; 2021]. No pathogenic variants were identified in the other genes analyzed.

Ophthalmologic and endocrine examinations did not reveal particular aspects concordant with diagnosis of Gardner syndrome.

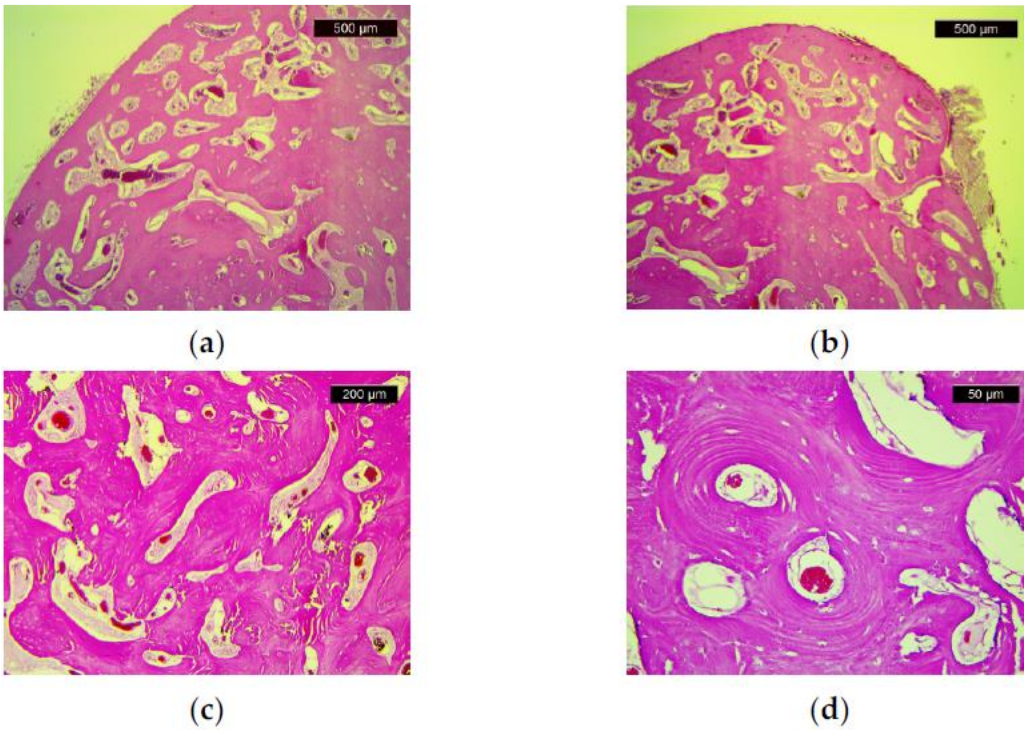


Figure I.19. Histologic features of osteoma: (a) Dense cortical lamellar bone tissue (HE, ×25); (b) Dense, compact bone tissue with marked paucicellular and congestive stromal fibrosis (HE, ×25); (c) Compact, dense bone with visible Haversian canals (HE, ×50); (d) Haversian canals (HE, ×200).

The diagnosis of Gardner syndrome was established based on the presence of multiple adenomatous polyps in the colon, the presence of extracolonic manifestations (multiple osteomas, dental abnormalities, soft tissue tumor), suggestive familial history for FAP, being confirmed by the discovery of a pathogenic variant in the *APC* gene.

So far, the female patient underwent surgical removal of the mandibular osteoma and orthodontic treatment. The male patient benefitted only from orthodontic treatment due to incomplete ossification. Both patients entered in the surveillance oncological program with an annual endoscopic evaluation of the digestive mucosa and biopsy of the lesions.

2.2.4. Discussion

The APC protein is a complex protein, having several domains with different cellular roles. The APC protein contains an oligomerization domain; an armadillo repeat-domain (implied in cell migration and cell adhesion by link of Asef, KAP3 and IQGAP1); a 15–20 aa repeats that fix β-catenin and participates in cell adhesion, proliferation and differentiation; SAM repeats that allow connection with axin; a basic region for microtubule binding and C-terminal domains for EB1 binding. The last two domains are implied in chromosomal segregation and mitotic progression [Zhang, Shay, 2017].

The majority of mutations are missense or frameshift, and produce a truncated protein, usually by modification of the last exon that contains 75% of the coding sequence of the gene [Morin, Weeraratna, 2003; Aitchison et al., 2020]. The region between codons 1250 and 1464 is considered a mutation cluster region (MCR) and changes of this region have a higher frequency in the general population (15.49% of total) [Cetta, Dhamo, 2007]. The significant consequences seem to be mutations in domains implied in binding β-catenin, EB1 and microtubules. These mutations disrupt cellular processes and generate the appearance of

tumors by stimulation of cell migration, activation of proliferation and inhibition of differentiation. Additionally, chromosomal instability and cell immortalization are produced [Zhang, Shay, 2017]. The most common mutations are in codon 1309 (10% of patients with FAP) and codon 1061 (5% of patients). About 25–30% of mutations are de novo, without evidence of FAP phenotype or mutations in the *APC* gene in family members. In some cases, the mutation exists only in the gametes (germinal mosaicism) [Half, Bercovich, Rozen, 2009]. The types of mutations in the *APC* gene included in the Human Gene Mutation Database (HGMD®) are: missense/nonsense (578 variants), splicing substitutions (125 variants), regulatory substitutions (13 variants), small deletions (20 pb or less; 796 variants), small insertions/duplications (20 pb or less; 343 variants), small indels (20 pb or less; 51 variants), gross deletions (143 variants), gross insertions/duplications (16 variants), complex rearrangements (13 variants) (HGMD® Professional 2021.1, accessed on 26 June 2021)[Stenson et al., 2017 - HGMD]. The distribution of germline mutations among exons is presented in Figure I.20.

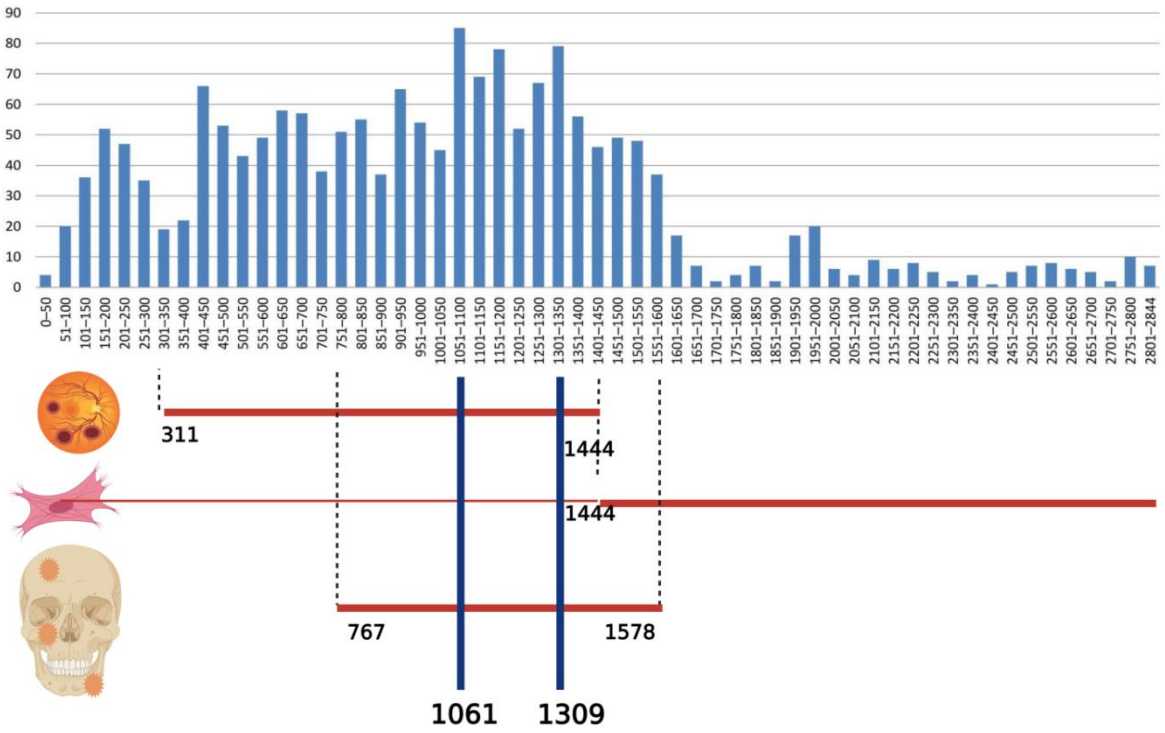


Figure I.20. Distribution of germline mutations in *APC* gene (data from the online database HGMD® Professional 2021.1, accessed on26.06.2021) and correlation with congenital hypertrophy of the retinal pigment epithelium, desmoid tumors and osteomas. The abscissa represents the APC codon number and the ordinate represents the number of different mutations. Created with BioRender.com.

The gene panel used for our patients contains genes involved in hereditary polyposis syndromes, some of which are relatively new but also genes involved in other types of hereditary cancers. Using such a panel offers an accurate differential diagnosis with the intestinal polyposis syndromes. Among those genes, we mention: *NTHL1* (nth like DNA glycosylase 1), *POLE* (DNA polymerase epsilon, catalytic subunit), *POLD1* (DNA polymerase delta 1, catalytic subunit), *MSH3* (mutS homolog 3), *AXIN2* (axin 2), *GREM1* (gremlin 1, DAN family BMP antagonist), *MUTYH* (mutY DNA glycosylase), *STK11*

(serine/threonine kinase 11), *SMAD4* (SMAD family member 4) and *BMPR1A* (bone morphogenetic protein receptor type 1A). All these genes are found in the panel of genes recommended for testing for inherited colorectal cancer and polyposis by the American College of Medical Genetics and Genomics [Mao et al., 2021]. The use of targeted gene panel-based next-generation sequencing technology is recommended for patients with FAP because it is cost effective, and less time consuming. This approach is more targeted but delivers less information than whole genome sequencing (WGS) as only a subset of genes is evaluated [Wang et al., 2019].

Extracolonic Manifestations in FAP

Penetrance is almost complete for colonic manifestations, but variable for extra colonic manifestations. The development of adenomatous polyps begins in childhood and adolescence. It is estimated that 50% of patients with FAP have colorectal adenomas by age 15 and the percentage increases to 95% by age 25 [Brosens et al., 2007]. The age of onset of colonic symptoms correlates with the location of the mutation: age 20 (variants at 1309 codon), age 30 (variants between 168 and 1580 codons except for the 1309 codon), age 52 (variants in 5′ of codon 168 and 3′ of codon 1580) [Friedl et al., 2001].

There are two categories of extracolonic manifestations in FAP: malignant and non-malignant. They are present in 70% of FAP cases [Ficari et al., 2000]. Non-malignant extra intestinal manifestations are represented by: osteomas, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium (CHRPE), benign cutaneous lesions, desmoid tumors and adrenal masses [Jasperson, Patel, Ahnen, 2021]. Malignant manifestations are located in the small bowel (duodenum, periampulla or distal to the duodenum), pancreas, thyroid, CNS, liver, bile ducts and stomach [Jasperson, Patel, Ahnen, 2021].

- *Osteomas*

Osteomas are present in 60–80% of patients with FAP and 50% of all osteomas occur in FAP [Dinarvand et al., 2019; Bülow, 1987]. The prevalence of osteomas is 1–2% in the general population [Groen et al., 2008]. Osteomas are benign tumors characterized by compact lamellar cortical or cancellous bone [Gundewar et al., 2013]. There are three types of osteomas: central (originates in the endosteum), peripheral (originates in the periosteum) and extra skeletal soft tissue osteoma (developing in the muscles) [Orabona et al., 2015]. They are most commonly located in the skull (jaw bones and paranasal sinuses), and rarely in long bones or muscles. Osteomas are more common in the mandible than in the maxilla [Debta et al., 2016].

In descending order of the frequency of osteomas at the level of the sinuses, we mention: frontal sinus, ethmoid, maxillary and sphenoid sinuses. The prevalence in the paranasal sinuses is different depending on the imaging method used for their detection: conventional examination shows a prevalence of about 1% in the general population, while the use of computed tomography has a higher detection rate showing a prevalence of about 3% in the general population [Erdogan et al., 2009]. The presence of multiple osteomas (more than three osteomas) is suggestive for Gardner syndrome [Erdogan et al., 2009; Payne, Anderson, Cook, 2002]. In a prospective study of paranasal osteomas, Erdogan et al. showed that in 7% of cases, osteomas were detected in more than one sinus [Erdogan et al., 2009].

Osteomas are important in the early management of FAP due to the fact that they occur before intestinal manifestations—the detection of osteomas precedes the diagnosis of FAP by 17 years [Bülow, 1987]. The appearance of osteomas is associated with mutations between codons 767 and 1578 [Groen et al., 2008]. In our case the mutation concerns the same region.

Another particularity of our patients is the presence of multiple osteomas in cranial bones, jaw bones and paranasal sinuses. However, osteoma of sphenoid sinus (present in patient III.2) is a less common location.

❖ *Dental Abnormalities*

Dental abnormalities are present in 30–75% of patients with FAP [Cankaya et al., 2012]. The meta-analysis performed by Almeida et al. shows a frequency of dental anomalies of 30.48% [Almeida et al., 2016]. Dental abnormalities in FAP are represented by: impacted teeth, congenitally missing teeth, supernumerary teeth, dentiferous cysts, compound odontomas, hypercementosis [Jasperson, Patel, Ahnen, 2021; Dinarvand et al., 2019]. Supernumerary teeth were identified in 11–27% of FAP patients compared to 0–4% in the general population. The most common location is anterior and around canines, in the alveolar bone between the teeth or attached to the follicle of an impacted tooth. However, this location is not specific to FAP [Wijn et al.,2007; Ida, Nakamura, Utsunomiya, 1981]. The literature is poor in studies about the correlation between the presence of dental abnormalities and the location of mutations in the *APC* gene. It was assumed that there was the same correlation as in the case of desmoid tumors and osteomas [Oku et al., 2004; Järvinen, Peltomaki, 2004].
Due to the existence of dental abnormalities, a dentist may be involved in the early diagnosis of familial adenomatous polyposis.

Supernumerary teeth can be an important clue for early diagnosis. We found dental modifications in our cases. The two siblings have impacted teeth and hypercementosis, while their mother and their maternal grandfather showed supernumerary teeth.

❖ *Desmoid Tumors*

Desmoid tumors (DT) appear in the musculoaponeurotic tissue in any region of the body. There are 3 common locations: extremities (proximal or at the girdle), abdominal wall and intra-abdominal (intestinal wall and mesentery) [Papagelopoulos et al., 2006]. They represent 3% of soft tissue tumors and 0.03% of all neoplasms [Papagelopoulos et al., 2006]. DT occurs in 12–15% of FAP patients [Sinha et al., 2011]. They generate a high mortality in patients with FAP, with a rate between 10% and 50% [Clark et al., 1999]. The patient with FAP has an 800-fold higher risk of developing DT and the risk increases if there is a mutation after codon 1399 in the *APC* gene [Sinha et al., 2011]. DT in FAP appear earlier than sporadic DT [Koskenvuo, Ristimaki, Lepisto, 2017]. In 80% of patients with DT, they appear until the age of 40 with a peak in the second and third decade of life [Sinha et al., 2011]; 65% of DT occur intra-abdominally [Sinha et al., 2011]. There are four risk factors for DT: a positive family history for DT, mutation location, surgical trauma, female sex [Sinha et al., 2011].

The correlation between the appearance of DT and the location of the mutation is not highly specific. Friedl et al. found a 60% frequency of DT in patients with FAP caused by mutations in codons 1445–1580, and only a 20% frequency of DT in patients with mutations before codon 1444 in the FAP gene [Friedl et al., 2001].

❖ *Congenital Hypertrophy of the Retinal Pigment Epithelium*

Congenital hypertrophy of the retinal pigment epithelium (CHRPE) generates a depigmented halo in the retina and is the most common extracolonic manifestation found in 74% of FAP patients compared to 1.2–4.4% in the general population [Tiret, Parc, 1999; Nusliha et al., 2014]. It has no clinical significance and it is asymptomatic. It is the earliest sign of disease, being present at birth or in the neonatal period [Nusliha et al., 2014]. It is associated with mutations in the codon 311–1444 [Nieuwenhuis, Vasen, 2007]; 70.74% of the mutations described in HGMD are in this codon range.

❖ *Malignant Extracolonic Manifestations*

Malignant extracolonic manifestations in FAP are represented by some rare entities (small bowel cancer distal to the duodenum) or others more common (such as thyroid cancer or small bowel cancer (duodenum or periampulla)). Lifetime risk for cancer in FAP differs among cancer types are presented in (Table I.9.) [Jasperson, Patel, Ahnen, 2021]. Studies concerning this type of cancer are rare, in correlation with their small frequency.

Table I.9. Extracolonic cancer in FAP—lifetime risk (modified after *Jasperson*) [Jasperson, Patel, Ahnen, 2021]**.**

Extracolonic cancer	Lifetime risk for cancer
Small bowel (duodenum or periampulla) cancer	4-12%
Small bowel (distal to the duodenum) cancer	rare
Pancreas cancer	1%
Tyroid cancer	1-12%
CNS cancer	<1%
Liver cancer	1.6%
Bile ducts cancer	Low, but increased
Stomach cancer	<1%

Mutations in codons 697–1224 of the *APC* gene are associated with a 3-fold higher risk of brain tumor and a 13-fold higher risk of medulloblastoma [Attard et al., 2007]. Duodenal adenomas have a 3–4 times higher risk of developing if the mutation in *APC* is between codons 976–1067 [Wiik, Talseth-Palmer, 2020].

❖ *Thyroid Cancer*

The incidence of thyroid cancer is 2–12% in cases with FAP and 10% in the Gardner variant with a cumulative risk of 2.8% by age 60 [Pichert, Jacobs, 2016]. This neoplasia is multifocal and bilateral. In cases with FAP, the majority of cases are cribriform morular variant of papillary CT [Steinhagen et al., 2012]. It is associated with germline mutations between the codons 1286 and 1513 in the *APC* gene [Pichert, Jacobs, 2016; Truta et al., 2003]. In this interval of the APC gene were reported 251 variants which represent 14.40% of the totally different mutations reported in HGMD up to date. It predominates in women and is diagnosed earlier than sporadic with an average diagnostic age of 29.2 years [Truta et al, 2003; Cetta et al., 2000; Septer et al., 2013]. In a systematic review, Septer et al. concluded that there is an increased risk of thyroid cancer associated with a mutation in codon 1061 [Septer et al., 2013].

2.2.5. Conclusions

The genetic syndromes predisposed to colorectal cancer (including FAP) are characterized by pleiotropy and genetic heterogeneity. Mutations in the *APC* gene, which later lead to

familial adenomatous polyposis manifest earlier as osteomas, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium, benign cutaneous lesions, desmoid tumors and adrenal masses. An early diagnosis via these early manifestations allows a proper oncological surveillance. Furthermore, a detailed family history in patients with these extracolonic manifestations is very important in identifying the family members at risk.

2.3. Genomics, epigenomics and biomarkers in cancer

Biomarkers can be classified, according to Food and Drug Administration and the National Institutes of Health, into the following several categories: susceptibility/risk; diagnostic; monitoring; prognostic/predictive; pharmacodynamics/response biomarker; safety biomarker, but some biomarkers can fit it into several classes of biomarkers [Califf, 2018].

2.3.1. Circular RNA

The aim of this review is to summarize the biology of circular RNA and to highlights the potential role as biomarker.

2.3.1.1. Circular RNA—Complex Molecule

Circular RNAs (circRNAs) are endogenous RNA molecules that form covalently closed continuous loops [Li et al., 2020; Sanger et al., 1976]. They are part of the long non-coding RNA (lncRNA). LncRNA can be classified in several categories: based on transcript length, association with annotated protein-coding genes, association with other DNA elements of a known function, protein-coding RNA resemblance, association with repeat elements, association with specific biochemical pathways, implication in biochemical stability, conservation of the sequence and structure of cellular elements, differential expression in different tissues, association with subcellular structures, and variable function [Laurent, Wahlestedt, Kapranov, 2015].

CircRNAs were discovered in others Metazoa (mouse, Zebrafish, Caenorhabditis elegans, Saccharamyces cerevisiae, Schizosaccararomyces pombe, Plasmodium falciparum, Dictyostelium discoideum) or vegetals (Arabidopsis thaliana Oryza sativa ssp. Japonica, Oryza sativa ssp. Indica, Nicotiana benthamiana etc) [Guria et al., 2020].

Depending on their composition, circRNA molecules can be classified in: exonic circRNAs (ecircRNAs)—formed by exons, circular intronic RNAs (ciRNAs)—formed by introns, and exon-intron circRNAs (EIciRNAs)—formed by both exons and introns [Zhou, Yang, Xiong, 2018]. Most circRNA molecules contain exons from genes encoding proteins, but they also originate from introns, intergenic regions, UTRs, ncRNA loci, and antisense locations of known transcripts. The other newly described categories are: fusion circRNA (f-circRNAs), read-through circRNAs (rt-circRNAs), and mitochondria-encoded circRNA (mecciRNAs). In this review, we summarise data on the classification, biogenesis, and functions of circRNAs and discuss their potential role as biomarkers, especially in the diagnosis of cancers.

Prevalence

Over 70% of the human genome gives rise to different RNA molecules with a role in maintaining tissue homeostasis and pathophysiological processes [Boeckel et al., 2015]. Only 2% of all RNA molecules encode proteins [Cheng et al., 2021]. Over 10% of expressed genes can produce circRNAs [Chen, 2016]. Of the active transcribed genes in humans, 5.8–23% of them give rise to circRNAs [Ebbesen, Hansen, Kjems, 2017]. A circRNA is expressed in a tissue-specific complex way, cell-type specific or developmental-dependent stage [Chen, Huang, 2018; Memczak et al., 2013; Belousova, Filipenko, Kushlinskii, 2018; Zhang et al., 2014]. They are found with increased frequency in the brain and during foetal development [Salzman, 2016]. Nearly 20% of protein-coding genes produce circRNAs in mammalian

brains [You et al, 2015]. In the most comprehensible database-circAtlas, a number of the 413,657 types of circRNAs were described in 2020. By categories in descending order, they were as follows: exonic—252,494; intronic—51,291; antisense—39,060; non-repeat—24,896; 3′UTR—1686; 5′UTR—754 [circAtlas2.0.]. Salzman et al. estimate that the circRNAs represent 1% of (polyA)RNA molecules [Salzman et al., 2012].

The newly described entity, mecciRNAs, represents only a small part of the total circRNA molecules [Liu, Yang, Shan, 2021]. Hundreds of circRNAs encoded by the mitochondrial genome have already been identified [Liu et al., 2020].

Properties of circRNA

CircRNA is resistant to RNaseR (an enzyme with 3' to 5' exonuclease activity), which degrades most linear RNAs [Meng et al., 2017; Mehta, Demsey, Vemuganti, 2020]. Thus, the half-life is 2.5 times longer than that of linear mRNA, which gives them characteristics used as diagnostic or therapeutic biomarkers [Enuka et al., 2016; Zhou et al., 2020]. They have no electric polarity and are not a polyadenylated tail [Qu et al., 2015]. The length varies between 100 and 4000 nucleotides [Belousova, Filipenko, Kushlinskii, 2018].

CircRNAs Localisation

Different types of circRNA are located in different cell compartments. ciRNAs are found in the nucleus, ecircRNAs are present in the cytoplasm and exosomes, EIciRNA is located in the nucleus, f-circRNAs are present in all cellular compartments, rt-circRNAs are found in cytoplasm, while mecciRNAs are located in the mitochondrial environment (inside and outside of organite) [He et al., 2021; Shang et al., 2019].

Stable circRNAs were discovered in exosomes with a role in the transcriptional and translational regulation, splicing regulation, miRNA sponge, and protein inhibition [Fanale et al., 2018]. They may have an oncogenic role or a tumour suppressor one [Pucci et al., 2018], being involved in tumour growth and metastasis, angiogenesis, modulation of the microenvironment, pre-metastatic niche formation, and immunomodulation and drug resistance [Ruivo et al., 2017]. Some exosomes survive in several biological products such as blood, urine, saliva, breast milk, synovial fluid, amniotic fluid, bronchoalveolar lavage fluid, malignant ascites, and semen [Fanale et al., 2018; Boukouris, Mathivanan, 2015]. More than 1000 exosomal circRNAs have been identified in human serum and allowed a differentiation between tumour patients and healthy ones [Fanale et al., 2018].

Biogenesis

Most circRNA molecules contain several exons, often two or three exons [Zhang et al., 2014]. Since the vast majority of exonic circRNAs contain exons placed in the middle of the reading frame, it has been suggested that the mechanism of formation of circRNAs is mainly related to RNA splicing [Zhang et al., 2014]. Alternative circularisation is the phenomenon by which multiple exon circularisation events occur from single loci genes [Zhang et al., 2014]. The intronic repeats can be a determining factor of the back-splicing process [Wilusz, 2015; Wilusz, 2018]. Approximately 90% of circRNAs appear to have complementary ALU elements in introns that flank the gene region from which they originate [Wilusz, 2015; Ivanov et al., 2015].

For exonic circular RNAs, the common mechanism is back-splicing in which 3' splice donors bind covalently to 5' splice acceptors but in reverse order. There are three models: exon skipping or lariat-driven circularization; direct back-splicing, or intron-pairing driven circularization and RNA-binding-protein-driven circularization (Figure I.21.) [Zhou et al., 2020; Meng et al., 2017].

Additionally, three mechanisms have been proposed in formation of intronic circRNA. In the first model the stages are the isolation of an exon, the assembly of the isolated exon

with the adjacent one and the formation, in this way, of a linear intron, followed by its circularisation and finally the loss of the 3'-tail of the lariat RNA. In the second model, the second exon is isolated (downstream exon), and from the upstream exon coupled with the downstream intron, the exon is detached, and the lariat is circulated. In the third spliceosome model, the 3'end of the intron is connected to the 5' end and forms the lariat from which the 3'-tail will be removed [Meng et al., 2017].

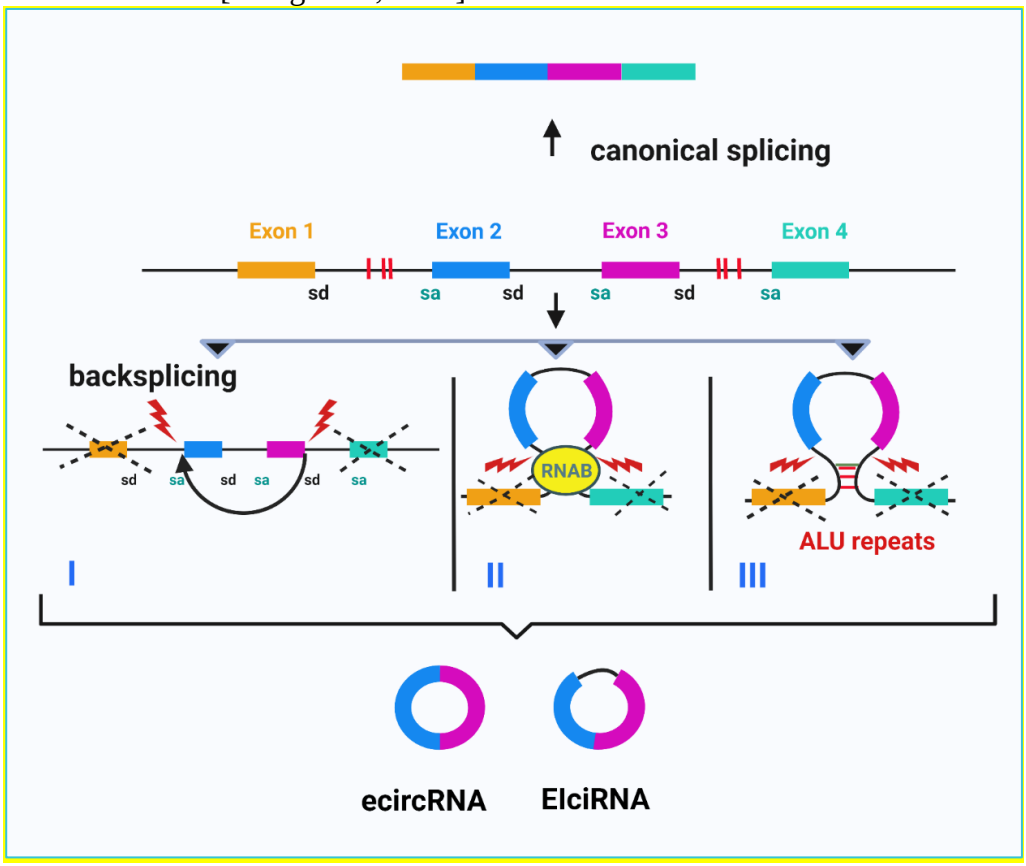


Figure I.21. Biogenesis of ecircRNAs and EIciRNAs. I: Back-splicing—the splice donor binds covalently to splice acceptor; II: RNA-binding-protein-driven circularisation; III: Intron-pairing driven circularisation based on intronic ALU repeats. Adapted from "circRNA in Cancer", by BioRender.com (2021). Retrieved from: https://app.biorender.com/biorender-templates (accessed on November 18,2021). (sd—splice donor; sa—splice acceptor; RNAB—RNA binding protein; red bars—ALU repeats).

The back-splicing efficiency seems to be lower than canonical splicing [Qu et al., 2015; Starke et al., 2015]. For circularisation, the presence of canonical splice signals is required [Starke et al., 2015]. The formation of circRNA by back-splicing is correlated with a minimal exon length of 353 nucleotides (nt) in the case of a single exon circularised by back-splicing and 112–130 nt per exon in the case of multi-exon back-splicing [Zhang et al.,2014; Qu et al., 2015]. In the majority of cases, exons that will be circularised are flanked by introns that contain reverse complementary sequences. These can be repetitive elements (ex-ALU elements) or non-repetitive complementary sequences. The number of nucleotides in these sequences is 30–40, but the longer the sequence is, the higher the circRNA production is [Zhang et al., 2014; Qu et al., 2015].

Circular RNAs are more common than linear transcripts in about 50 human genes [Salzman et al., 2012]. Factors influencing circRNA formation are the intron and exon length, repetitive sequences and RNA-binding proteins (RBPs) such as quaking (QKI), RNA-specific adenosine deaminase (ADAR1), NF90/NF110, heterogenous ribonucleoprotein L

(HNRNPL), and muscle blind (MBL/MBNL1) [He et al., 2021; Fu et al., 2018; Ashwal-Fluss et al., 2014].

Most circRNAs are synthesized in a sense orientation, but there are also molecules of circRNAs synthesized in an antisense orientation [Ma et al., 2021].

Jeck et al. show that circularised exons are flanked by introns that have ALU repeats (twice as common as uncircularised exons) [Jeck et al., 2013]. These ALU elements are in inverted orientation rather than complementary. The proportion of introns that flank ecircRNA and have complementary ALU pairs and those that have non-complementary pairs is 20 and 8%, respectively [Jeck et al., 2013]. Circularised exons are six times more likely to contain complementary ALUs than noncircularised exons, and ecircRNAs may be 10 times (> 10-fold) more common than associated linear transcripts [Jeck et al., 2013]. Such a particularity was identified in the central nervous system [Wilusz, 2018].

F-circRNAs are formed by a linear fusion transcript derived from genome rearrangements, while rt-circRNAs are formed by the coding exons of two adjacent genes with similar orientations (read-through transcriptions) [Zhou, Yang, Xiong, 2018; He et al., 2021; Guarnerio et al., 2016; Vidal, 2020]. Over 95% of human genes are alternately spliced, a process regulated by both cis-regulatory elements and trans-acting factors [Wang et al., 2008]. Serine and arginine-rich (SR) proteins are RNA-binding proteins that act as regulators of constitutive and alternative splicing [Jeong, 2017]. Heterogeneous nuclear ribonucleoproteins (hnRNPs) represent key proteins in the cellular nucleic acid metabolism intervening in alternative splicing, mRNA stabilisation, and transcriptional and translational regulation [Geuens, Bouhy, Timmerman, 2016].

CircRNA Functions

CircRNA functions are manifested at the molecular level, the cellular level, and the organism level, intervening in both normal physiological processes and in pathological processes. CircRNAs modulate the expression of hereditary information by intervening in the following molecular mechanisms of gene expression: transcription, splicing, microRNA sponge, modulation of protein–protein interaction and protein (RBP) sponges, as scaffolds for the assembly of the components' translation (Figure I.22.) [Altesha et al., 2019]. The following two alternative mechanisms have been proposed for initiating translation: the presence of an Internal Ribosome Entry Site (IRES) and the presence of N6-methyladenosine (m6A) residues, both pathways being activated under cellular stress conditions [Diallo et al., 2019]. Proteins encoded by circRNAs are truncated proteins with the same function as full-length proteins, others have independent or opposite functions [Zhou et al., 2020].

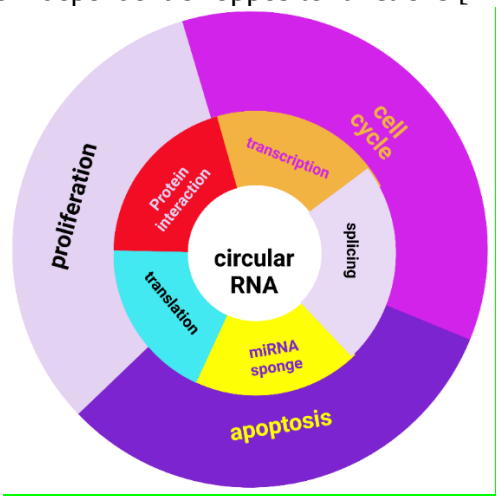


Figure I.22. CircRNA functions and involvement in cellular processes. Created with BioRender.com.

- m6A Modifications

At least 13% of all circRNAs have m6A modifications and a single m6A is sufficient to trigger translation in a cap-independent manner [Diallo et al., 2019; Yang et al., 2017]. An m6A modification regulates the circRNA metabolism precisely by modulating biogenesis, translation, degradation, and cellular localization [Wu et al., 2021]. M6A modification appears to increase the biogenesis of circRNAs in a METTL3/YTHDC1 (methyltransferase-like 3 protein/YTH domain containing 1)-dependent manner [Wu et al., 2021; Chen et al., 2021]. In the human heart, 9% of expressed genes can produce circRNAs, but, in the brain, the percentage is higher: 20% [Wu v 2021; Aufiero et al., 2018]. m6A sites in mRNAs are more common in the last exon, but the last exon does not enter in the circularisation process, which suggests that the pattern of m6A modification in mRNAs and circRNAs is different [Zhang et al., 2014; Wu et al., 2021]. The functions of m6A on mRNA and on circRNAs are mediated by the following three categories of factors: methyltransferase (acts as "writers"), demethylase (acts as "erasers"), and factors involved in recognition (acts as "readers") [Wu et al., 2021].

- CircRNA–Proteins Interactions

In a hypothetic situation of two proteins, A and B, which interact with each other, the interaction of circRNA with them can be in one of the following ways:

a. It binds to both proteins and strengthens the interaction between them (cements). This effect is achieved by the following two mechanisms: circRNA mediates the post-translational changes (ubiquitination and phosphorylation) of protein A catalysed by protein B or the transactivation of protein A by protein B followed by subsequent changes;
b. It binds only to one of the proteins that strengthen or dissociate the interaction between the two proteins;
c. It binds to both proteins and dissociates them (normally they combine) [Zhou et al., 2020].

- Binding or Sequestration of Proteins

The main functions of circular RNA are the sequestration of microRNA/proteins, the modulation of transcription-modulating RNA polymerase II (Pol II) transcription, interference with splicing, and translation to produce polypeptides [Li et al., 2018].

CircRNAs bind to cis elements and control TFs (transcription factors) and modulate epigenome. There are the following three categories of actions: recruiting TFs, recruiting modifying enzymes, and recruiting chromatin remodelers [Zhou et al., 2020]. Intronic circRNA (especially present in the nucleus) may be a positive regulator of RNA polymerase II (Pol II) transcription. EIciRNAs (also located in the nucleus) interact with U1 small nuclear ribonucleoprotein particles (U1snRNP) and thereby promote the transcription of parental genes [Li, 2015].

Exonic circRNA reaches the cytoplasm in the following two ways: by a nuclear export system or by escaping from nuclei during cell division [Suzuki, Tsukahara, 2014; Liu et al., 2017]. Different factors intervene in the nuclear export depending on the size of the circRNA: UAP56 is involved in the export of circular RNAs' molecules with a length > 1300 nt and URH49 in the nuclear export of short circular RNAs (<400 nt) [Zhou et al., 2020]. UAP56 is an ATP-dependent helicase that is an essential splicing factor also important for mRNA export

[Shi et al., 2004]. URH49 (which shares a sequences identity of 90% and similarity of 96% with UAP56) have an increased level of expression in the cell proliferation phase. Thus, it appears that a URH49-dependent mRNA export is related to cell proliferation [Fujita et al., 2020].

The regulation of circRNA production is performed by cis and trans elements. The cis elements are represented by intronic complementary sequences (ICSs), and the trans elements are represented by RNA binding proteins (RBPs). CircRNA in the cytoplasm acts mainly by binding and trapping microRNAs. CircRNAs in the nucleus regulate expression and splicing in host genes [Boeckel et al., 2015; Memczak et al., 2013; Hansen et al., 2013]. circRNAs have specific binding sites for miRNAs and, thus, act as a "trap" for miRNAs that no longer interfere with mRNA expression [Cen et al., 2021].

Intronic and exon-intron circRNAs act as regulatory factors for parental gene transcription through interactions with the RNA polymerase II, snRNPs, and hnRNPs [Diallo et al., 2019; Li et al., 2015].

- *Circular RNA in Essential Processes—Cell Cycle, Proliferation, Apoptosis*

Circular RNAs are involved in the following key cellular processes: proliferation, apoptosis, cell cycle [Belousova, Filipenko, Kushlinskii, 2018]. They can intervene in the cell cycle: circ-Foxo3 interacts with CDK2 (cyclin-dependent kinase 2) and cyclin-dependent kinase inhibitor 1 (p21) and this circ-Foxo3-p21-CDK2 complex blocks cell cycle progression and represses all proliferation [Du et al, 2016].

Intronic circRNAs can accumulate in the cytoplasm and bind to TAR DNA binding protein 43 (TDP43) and this could be a beneficial effect in the treatment of amyotrophic lateral sclerosis because it suppresses TDP43 toxicity [Armakola et al., 2012].

2.3.1.2. Circular RNA—A Potential Biomarker in Cancer

CircRNAs are involved in the hallmarks of cancer-sustaining proliferative signalling, the evasion of growth suppressors and/or the impairment of differentiation signals, avoiding immune destruction, enabling replicative immortality, tumour-promoting inflammation, activating invasion, metastasis and angiogenesis, genome instability and mutation, evading cell death and senescence, deregulating cellular energetics, and therapeutic resistance in human cancers [Bach, Lee, Sood, 2019; Wang et al., 2021]. In some cancers, several circRNA molecules have been analysed, some with upregulation effects, others with downregulation effects. Table I.10. presents statistics of the number and effect of circRNAs registered and characterised in the CircAtlas database as being associated with some pathologies.

For diagnostic accuracy, a biomarker should have an AUC (Area Under the Curve) greater than 0.5 on ROC (receiver operating characteristic) curves.

The potential circRNA diagnostic biomarkers in cancers are summarized in Table I.11.

Table I.10. CircRNA in cancer [circAtlas2.0].

Cancer Type	circRNAs (No)	circRNAs Downregulated (No)	circRNAs Upregulated (No)	circRNAs N/A
Acute Lymphoblastic Leukaemia	1	-	-	1
Acute myeloid leukaemia	11	4	5	2
Basal cell carcinoma	18	7	10	1
Bladder cancer	19	9	10	-
Breast cancer	82	18	28	36
Cervical cancer/carcinoma	18	-	17	1
Cholangiocarcinoma	1	1	-	-
Clear cell renal cell carcinoma	2	2	-	-
Kidney clear cell carcinoma	1	-	1	-
Colon cancer	6	5	-	1
Colorectal cancer	42	18	20	4
Cutaneous squamous cell carcinoma	5	2	3	-
Endometrial cancer	2	-	2	-
Epithelial Ovarian carcinoma	3	-	-	3
Oesophageal cancer	7	4	3	-
Oesophageal squamous cell carcinoma	34	12	22	-
Gastric cancer	78	41	27	10
Glioblastoma	46	19	8	3/16*
Hepatoblastoma	17	9	8	-
Hepatocellular carcinoma	22	13	4	5
Hypopharyngeal squamous cell carcinoma	6	3	3	-
Laryngeal squamous cell cancer tissues	4	1	1	2
Liver cancer	5	-	3	2
Lung adenocarcinoma	4	1	3	-
Lung cancer	8	3	4	1
Non-small cell lung cancer	9	1	6	2
Malignant melanoma	1	-	-	1
Oral squamous cell carcinoma	9	1	1	7
Osteosarcoma	11	1	7	3
Pancreatic cancer	4	1	3	-
Pancreatic ductal adenocarcinoma	12	5	7	-
Papillary thyroid carcinoma	17	3	13	1
Prostate adenocarcinoma	1	1	-	-
Prostate cancer	1	-	1	-

N/A—not available; * unclear.

Table I.11. CircRNAs in cancers.

Cancer Type	circRNAs	AUC	References
Acute Lymphoblastic Leukaemia	hsa_circ_0012152	0.8625	[Guo et al., 2020]
	hsa_circ_0001857	0.909	
Acute myeloid leukaemia	hsa_circ_0004277	0.957	[Li et al., 2017]
Bladder cancer	hsa_circ_0018069	0.709	[Cheng et al., 2021]
	circASXL1	0.77	
	hsa_circ_0077837	0.775	
	hsa_circ_0004826	0.79	
	circ0006332	0.86	
	circ_0137439	0.89	
Breast cancer	hsa_circ_103110	0.63	[Lü et al., 2017 ; Yin et al., 2018 ; Li et al., 2020]
	hsa_circ_104689	0.61	
	hsa_circ_104821	0.60	
	hsa_circ_006054	0.71	
	hsa_circ_100219	0.78	
	hsa_circ_406697	0.64	
	hsa_circ_0001785	0.771	
	hsa_circ_0104824	0.823	
Cholangiocarcinoma	hsa_circ_0000673	0.85	[Zhao et al., 2020 ; Jiang et al., 2018]
	Cdr1as	0.740	
Clear cell renal cell carcinoma	circEHD2	0.757	[Frey et al., 2021]
	circNETO2	0.705	
	circEGLN3	0.879	
Colorectal cancer	circ_0001178	0.945	[Li et al., 2021]
	circCDC66	0.884	
	circITGA7	0.879	
	circ_0000567	0.865	
	circ_0001649	0.857	
	circ_0003906	0.818	
	circ_0000826	0.816	
	circ_0000711	0.810	
	circ_001988	0.788	
Endometrial cancer	circ_0001776	0.7389	[Jia, Liu, Wang, 2020]
Epithelial Ovarian carcinoma	circBNC2	0.923	[Hu et al., 2019]
Oesophageal squamous cell cancer	hsa_circ_0001946	0.894	[Fan et al., 2019]
	hsa_circ_0062459	0.836	
Gastric cancer	hsa_circ_0007507	0.832	[Zhang et al., 2021 ; Chen et al., 2017 ; Wang et al., 2018]
	hsa_circ_0000190	0.75	
	hsa_circ_0000096	0.82	
Glioblastoma	circFOXO3	0.870	[Chen et al., 2020 ; Stella et al., 2021]
	circ_0029426	0.730	
	circ-SHPRH	0.960	

Cancer Type	circRNAs	AUC	References
	circHIPK3	0.855	
	circSMARCA5	0.823	
Hepatoblastoma	circHMGCS1	0.8971	[Zhen et al., 2019]
Hepatocellular carcinoma	exo_circ_0006602	0.907	[Wang et al., 2018 ; Guo et al., 2021; Li et al., 2019]
	circSMARCA5	0.938	
	hsa_circ_0005075	0.94	
Hypopharyngeal squamous cell carcinoma	circMORC3	0.767	[Guo et al., 2020]
Laryngeal squamous cell cancer tissues	hsa_circ_0036722	0.838	[Guo et al., 2020]
Liver cancer	circZFR	0.7069	[Ren et al., 2017]
	circFUT8	0.7575	
	circIPO11	0.7103	
Lung adenocarcinoma	hsa_circ_0005962	0.73	[Liu et al., 2019 ; Zhu et al., 2017 ; Li et al., 2018]
	hsa_circ_0086414	0.78	
	hsa_circ_0013958	0.815	
	hsa_circ_0000729	0.815	
Lung cancer	circ_102231	0.9	[Huang, Zhang, Shao, 2019]
Non-small cell lung cancer	circFARSA	0.71	[Huang, Zhang, Shao, 2019; Xian et al., 2020]
	circ_0079530	0.76	
	circRNA-FOXO3	0.78	
	circ_0014130	0.89	
	circ_0047921	0.757	
	circ_0056285	0.625	
	circ_0007761	0.750	
Oral squamous cell carcinoma	hsa_circ_0001874	0.922	[Wang et al., 2021]
	hsa_circ_0001971	0.922	
	circ-MMP9	0.91	
	circMAN1A2	0.799	
	circSPATA6	0.7748	
Osteosarcoma	circ_0000190	0.889	[Zhang et al., 2021]
	circ_0000885	0.783	
	circ_HIPK3	0.783	
	circPVT1	0.871	
	circ_0081001	0.898	
Pancreatic cancer	circ-LDLRAD3	0.67	[Yang et al, 2017; Xu et al., 2021]
	hsa_circ_0013587	0.6995	

AUC—Area Under the Curve.

Some of the cRNA molecules have been shown to be more effective biomarkers than conventional tumour serum markers. The use of a combination of circRNAs or circRNAs and conventional tumour serum markers is also more efficient in some cancers or to discriminate between cancer and other pathologies.

In gastric cancer, the combined use of hsa_circ_0007507, CEA, and CA19-9 has the best diagnostic accuracy (AUC = 0.849) of distinguishing between gastric and healthy cancer patients. The use of only the serum tumour marker Carcinoembryonic antigen (CEA) has an

AUC = 0.765, and the use of Carbohydrate antigen 19-9 (CA19-9) has a lower efficacy [Zhang et al., 2021].

In hepatocellular carcinoma, the exo_circ_0006602 was a more efficient diagnostic biomarker than the classic tumour serum markers: alpha-fetoprotein (AFP) (AUC = 0.694, $p < 0.002$) and CEA (AUC = 0.589, $p = 0.146$). The combined use of exo_circ_0006602 and AFP increases the AUC to 0.942 ($p < 0.00011$) [Guo et al., 2021].

CircSMARCA5 plays a role in apoptosis, proliferation, invasion, and metastasis and has been shown to be used in the diagnosis of hepatocellular carcinoma with good accuracy: AUC = 0.938. It is also effective (alone or in combination with AFP) in differentiating HCC vs. Hepatitis and HCC vs. Cirrhosis. Thus, AUC values of 0.853 and 0.903 were obtained for circSMARCA5 and circSMARCA5 + AFP, respectively, in the discrimination of HCC vs. Hepatitis. For the differentiation of HCC vs. Cirrhosis, the use of circSMARCA5 brought an AUC of 0.711, and this value increased to 0.858 when combined with AFP. An important aspect is that in patients with AFP levels below 200 ng/mL, the diagnostic efficiency was good because the use of circSMARCA5 achieved an AUC of 0.847 in discriminating HCC vs. Hepatitis patients and an AUC of 0.706 in the HCC vs. Cirrhosis group. This is important because in 15–30% of patients with advanced HCC, the alpha-fetoprotein remains within the normal limits [Li et al., 2019].

In their review, Cheng et al. summarised the following prognostic factors in bladder cancer: circLPAR1, circASXL1, circRIP2, circPICALM, circ_403658, circHIPK3, hsa_circ_0077837, and circEHBP1 [Cheng et al., 2021].

In colorectal cancer, early diagnosis is very important because 20% of cases at the time of diagnosis already have metastases. The use of a panel of three circRNAs (circ-CCDC66, circ-ABCC1, circ-STIL) has a diagnostic accuracy expressed by an AUC of 0.78, and the association of carcinoembryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19–9) markers increases the AUC at 0.855 [Li et al., 2021; Lin et al., 2019].

CircRNAs with potential therapeutic targets are those involved in activating proliferation, growth, invasion, migration, metastasis. This includes circRNAs with elevated levels, like: CDR1as/CiRS-7 (via miR-7), circHIPK3/circ_0000284 (via miR-7), circRNA-ACAP2 (via miR-21-5p), circ_000984 (via miR-106b), circ_0001955 (via miR-145), circ_0055625 (via miR-106b), circ-BANP, circ_0000826, circPPP1R12A/circ_0000423, circ_0001178 (via miR-382/587/616), circ-NSD2 (via miR-199b-5p), circ_001569 (miRNA-145), and circNSUN2. Vice versa, low-level circRNAs are those that promote apoptosis and have a negative effect on growth, proliferation, migration, and metastasis. Most of these molecules act by interacting with miRNA molecules. This category includes circ-ITGA7 (via miR-370-3p), circDDX17, circ_0026344 (via miR-21, miR-31), circ-FBXW7, and circITCH (via miR-20a, miR-7, and miR-214) [Li et al., 2021].

hsa_circ_0001946 also seems to be a potential biomarker prognosis for oesophageal squamous cell cancer (ESCC). Hsa_circ_0001946 intervenes in ESCC oncogenesis through its role in the miRNA–mRNA network and, thus, in cell proliferation, migration, and invasion [Fan et al., 2019].

Sand et al. found 23 circRNAs with significantly elevated levels in basal cell carcinoma and 48 circRNAs with low levels compared to healthy subjects. Different molecules of circRNAs come from the same *CASC15* gene (previous symbol *LINC00340*) and have different growth levels, ranging from 5.95 to 3.14 compared to levels in healthy subjects. Very low levels were recorded for the circRNAs derived from *FADS2* (has_circ_0022383 with -54.3; has_circ_0022392 with -41.36) or *ASAP2* (fold change -9.82) [Sand et al., 2016].

2.3.1.3. Conclusions

CircRNAs are abundant, stable, expressed in a cell-type and tissue-specific manner, and are present in accessible tissues, which makes them useful as biomarkers in cancer and other diseases. As these molecules are positively or negatively correlated with different stages and mechanisms in cancer, they could be used in diagnosis, risk, and prognostic stratification or therapeutic targets (e.g., via microRNA). Therefore, the use of circRNAs as biomarkers is another step towards personalised medicine. Further studies are necessary to validate some of these circRNAs as different types of biomarkers.

2.3.2. Melanoma – genomics and epigenomics

2.3.2.1 Introduction

Melanoma is a malignant tumor originating from melanocytes, the cells specialized in producing the melanin pigment. Melanocytes emerge from the neural crest, an embryonic structure consisting of migratory pluripotent cells from which several different cell types originate [Uong, Zon, 2010]. In the maturation process, melanocyte progenitors migrate, differentiate, and colonize the skin, hair follicles, uvea, and mucous membranes throughout the body. Accordingly, melanoma can arise in any of these sites, leading to a genetically, histologically, phenotypically, and clinically heterogeneous disease.

In Caucasian populations, the most common type of melanoma is cutaneous melanoma (CM). In recent decades, a continuous increase of CM frequency rates has been observed in Caucasian populations worldwide, making CM the cancer with the most rapidly increasing occurrence [Eggermont, Spatz, Robert, 2014]. Melanoma is dangerous because it poses a greater risk for metastasizing than other skin cancers [Wagstaff, 2022].

Cutaneous melanoma comprises four main subtypes: superficial spreading melanoma, lentigo melanoma, acral lentiginous melanoma, and nodular melanoma. Superficial spreading melanoma accounts for about 70% of melanoma cases. It is more frequent in fair-skinned patients, the locations are the trunk and legs, and the excessive UV exposure is a risk factor [Bolognia, Schaffer, Cerroni, 2017]. Lentigo melanoma targets older patients with sun damaged skin and the affected skin areas are the face, ears, and neck [Goydos, Shoen, 2016]. Acral lentiginous melanoma is typically found in individuals with darker skin. Clinical presentation is a dark spot on the sole or palms. Subungual melanoma represents a subcategory of acral lentiginous melanoma. Clinically, the lesions are localized under the nail beds and appear as dark vertical streaks. Sun exposure is not a risk factor for this rare form of melanoma [Goydos, Shoen, 2016]. Nodular melanoma accounts for 15% of cases. It is the most aggressive subtype of melanoma and usually affects fair-skinned people over 65. All races could be affected and clinical appearance is a firm lump/node that arises on the skin's surface [Menzies et al., 2013].

The American Joint Committee on Cancer (AJCC) proposed scoring parameters for clinical classification of melanoma. This classification takes into consideration the thickness of the primary tumor (T) and its subcategory, the presence of ulceration, the regional lymph node involvement (N), and the presence of distant metastases (M). In patients with distant metastases, there are two categories according to serum lactate dehydrogenase (LDH) levels: 0—with low LDH level and 1—with elevated LDH level. However, TNM classification is the most important prognostic factor, and mitotic rate, age, and gender are also valuable prognostic factors.

Well-known clinicopathologic characteristics define traditional parameters for melanoma staging and prognosis. Regarding primary tumors, tumor thickness and ulceration are strong predictors of survival. The degree of vascular invasion also significantly influences outcome, but only in the set of thin melanomas (<1 mm). In the N category, three independent

elements have prognostic value: the number of metastatic nodes, whether nodal metastases were clinically occult or apparent, and the presence or absence of musculoskeletal injury. Moreover, patients with clinically palpable nodes have a shorter survival than patients with non-palpable disease. The M status, as well as the localization and the number of metastatic sites, is also of great importance for determining the prognosis of a patient [Barreiro-Capurro et al., 2021].

LDH is important for the prediction of survival in advanced stages of melanoma. An elevated serum level of this marker is independent and significant for such a predictor [Tonella et al., 2021]. CM development is a complex multi-factorial process, arising from multiple etiologic pathways and involving the interplay of genetic and environmental risk factors. The two main causes of melanoma are extrinsic UV exposure and genetic predisposition (family history and phenotypic traits carrying a strong genetic component, including hair and eye color and the number of common and atypical melanocytic nevi) [Potrony et al., 2015; Nikolaou, Stratigos, 2014].

Recent decades have brought a major impact on improving survival in melanoma patients. Immunotherapy (independently of genomic mutations) and the combination of BRAF and MEK inhibitors (in patients with BRAF V600 mutations who have a poorer prognosis) improved overall survival [Leonardi et al., 2020; Giunta et al., 2020]. At the same time, new molecular profiles were identified. However, mortality remains high in conditions where more than half of the patients with metastases die within five years [Michielin et al., 2020].

The research studies in recent years in the field of oncology, especially using high-throughput technologies, constitute a premise for personalized medicine and precision medicine at the same time. Thus, the determination of gene expression profile, disease- related genes, and gene signature allows for a better grouping of patients for the purpose of targeted individualized treatment. For example, some studies demonstrated the association between mRNA-signatures and prognosis in melanoma patients [Narrandes, Xu, 2018; Zager et al., 2018].

2.3.2.2. Genomics in Melanoma and Implications for Biomarkers

Genetic aberrations, concurrently with epigenetic characteristics and the tumor microenvironment (TME), lead to tumor progression that eventually results in uncontrolled cell proliferation, an escape of immune annihilation, and induces a metastatic potential. Discovery of the genomic modifications of melanocytic tumors has remarkably enhanced our understanding of pathogenesis. Thus, we established correlations between the genetic abnormalities and the clinical and pathologic characteristics. The uncontrolled cell proliferation of melanocytes depends on the mutations in some oncogenes, such as *MC1R*, *CDK4*, *BRAF*, *CCND1*, *RAS*, *NRAS*, *c-KIT*, *GNAQ*, and *GNA11* or in tumor suppressor genes, such as, *TP53*, *BCORL1*, *PPP2R3B*, *RASA2*, *PTEN*, and *CDKN2A* [Fath et al., 2022]. The main signalling pathways of melanoma are: MAPK (mitogen-activated protein kinase), AKT (protein kinase B) pathway (PI3K/PTEN/AKT), cell-cycle regulation pathway, pigmentation-related pathway (MITF signalling pathways), and p53 pathway [Guo, Wang, Li, 2021].

The progression to melanoma involves a minimum of three key mutations, but the number of genetic abnormalities is high in melanoma. Sometimes, the occurrence of mutations is so fast that a precursor lesion exists only for a short time, which is not enough to detect them [Stephens et al., 2011; Shain et al., 2015; Akbani et al., 2015; Hayward et al., 2017].

Genomic classification of cutaneous melanoma includes four main categories: *BRAF*-mutated melanoma (50% of all cases); *RAS*-mutated melanoma (25%; *NRAS* is the most commonly mutated); *NF1*-mutated melanoma (10–15%); and the triple-wild-type melanoma (10%; rare variants in *KIT*, *GNAQ*, *GNA11,* or translocations of kinases) [Hayward et al.,

2017].

The MAPK pathway plays a major role in the regulation of cell proliferation, cell survival, invasion, the angiogenesis process, and metastasis. Mutant Ras proteins play a role in oncogenesis by activation of the downstream cascade without the stimulation of the respective upstream pathway. Furthermore, it has been suggested that the activation of BRAF, NRAS, and PI3K can occur in various stages of melanoma development [Cheng, Zhang, Li, 2013].

Up to 30% of melanomas are linked to melanocytic precursor lesions, including nevi or intermediate melanocytic tumors [Pampena et al., 2017; Shain, Bastian, 2016]. It is believed that the initial mutations affect the MAPK pathway, leading to the formation of nevi. When no other genomic aberration is present, melanocytes with a MAPK pathway-activating mutation allow for an oncogene- induced senescence. If benign or intermediate melanocytic tumors develop into further genetic aberrations in cancer-related genes, they are liable to progress and acquire metastatic prospects [Shain et al., 2018].

The genes from MAPK pathways involved in melanoma pathogenesis are summarized in Table I.12.

Table I.12. Genes from MAPK pathways involved in melanoma pathogenesis [Al Mahi, Ablain, 2022; HGNC].

Approved Gene Symbol	Approved Gene Name	Protein	Frequency of Mutations In Melanoma (%)
BRAF	B-Raf proto-oncogene, serine/threonine kinase	Serine/threonine-protein kinase B-raf	53
NRAS	NRAS proto-oncogene, gtpase	GTPase NRas	32
NF1	Neurofibromin 1	Neurofibromin	19
KIT	KIT proto-oncogene, receptor tyrosine kinase	Mast/stem cell growth factor receptor Kit	8
MAP2K1	Mitogen-activated protein kinase kinase 1	Dual specificity mitogen-activated protein kinase kinase 1	7
SPRED1	Sprouty related EVH1 domain containing 1	Sprouty-related, EVH1 domain-containing protein 1	6

❖ *BRAF*

BRAF is a *RAF* gene. The connection in the signaling pathway is with the small G protein RAS and through MEK1/2 activate ERK1/ERK2 [Wagstaff, 2022].

BRAF mutations represent a frequent event in oncogenesis, mainly in melanoma [Davies et al., 2002]. The most common mutation (75%) is V600E, when the valine is replaced with glutamic acid in position 600.

BRAF mutations appear in the first stages of melanocytic tumors [Pollock et al., 2003], whereas individual studies indicated that *BRAF* mutations have an unfavorable prognosis in patients with melanoma [Long et al., 2011; Mann et al., 2013], which has not been definitively shown in other studies [Bhatia et al., 2015].

BRAF mutation is implicated in melanoma progression, sustained angiogenesis, tissue invasion, and metastasis, as well as the evasion of the immune response. Multiple studies assessed the prognostic importance of *BRAF* mutations but their role remains controversial. Most studies found a positive association between *BRAF* mutation and poor clinical outcome. The analysis performed in the phase III Keynote 054 trial showed that there are differences between the relapse-free survival (RFS) in patients with BRAF mutations compared to those

without BRAF mutations only in the group treated with a placebo. There is no difference in patients treated with pembrolizumab [Eggermont et al., 2020]. Other studies suggested an opposite correlation between BRAF mutation and patient outcome prediction. For example, Tas and Ertuk investigated the prognostic value of BRAF V600E mutation in 151 stage III patients. The mutation was present in 51% of patients and was associated with better overall survival (OS) and longer disease-free survival [Tas, Erturk, 2020].

A recent study indicates that extracellular vesicles (EVs) could be a promising source of mutant DNA for *BRAF* mutation status for evaluating BRAF therapy [Zocco et al., 2020].

❖ *NRAS*

NRAS is a member of the *RAS* family of oncogenes and was originally found in oncogenic viruses [Hall et al., 1983]. *NRAS* was found to be mutated in melanoma cell lines [Albino et al., 1984]. *NRAS* mutations emerge in about 20% of melanomas and are evenly spread among cutaneous, acral, and mucosal melanomas [Akbani et al., 2015; Hayward et al., 2017]. In opposition, variants in *HRAS* (HRas proto- oncogene, GTPase) and *KRAS* (KRAS proto-oncogene, GTPase) are less common: 2% and 3%, respectively, of all melanoma [Al Mahi, Ablain, 2022]. These mutations are recurrent and they are mutually exclusive with other *RAS* gene mutations [Fernández-Medarde, Santos, 2011].

Mutations in *RAS* genes generally concern codons 12, 13, and 61. Whereas most aberrations in *NRAS* involve the glutamine on position 61 (Q61), mutations in *KRAS* and *HRAS* are usually found at the glycine 12 and 13 (G12, G13). All three hotspot mutations (Q61, G12, and G13) contribute to GTPase inactivation, resulting in a constitutively functional GTP-bound protein. Activation of the RAS protein signals is generated via multiple oncogenic downstream paths, including activation of the MAPK signaling pathway by the RAS/RAF/MEK/ERK signaling cascade and activation of the PI3K/AKT pathway through PI3K (phosphatidylinositol 3OH-kinase) phosphorylation [Albino et al., 1984].

❖ *NF1*

NF1 (neurofibromin 1) is a tumor suppressor gene [Wallace et al., 1990]. It intervenes as an oncogene in different cancers and it was seen to be non-functional in multiple human malignancies, including lung cancers, neuroblastomas, and glioblastomas [Ding et al., 2008; Parsons et al., 2008]. *NF1* encodes the protein neurofibromin, which negatively regulates RAS by hydrolysis of RAS-bound GTP to GDP [Martin et al., 1990]. Functional inactivation of *NF1* contributes to the activation of *RAS* and its downstream signaling pathways, including the MAPK, PI3K/AKT, and mTOR pathways [Dasgupta et al., 2005].

It has been observed that *NF1* inactivating mutations occur in melanomas without *BRAF* and *NRAS* mutations and cause the activation of the MAPK pathway. *NF1* mutations are typically inactivating, often truncating mutations or losses, and there are no mutation hotspots. Usually, melanomas occurring in heavily sun-damaged skin or desmoplastic melanomas show a significantly higher rate of *NF1* mutations [Krauthammer et al., 2015; Shain et al., 2015; Wiesner et al., 2015]. *NF1* mutations are more frequent in acral and mucosal melanomas [Cosgarea et al., 2017; Moon et al., 2018].

Studies have pointed out that *NF1* could be associated with resistance to BRAF and MEK inhibitors [Maertens et al., 2013; Whittaker et al., 2013]. Nevertheless, *NF1*-mutated melanomas have been associated with tumors with a high tumor mutation burden (TMB) that respond well to immunotherapy [Cirenajwis et al., 2017; Eroglu et al., 2018]. These discoveries demonstrate that the *NF1* mutational status could be important for therapeutic decision making.

❖ *MAP2K1/MAP2K2 (MEK1/MEK2)*

MAP2K1 and *MAP2K2* encode the protein kinases MEK1 and MEK2. They function

specifically in the MAPK/ERK cascade and determine the activation of MAPK3/ERK1 and MAPK1/ERK2 and further transduction of the signal within the MAPK/ERK cascade [Caunt et al., 2015]. *MAP2K1/MAP2K2* mutations have been associated with resistance to RAF and MEK inhibitors [Nikolaev et al., 2011; Van Allen et al., 2014].

❖ *KIT*

The *KIT* gene encodes the mast/stem cell growth factor receptor Kit [Curtin et al., 2006]. *KIT* gene modifications (mutations, copy number variations) are mutually exclusive to *BRAF*, *NRAS*, and *NF1* mutations. The most involved exons are 11 and 13. The L576P and K642E mutations have a positive response to KIT inhibitors, but this is a temporary answer [Beadling et al., 2008; Hodi et al., 2008; Carvajal et al., 2011; Guo et al., 2011].

Translocations represent another mechanism that activates the MAPK and other oncogenic pathways. They are more frequent in tumors with spitzoid morphology. In these cases, fusion gene results in *ALK*, *ROS1*, *RET*, *BRAF*, *NTRK1*, *NTRK3*, and *MET* genes [Wiesner et al., 2014; Yeh et al., 2015]. These translocations were determined as mutually exclusive of each other and other MAPK-activating mutations, sustaining the pinpointed translocations as being critical driver events. *BRAF* translocations occur in melanomas [Botton et al., 2013]. In this way, the MAPK and PI3K signaling pathways are activated [Wiesner et al., 2014].

❖ *CDKN2A*

CDKN2A was first associated with familial melanoma predisposition [Cannon-Albright et al., 1992]. Latter studies recognized *CDKN2A* as an essential gene in this locus, responsible for controlling the cell cycle [Kamb et al., 1994]. *CDKN2A* is the most often concerned tumor suppressor gene in sporadic melanoma [Curtin et al., 2005]. *CDKN2A* losses, frequently biallelic, are seen in 50–80% of sporadic melanoma [Akbani et al., 2015; Gast et al., 2010]. The most common abnormalities are inactivating mutations and promoter methylation [Bennett, 2008].

The *CDKN2A* gene encodes for p16 and p14ARF. p16 is important in cell cycle control. It acts on CDK 4/6 and blocks the phosphorylation of the retinoblastoma protein (Rb) [Koh et al., 1995; Lukas et al., 1995]. Rb phosphorylation releases the E2F transcription factor with cell cycle progression from G1 to the S phase [Weinberg, 1995]. p14ARF inhibits the ubiquitin ligase MDM2, which targets proteasomal degradation of TP53 [Lukas et al., 1995].

❖ *PTEN*

PTEN is a tumor suppressor gene with inactivating action in the PI3K signaling pathway. The PTEN mainly determines the inhibition of the AKT pathway.
PTEN has been reported to be mutated or nonexistent in up to 70% of melanomas [Akbani et al., 2015]. Epigenetic silencing of *PTEN* may likewise play a role in melanoma [Lahtz et al., 2010]. *PTEN* loss is more often found in *BRAF*-mutant than in *NRAS*-mutant melanoma [Gast et al., 2010], a finding compatible with PI3K/AKT pathway activation by *NRAS* but not *BRAF* mutations, which need an additional hit. *PTEN* inactivation has been linked to resistance to BRAF inhibitors [Zuo et al., 2018] and immunotherapy [Peng et al., 2016] in melanoma patients.

❖ *TERT Promoter Mutations*

Mutations in the telomerase reverse transcriptase (*TERT*) promoter melanomas are activatied in melanoma [Huang et al., 2013]. The effects are an increased gene expression, and cell proliferation without senescence or apoptosis [Bell et al., 2015]. *TERT* promoter mutations were demonstrated to promote tumorigenesis in two phases, mainly by repairing the shortest telomeres [Chiba et al., 2017]. These mutations are markers of aggressive

behavior and also of inferior prognosis [Lu et al., 2018].

❖ *TYRP1*

The microarray analysis of melanoma metastasis showed that the gene *TYRP1* was associated with shorter survival. *TYRP1* expression in the validation group demonstrated a powerful correlation between TYRP1 protein level and distant metastasis-free survival and OS. This discovery indicates *TYRP1* as a possible prognostic marker for stage III melanoma patients [Journe et al., 2011].

Even though nowadays we know that *TYRP1* plays an important role, the link with patient survival and how its expression affects cell behaviour is still unclear [Narrandes, Xu, 2018].

❖ *ctDNA and CTC*

In a recent systematic review, Gandini et al. summarized the studies about the link between ctDNA and survival in over 2000 stage III and IV melanoma patients. They observed that detectable ctDNA before treatment and during follow-up correlated to poorer progression-free survival (PFS) and OS, with no differences by tumour stage or systemic regimen. Even though ctDNA has a high potential as a prognostic biomarker, the standardization of a methodology is necessary before introducing liquid biopsy in clinical practice [Gandini et al., 2021].

❖ *Common Variants*

A recent meta-analysis genome-wide association study identified 54 significant loci for melanoma and 68 independent SNPs (Single nucleotide polymorphisms) in the meantime. These variants concern genes or loci located in the vicinity of genes involved in pathogenic pathways of melanoma such as DNA repair and telomere length, differentiation of melanocyte and cell adhesion, and immunity [Landi et al., 2020].

The most common variants associated with melanoma that present a DNA repair pathway modification are rs78378222 and rs161548 at the TP53 locus. In this case, the absence of the normal response of TP53 to cellular stress determines the cellular hyperproliferation of mutant cells [Landi et al., 2020]. The loci and the SNPs associated with telomere maintenance correspond to the following genes: *POT1* (rs4731207), *TERC* (rs3950296), *RTEL1* (rs143190905), *MPHOSPH6* (rs2967383), *STN1* (rs7902587), *CCND1* (rs4354713), *ATM* (rs1801516), and *PARP1* (rs2695237) [Landi et al., 2020].

SNPs identified in following genes (or genetic regions located in their proximity) are implied in melanocyte development and differentiation pathway: *FOXD3* (rs670318), *NOTCH2* (rs2793830), *MITF* (rs149617956), *NOTCH1*, and *SOX10* [Landi et al., 2020].

E-cadherin encoded by the *CDH1* gene (cadherin 1) plays a major role in the adhesion between melanocytes and keratinocytes. E-cadherin expression is lost in the stage of melanoma progression. SNP rs4420522 in the *CDH1* gene is a risk allele [Landi et al., 2020]. Common variants in immunity genes associated with melanoma susceptibility have been identified, for example, rs28986343 at the HLA locus and association between rs408825 and *MX2* gene. On the other hand, rs1126809 in the *TYR* gene or rs6059655 in the *ASIP* gene are protective effects [Landi et al., 2020].

❖ *Copy Number Variations (CNV)*

Numerous numerical and structural chromosomal aberrations, unlike benign proliferations that do not have such changes, were discovered in malignant melanomas [Bastian et al., 1998]. Chromosomes most frequently involved are 1, 6, 7, 9, 10, and 11. These

chromosomal regions contain genes [*GNAQ* (9q21.2), *BRAF* (7q34), *PTEN* (10q23.31), *CCND1* (11q13.3), *RREB1* (6p24.3), *MYB* (6q23.3), and *CDKN2A* (9p21.3)] involved in the MAPK pathway, which is consistent with the involvement of this pathway in the pathogenesis of melanoma [Bastian et al., 1998; James et al., 2014].

Copy number variations can be generated by loss or gain of genetic material and these correlate with the stage of tumorigenesis, patient age, and histological type [Bastian et al., 1998; James et al., 2014].

The most frequent anomalies are losses of chromosome 9 (81% of tumours) and chromosome 10 (63% of tumours), with both anomalies appearing in the early stage of tumorigenesis. At the level of chromosome 9, usually, region 9p21 where the *CDKN2A* gene is located, which encodes cyclin-dependent kinase inhibitor 2A, is concerned. It interacts with CDK4 (cyclin-dependent kinase 4) and CDK6 (cyclin-dependent kinase 6) and acts as a negative regulator of normal cell proliferation [Bastian et al., 1998]. For chromosome 10, the frequently involved regions are 10q21-22 and 10q24-qter [Bastian et al., 1998]. In final stages of melanoma and in metastasis, gains on chromosome 6, 7, 8, and 1q were observed. Also, was discovered the association between gains on chromosome 1q and 6p with lost on chromosome 10q [Bastian et al., 1998]. Loss of heterozygosity frequently concerns regions 1p, 9q, and 10q and is correlated with cell proliferation [Bastian et al., 1998; James et al., 2014]. Chiu et al identified 249 copy number variations in circulating tumor cells (139 copy number gains and 110 copy number loss) found in more than 50% of the studied cases. A panel of five such CNV proved to have a negative prognostic impact (copy number gains: 1p35.1, 2q14.3, 14q32.33, and copy number loss: 14q32.11, 21q22.3) [Chiu et al., 2014].

❖ *Hereditary Melanoma*

Hereditary melanomas represent 5–12% of all melanomas [Ransohoff et al., 2016]. Germline mutations are characterized by high penetrance and are associated with other cancers or other locations [Ransohoff et al., 2016]. The mechanisms of oncogenesis include: activation of oncogenes, loss of tumor suppressor genes, and chromosomal instability. Most cancer predisposition syndromes are transmitted in an autosomal dominant manner [Ransohoff et al., 2016; Pho et al., 2016; Soura et al., 2016]. Table I.13. summarizes the genes and the syndromes with predisposition to cancer and risk for melanoma. Lifetime melanoma risk is different in these syndromes: 60–90% in FAMMM or melanoma-pancreatic cancer syndrome, up to 80-fold in hereditary retinoblastoma, and 2000-fold in xeroderma pigmentosa [Pho et al., 2016].

Table I.13. Syndromes with predisposition to cancer and risk for melanoma and their related genes/susceptibility locus

[Ransohoff et al., 2016; Pho et al., 2016; Soura et al., 2016; OMIM]

Syndrome (Phenotype MIM number)	Gene symbol/ susceptibility locus	Gene name	Inheritance
Familial atypical multiple mole melanoma syndrome (FAMMM)	*CDKN2A* (9p21.3) *CDK4* (12q14.1) *MC1R* (16q24.3) *XRCC3* (14q32.33) *MITF* (3p13) *TERT* (5p15.33) *POT1* (7q31.33) 1p36, 1p22, 20q11	cyclin dependent kinase inhibitor 2A cyclin dependent kinase 4 melanocortin 1 receptor X-ray repair cross complementing 3 melanocyte inducing transcription factor telomerase reverse transcriptase protection of telomeres 1	AD

Syndrome (Phenotype MIM number)	Gene symbol/ susceptibility locus	Gene name	Inheritance
Melanoma-pancreatic cancer syndrome (OMIM #606719)	*CDKN2A* (9p21.3)	cyclin dependent kinase inhibitor 2A	AD
Melanoma-astrocytoma syndrome (OMIM #155755)	*CDKN2A* (9p21.3)	cyclin dependent kinase inhibitor 2A	AD
Susceptibility to uveal melanoma 2 (OMIM #606661) **Tumor predisposition syndrome-1 (OMIM# 614327)**	*BAP1* (3p21.1) *BAP1* (3p21.1)	BRCA1 associated protein 1 BRCA1 associated protein 1	AD
Xeroderma pigmentosa XPA (OMIM#278700) XPB (OMIM#610651) XPC (OMIM#278720) XPD (OMIM#278730) XPE (OMIM#278740) XPF (OMIM#278760) XPG (OMIM#278780) XPV (OMIM#278750)	 *XPA* (9q22.33) *ERCC3* (2q14.3) *XPC* (3p25.1) *ERCC2* (19q13.32) *DDB2* (11p11.2) *ERCC4* (16p13.12) *ERCC5* (13q33.1) *POLH* (6p21.1)	 XPA, DNA damage recognition and repair factor ERCC excision repair 3, TFIIH core complex helicase subunit XPC complex subunit, DNA damage recognition and repair factor ERCC excision repair 2, TFIIH core complex helicase subunit damage specific DNA binding protein 2 ERCC excision repair 4, endonuclease catalytic subunit ERCC excision repair 5, endonuclease DNA polymerase eta	AR
Oculocutaneous albinism type 2 (OMIM#203200)	*OCA2*(15q12-q13.1)	OCA2 melanosomal transmembrane protein	AR
Hereditary Retinoblastoma (OMIM#180200)	*RB1* (13q14.2)	RB transcriptional corepressor 1	AD
Li-Fraumeni syndrome (OMIM#151623)	*TP53* (17p13.1)	tumor protein p53	AD
PTEN hamartoma tumor syndromes	*PTEN* (10q23.31)	phosphatase and tensin homolog	AD
Hereditary breast and ovary cancer syndrome	*BRCA1* (17q21.31) and *BRCA2* (13q13.1) (especially BRCA2)	BRCA1 DNA repair associated BRCA2 DNA repair associated	AD

AD – autosomal dominant; AR – autosomal recessive

2.3.2.3. Epigenomics in Melanoma and Implications for Biomarkers

Epigenetic changes relate to gene expression independent of changes in the DNA sequence that persist over several cell divisions [Chang et al., 2008].

Modifications of the epigenome are involved in cancer initiation, progression, and resistance to antitumor drugs [Giunta et al., 2021]. Epigenetic marks are important as biomarkers for diagnosis, prognosis, predictive for disease recurrence, and therapeutic targets [Hsu et al., 2021; Hatzimichael et al., 2014]. The epigenetic changes are reversible [Yoo, Jones, 2006; Wilting, Dannenberg, 2012].

MicroRNAs, non-coding RNAs, histones modifications, and abnormal DNA methylations were associated with the stages of melanoma progression [Mannavola et al., 2019].

❖ *Histones Modifications*

In histone modifications, 3 types of proteins are involved: histone writer proteins, eraser proteins, and reader proteins. The role of the first category is to add different chemical groups to histones through various chemical processes (acetylation, methylation, phosphorylation, ubiquitylation, glycosylation, ADP-ribosylation, carbonylation, and SUMOylation) [Orouji, Utikal, 2018]. Eraser proteins remove chemical groups. The reader proteins modulate gene expression by recruiting transcription factors or transcription repressors [Giunta et al., 2021; Gallagher, Tiffen, Hersey, 2015; Yun et al., 2011]. Chromatin remodelling occurs by modifying histones. These changes produce gene hyperexpression or gene inactivation [Giunta et al., 2021]. The most important histone modifications are histone methylation and histone acetylation.

Methyltransferases and histone demethylases

Methylation of histones occurs most frequently at the level of lysine residues and/or arginine residues [Orouji, Utikal, 2018]. Lysine methylation consists of the addition of one to three methyl groups. The conversion from the unmethylated form to the methylated form is done under the action of methyltransferases (writer) and the reverse demethylation process is carried out by histone demethylases (eraser) [Orouji, Utikal, 2018; Grønbaek, Hother, Jones, 2007]. Figure I.23 summarize methyltransferases and histone demethylases, their action, and their place of action.

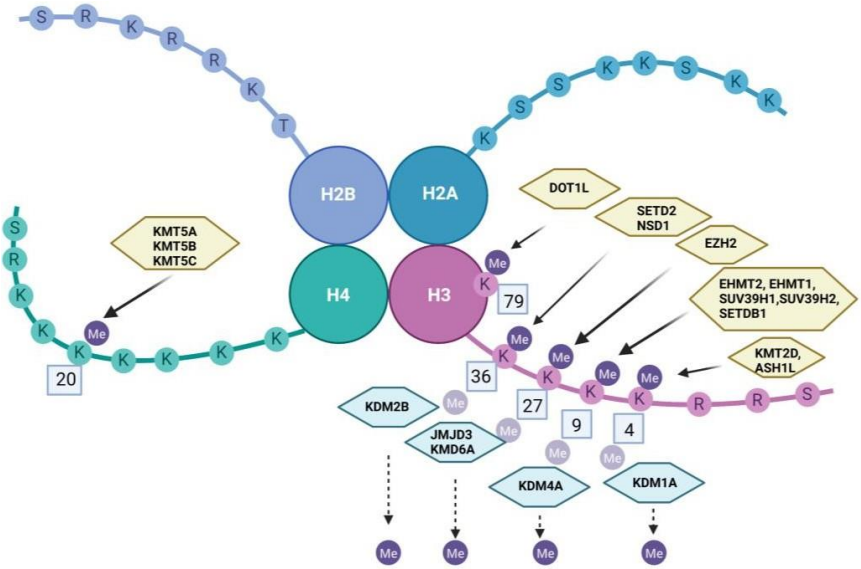


Figure I.23. Methylation and demethylation of histones. Created with BioRender.com.

Histone methylation has two effects: silencing signatures and actively transcribed chromatin.

Silencing signatures are translated by the trimethylation of lysine 9, 27, and 36 at the N-terminal tail of histone H3 (H3-K9, H3-K27, H3-K36) and lysine 20 on histone H4 (H4-K20) [Gallagher, Tiffen, Hersey, 2015]. H3-K9 trimethylation is a condition for establishing and maintaining a stable heterochromatin status [Grønbaek, Hother, Jones, 2007]. Histone H3 acetylation at the level of 27 lysine residue (H3K27ac) is an important regulator of *MITF* (melanocyte inducing transcription factor) expression and is associated with an increased metastatic potential to melanoma cells [Mannavola et al., 2019; Verfaillie et al., 2015].

Actively transcribed chromatin appears as a result of the trimethylation of lysine 4 and 79 of histone H3 (H3-K4, H3-K79) [Grønbaek, Hother, Jones, 2007].
The most important methyltransferase with a role in melanoma is EZH2 (Histone- lysine N-methyltransferase EZH2) encoded by the *EZH2* gene (enhancer of zeste 2 polycomb repressive complex 2 subunit). EZH2 represents the catalytic subunit of Polycomb Repressive Complex 2 (PRC2). PRC2 has a role in (mono-, di-, or tri-) the methylation on H3 lysine 27 and in such a way in the silencing of target genes [UniProt]. In total, 3% of melanomas are associated with activating mutations in *EZH2*, with a role in melanoma progression [Gallagher, Tiffen, Hersey, 2015; Tiffen et al., 2015].

Acetylation is done exclusively at the level of lysine residues [Wilting, Dannenberg, 2012]. The balance of histone acetyl transferases and histone deacetylases determines the level and state of acetylation [Gallagher, Tiffen, Hersey, 2015].

Histone Deacetylases (HDACs) and Histone Acetyltransferases (Lysine Acetyltransferases—KATs)

Histone deacetylases (HDACs) are enzymes whose main function is removing acetyl groups from histones and secondary functional consequences appear on chromatin remodelling and gene expression. HDACs are classified into four classes: class I (HDAC1; HDAC2; HDAC3; HDAC8 – in nucleus); class II with subclass IIa (HDAC4; HDAC5; HDAC7; HDAC9 – in cytoplasm/nucleus) and subclass IIb (HDAC6; HDAC10 – in cytoplasm); class III (SIRT 1–7: cytoplasm/nucleus); and class IV (HDAC11 – in cytoplasm/nucleus) [Orouji, Utikal, 2018]. Class I, II, and IV are Zn2+ dependent, while class III are NAD-dependent [Milazzo et al., 2020].

Several HDACs can act to deacetylate certain histones in different forms of cancer: H3K9 (HDAC3), H3K14 (HDAC1, HDAC3), H3K56 (HDAC1, HDAC2), H4K5 (HDAC1, HDAC2, HDAC3), H4K8 (HDAC1, HDAC2), H4K12 (HDAC1, HDAC2, HDAC3), and H4K16 (SIRT1, SIRT2, HDAC1, HDAC2, HDAC3) [Wilting, Dannenberg, 2012].
Several acetylases acetylate a lysine residue in different cancers: H3K9 (KAT2, KAT12), H3K14 (KAT2, KAT3A, KAT3B, KAT6, KAT10, KAT12), H3K18 (KAT2, KAT3, KAT12), H3K56 (KAT3B), and H4K16 (KAT5, KAT8) [Wilting, Dannenberg, 2012].

❖ Histone Variants

Histone variants contribute to epigenome plasticity [Hsu et al., 2021].

The canonical histones are H2A, H2B, H3, and H4 [Gallagher, Tiffen, Hersey, 2015; Vardabasso et al., 2014]. The histone variants have different sequences and properties and can replace canonical histones. The effect is altered chromatin structures and gene transcription [Gallagher, Tiffen, Hersey, 2015; Vardabasso et al., 2014].

For the canonical H2A core histones, the expression is replication-dependent and the function is the core component of nucleosome. For non-canonical H2A core histones (H2A.X, H2A.Z, H2A.Z.2.1, H2A.Z.2.2, H2A.Bbd type 1, H2A.Bbd type 2 and H2A.Bbd type 3, macroH2A1.1, macroH2A1.2 and macroH2A2), the expression is replication-independent [Amatori et al., 2021]. The histone variants macroH2A, H2A.Z, and H3.3 are important in melanoma [Konstantinov, Ulff-Møller, Dimitrov, 2016].

The macroH2A has two regions: histone-like H2A domain and NHR (non-histone region); two isoforms (macroH2A1 and macroH2A2) and macroH2A1 have two spliced variants, macroH2A1.1 and macroH2A1.2. The role of macroH2A is a regulatory factor of tran- scription, cell differentiation, and reprogramming [Konstantinov, Ulff-Møller, Dimitrov, 2016]. MacroH2A suppresses melanoma progression and its expression in melanoma is generally lost [Gallagher, Tiffen, Hersey, 2015; Kapoor, et al., 2010]. H2A.Z.2 and histone 3 variant H3.3 are highly expressed in melanoma. Overexpression of H2A.Z.2 produced activation of E2F (E2 factor of transcription) targets genes and consecutively promotes

melanoma cell proliferation [Gallagher, Tiffen, Hersey, 2015; Vardabasso et al., 2015; Ávila-López et al., 2021].

❖ *Long Non-Coding RNAs*

The majority of the human genome transcript (90%) is not transcribed into proteins and has a role in the regulation of gene expression. Long non-coding RNA (lncRNAs) is represented by DNA sequences that has a length over 200 pb. It modifies the regulation of gene expression by transcription and translation regulation, chromatin changes, RNA changes through editing or splicing or degradation, and miRNA sequestration. At the cellular level, the modification of gene expression translates into the regulation of cell proliferation, differentiation, migration, and invasion [Yu et al., 2018].

Guo et al. synthesized the roles of lncRNAs in melanoma pathogenesis. Most lncRNA molecules are upregulated with a role in promoting melanoma pathogenesis: BASP1- AS1, SAMMSON, NCK1-AS1, LINC00470, LINC01291, MIR205HG, LINC00518, NEAT1, LHFPL3-AS1, TTN-AS1, LHFPL3-AS1, LINC00520, SRA, LINC00518, FOXD3-AS1, LN-MAT1, SLNCR1, OIP5-AS1, CASC15, LncRNA-ATB, KCNQ1OT1, HOXD-AS1, FALEC, BANCR, CCAT1, PVT1, RHPN1-AS1, ANRIL, HEIH, TSLNC8, KCNQ1OT1, LINC01158, MALAT1, and ZEB1-AS1 [Guo, Wang, Li, 2021].

lncRNA molecules that are downregulated with a suppressor role in melanoma pathogenesis have also been described: FUT8-AS1, TINCR, MEG3, LINC-PINT, DIRC3, ZNNT1, Linc00961, CPS1-IT1, CASC2, NKILA, CDR1as, GAS5, and LINC00459 [Guo, Wang, Li, 2021].

In more than 90% of melanomas, a lncRNA SAMMSON, which plays an oncogenic role in association with MITF, is present [Leucci, 2016].

Several lncRNAs were studied because of the high expression levels in melanoma patients, including SPRY4-IT1, MALAT-1, BANCR, UCA1, HOTAIR, and SNHG8. The levels of UCA1 and MALAT-1 were remarkably more elevated in patients with melanoma compared to healthy controls, and their levels were associated with the stage of the disease [Lazăr, Dinescu, Costache, 2020; Shan, Kahn, Pusztai, 2022]. The expression of LINC02249 was found to be elevated in cutaneous melanoma.

The high expression corresponds to poor OS and disease-specific survival, which is an independent prognostic factor [Du et al., 2022].

❖ *MicroRNAs*

Small ncRNAs includes miRNAs, piwi-interacting RNAs—piRNAs, and small nucleolar RNAs—snoRNAs. Their length is up to 200 nucleotides [He, Hannon, 2004; Watson, Belli, Di Pietro, 2019]. MiRNA (single stranded DNA molecules 18–25 nucleotides long) is involved in cancer by regulating oncogenes and tumour suppressor genes. The premature miRNAs are exported to the cytoplasm where mature miRNAs are formed. These molecules will bind to the target mRNA because of the complementarity of the bases. Mechanisms for regulating gene expression involve the degradation of the target mRNA or inhibition of translation into proteins [He, Hannon, 2004; Mohr, Mott, 2015].

Neagu et al. summarized the main miRNA in melanoma tissue and/or in circulation of patients with cutaneous melanoma: miR-214, miR-148a, miR-221, miR-16, miR-29c, miR-146a-5p, miR-205, pattern miR-142-5p, miR-150-5p, miR-342-3p, miR-155-5p, miR-146b-5p, miR-10b, miR-203, let-7a, and let-7b, miR-148, miR-155, miR-182, miR-200c, miR-211, miR-214,miR-221, miRNA-222, miR-106b, and 7 miRNAs (MELmiR-7). The following panel of oncomirs contains the miRNAs (miR-21, miR-10b, miR125b, miR-135b, miR-146a, miR-150, miR-155, miR-205, miR-211, miR-221, miR-222) that regulate tumour promoter genes associated with cutaneous melanoma (*RAS/MAK, MITF, PI3K-AKT, P27Kip, NRAS,*

TYRP1, *WEE1*, *LATS2*). Some miRNAs function as tumour suppressors in melanoma: miR-16, miR-29c, miR-203, miR-205, miR-206, and miR-675. The genes targeted by these miRNAs are *DMMT3B*, *B7-H3*, and *MTDH* [Neagu et al., 2020].

Huber et al. found a set of miRNAs (let-7e, miR-99b, miR-100, miR-125a, miR-125b, miR-146a, miR-146b, and miR-155) implicated in the transformation of monocytes into immunosuppressive MDSCs. MiRNAs such as miR-28 and miR-17-5p seem to interfere with PD-1 and PD-L1 expression at a post-transcriptional level, enabling resistance to immunotherapy [Huber et al., 2018].

Kanemaru et al. suggested that circulating miR-221-3p could be used as a melanoma biomarker, showing significantly distinct expressions between stage I/IV melanoma patients and healthy controls. Their work explained how miR-221-3p levels reduced after surgical removal of the primary tumor and increased upon disease recurrence, suggesting that circulating miRNA-221-3p could be a new tumor marker. High levels of miRNA-221 have been found in early-stage melanomas compared to healthy individuals. The levels of expression were also found to be proportionate with the stage of the disease [Kanemaru et al., 2011].

- *Circular RNA*

Circular RNAs (circRNAs) are endogenous RNA molecules with covalently looped structures [Tang et al., 2021; Caba et al., 2021]. There are several types of circRNA molecules according to their composition: exonic circRNAs (formed by exons), circular intronic RNAs (formed by introns), and exon-intron circRNAs (formed by both exons and introns) [Zhou, Yang, Xiong, 2018].

CircRNA intervenes in both normal physiological processes and pathological processes. CircRNAs modulate transcription, splicing, microRNA sponge, modulation of protein–protein interaction, and protein sponges [Caba et al., 2021].

Tang et al. summarized the circRNA involved in the inhibition of apoptosis, hyper-proliferation, invasion, migration, carbohydrate metabolism, and metastasis. Some of circRNA have an oncogenic function and activate several physiological processes in melanoma: circ_0084043 and circ-FOXM1 (proliferation, apoptosis, invasion). Other circRNA molecules have suppressive functions: circ_0023988 and circ_0030388 (proliferation, invasion, migration) [Tang et al., 2021].

Some circRNA molecules with low or increased expression have been described: circ_0016418 (up; promotes cell proliferation, migration, invasion, and epithelial to mesenchymal transition), ciRS-7 (CDR1as) (down; promotes cell invasion and metastasis, correlates with progression-free, overall survival and distinct therapeutic responses), and circ_0079593 (up; promotes cell proliferation, metastasis, glucose metabolism, inhibits apoptosis) [Caba et al., 2021; Zhou,Yang, Xiong, 2018; Lu, Li, 2020; Hanniford et al., 2020]. CDR1as has been identified as a marker of progression in melanoma [Ghafouri-Fard et al., 2022].

- *Abnormal DNA Methylation*

DNA methylation is the biochemical process in which a methyl group is added to a cytosine or adenine at the 5-position of carbon where the DNA base thymine is located. The cytosine is converted to methylcytosine [Bernstein, Meissner, Lander, 2007; Fu et al., 2017]. In total, 60% of gene promoters are associated with unmethylated CpG islands [Fu et al., 2017].

DNA methylation is a mark of suppression of gene expression. The effects are on cell differentiation and cell proliferation. Fu et al. summarized the genes hypermethylated in melanoma: *LINE-1*, *CLDN11*, *TERT*, *MGMT*, *KIT*, *TNF*, *MITF*, *RASSF6*, *RASSF10*, *GPX3*, *MMP-9*, *SYNPO2*, *CDKN1C*, *LXN*, *ASC/PYCARDC/PYCARD*, *Col11A1*, *SOCS1*, *Caspase 8*,

CDH1, *MGMT*, *RAR-b2*, *CIITA-PIV*, *SOCS2*, *TNFRSF10C (DcR1/2)*, *TPM1*, *TIMP3*, *CDKN2A*, *DPPIV*, *FRZB*, *SOCS3*, *THBS1*, and *TM* [Fu et al., 2017].

Sigalotti et al. studied the importance of DNA methylation as a prognostic biomarker in stage III melanoma. They assessed the genome-wide methylation profiles from 45 patients. Based on global methylation, the cohort was split into a favorable group, with a median survival of 31.5 months, and an unfavorable group, with a median survival of 10.3 months, with a 5-year overall survival of 41.2 and 0%, respectively. The group identified a 17-gene methylation signature sufficient to distinguish the good prognosis group, characterized by low methylation density. Hypomethylation was a significant predictor of increased OS [Sigalotti et al., 2012].

2.3.2.4. Conclusions

Cutaneous melanoma is the result of multiple genomic and epigenomic changes, some of them in close correlation. The negative prognosis of melanoma has raised interest in the discovery of prognostic and predictive biomarkers in order to improve life expectancy. For these reasons, the approach to the patient must be in relation to genetic and epigenetic changes. Prevention targeted the approaches of modifiable and non-modifiable risk factors. The existence of genetic and epigenetic biomarkers validated in various evolutionary stages would allow for a more precise molecular framing and a targeted approach to the changes present in the patient/group of similar patients. The general objective must be to avoid the metastatic stage.

Chapter 3. GENETIC HETEROGENEITY AND PLEIOTROPY IN BARDET-BIEDL SYNDROME – EXAMPLE OF THE MULTIDICIPLINARITY APPROACH IN CILIOPATHIES

The results of researches about Bardet-Biedl Syndrome were published in the following papers
ISI ARTICLES
Florea, L., **Caba, L.*** and Gorduza, E.V. *Bardet–Biedl Syndrome—Multiple Kaleidoscope Images: Insight into Mechanisms of Genotype–Phenotype Correlations.* Genes (Basel) 2021, 12(9): 135. **(corresponding author). IF=4.141** • awarded in the **UEFISCDI** competition "Premierea Rezultatelor cercetarii - Articole PRECISI 2021"
Caba, L., Florea, L., Braha, E. E., Lupu, V. V., & Gorduza, E. V. *Monitoring and Management of Bardet-Biedl Syndrome: What the Multi-Disciplinary Team Can Do*. J Multidiscip Health 2022,15, 2153-2167. **IF=2.919**
Khan, S., Focșa, I. O., Budișteanu, M., Stoica, C., Nedelea, F., Bohîlțea, L., **Caba, L.**, Butnariu, L., Panzaru, M., Rusu, C., Jurca, C., Chirita-Emandi, A., Banescu, C., Abbas, W., Sadeghpour, A., Baig, S. M., Balgradean, M., Davis, E. E. *Exome sequencing in a Romanian Bardet-Biedl syndrome cohort revealed an overabundance of causal BBS12 variants.* Am J Med Genet 2023, 1–16. **IF=2.578**
BDI ARTICLES
Focsa, I.O., Budisteanu, M., Stoica, C., Nedelea, F., Jurca, C., **Caba, L.**, Butnariu, L., Panzaru, M., Rusu, C., Balgradean, M. *Clinical Aspects of a Rare Disease: Bardet Biedl Syndrome.* Modern Medicine 2022, 29(1): 37.

3.1. Introduction

Ciliopathies are diseases caused by the dysfunction of motile and non-motile primary cilium [Strong et al., 2021]. Primary cilia are involved in numerous cellular processes such as cell cycle control, development, migration, polarity, differentiation, stimuli transduction, proliferation, and maintenance of stem cells [Cardenas-Rodriguez, Badano, 2009; Christensen et al., 2017; Alvarez-Satta, Cástro-Sánchez, Valverde, 2017]. Primary cilia also intervene in the cellular signaling pathways of development and homeostasis: hedgehog, Wnt (wingless-related integration site), Notch, Hippo, GPCR (G protein-coupled receptors), PDGF (platelet-derived growth factor), mTOR (mammalian target of rapamycin), and TGF-βeta (βeta transforming growth factor-β) [Wheway, Nazlamova, Hancock, 2018; Singla, 2006].

Cilia are microtubule-based organelles. They are anchored to the cytoskeleton and protrude at the cell surface [Adamiok-Ostrowska, Piekiełko-Witkowska, 2020]. They have three main components: axoneme, basal body and transition zone. The axoneme consists of nine peripheral microtubule doublets, and a central part that could be formed by a pair of microtubules (model 9 + 2) or by the absence of this pair of microtubules (model 9 + 0) [Mitchison, Valente, 2017]. The transport in and out of cilia is allowed by three complexes: intra-flagella transport (IFT) complex A (IFT-A), IFT complex B (IFT-B) and BBSome [Alvarez-Satta, Cástro-Sánchez, Valverde, 2017]. IFT-A is involved in the retrograde transport (tip to the base), IFT-B is involved in the anterograde transport (base to the tip) while BBSome is a multiprotein complex implied in ciliary trafficking activity [Ma et al., 2021].

Ciliopathies are characterized by a high clinical and molecular heterogeneity and a large clinical overlap between entities [Zaki et al., 2011]. The clinical expression of the cilia dysfunction is correlated with the activity of cilia. Motile cilia dysfunction causes

hydrocephalus, infertility, chronic respiratory issues, but also congenital heart defects and organ laterality defects (the last two are common manifestations of non-motile cilia dysfunction) [Reiter, Leroux, 2017]. Non-motile cilia dysfunction determines: retinal dystrophy, anosmia, hearing loss, central obesity, skeletal abnormalities (polydactyly, rib cage), hypogonadism, genital abnormalities, ataxia, epilepsy, mental disability, brain malformations, facial abnormalities, renal abnormalities (polycystic kidney disease-PKD, nephronophthisis-NPHP), and liver disease (liver fibrosis) [Reiter, Leroux, 2017].

3.2. Pleiotropy and Variable Expressivity in Bardet–Biedl Syndrome

3.2.1. Background

Bardet–Biedl syndrome (BBS, OMIM 209900) is a rare autosomal recessive multisystem non-motile ciliopathy primarily characterized by heterogeneous clinical manifestations. The prevalence of BBS is high in inbred/consanguineous populations. In the general population, BBS has a prevalence of 0.7/100,000 with a prevalence at birth of 0.5/100,000 [Orphanet]. However, the incidence of BBS is variable: 1 in 160,000 in North America and Europe, 1 in 17,000 in Kuwait—Bedouin populations; 1 in 3700 individuals in the Faroe Islands [Khan et al., 2016; Ajmal et al., 2013; Farag, Teebi, 2008; Hjortshøj et al., 2008].

BBS is characterized by a high genetic heterogeneity (locus, mutational, clinical), variable expressivity and pleiotropy. There are more than 24 loci involved and the mutational profile is diverse. There are modifying variants and numerous interactions between the BBS proteins (interactions disease protein—disease protein) or between the BBS protein and another protein (interactions disease protein—non disease protein). These explain the extremely polymorphic clinical picture that includes both major and minor features (Figure I.24.).

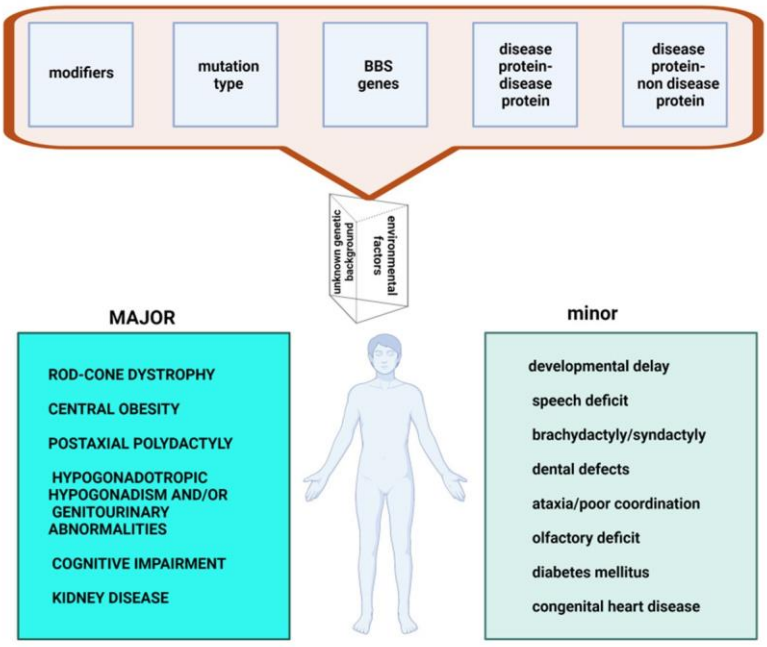


Figure I.24. Heterogeneity in Bardet Biedl Syndrome-determinants and clinical effects. Created with BioRender.com

3.2.2. Clinical picture

This pleiotropic disorder has a constellation of features, which are divided into major features (rod-cone dystrophy, central obesity, postaxial polydactyly, hypogonadotropic hypogonadism and/or genitourinary abnormalities, cognitive impairment, kidney disease) and minor features (developmental delay, speech deficit, brachydactyly or syndactyly, dental defects, ataxia or poor coordination, olfactory deficit, diabetes mellitus, and congenital heart disease) [Sheffield et al., 1994; Beales et al., 1999; Sheffield et al., 2016; Forsythe, Beales, 2013].

Before the discovery of BBS genes, Beales et al. established a diagnostic algorithm based on phenotypic presentations of this syndrome [Forsythe, Beales, 2013].

Major Features

One of the most important features of BBS is the ***rod-cone dystrophy*** (RCD), affecting 94 - 100% of individuals [Denniston et al., 2014; Niederlova et al., 2019]. Rod and cone photoreceptor cells are modified ciliated cells responsible for the night and day vision. The main protein of rod cells is rhodopsin. The transport of this protein from the inside to the outside of rod cells is allowed by BBSome complex. Mutations in BBSome genes lead to mislocalization and accumulation of rhodopsin in rod cells. In this way the cellular homeostasis is disturbed and the degeneration of the photoreceptors is produced [Mockel et al., 2011]. The retinopathy pattern is a pigmentary one [Forsythe, Beales, 2013; Denniston et al., 2014]. RCD is the second major cause of syndromic retinal degeneration [Weihbrecht et al., 2017; Stone et al., 2017]. Other ophthalmic phenotypes described in the literature are: central cone-rod dystrophy, global severe retinal dystrophy, choroidal dystrophy [Denniston et al., 2014; Mockel et al., 2011]. The symptoms appear in the first decade of life with nyctalopia, which is usually evident by the age of 7–8 years old [Denniston et al., 2014; Berezovsky et al., 2012]. Progressive peripheral vision loss, decreased colour discrimination, impairment and loss of visual acuity can be observed throughout the evolution [Forsyth, Gunay-Aygun, 2003; Weihbrecht et al., 2017]. Most patients are legally blind in the second or third decade of life [Adams, Awadein, Toma, 2007]. Other ocular anomalies are posterior subcapsular cataract and refractive errors, strabismus, nystagmus [Forsythe et al, 2013; Carey et al., 2020; Berezovsky et al., 2012].

Obesity is a frequent feature (89%), it affects the thorax and abdomen and has an early onset (from the age of 2–3 years) [Niederlova et al., 2019]. Weight at birth is often within normal limits, although it is often towards the upper limit of normal. More than a third of the patients with Bardet-Biedl syndrome and normal birth weight develop overweight or obesity by the age of 1 year old. In adults, obesityis especially truncal, but in children it is evenly distributed throughout the body [Forsythe et al, 2013]. The average value of BMI is 35.7 ± 7.8 kg/m^2 [Mujahid, 2018].

A recent study of people with Bardet-Biedl syndrome found the prevalence of overweight and obesity according to WHO standards for raising children as follows in Table I.14. [Pomeroy et al., 2021].

Hunger, satiety and energy consumption are deeply controlled processes in which neuroendocrine factors play a significant role. Common adult obesity was associated with nucleotide polymorphism in *BBS2* gene (rs4784675). Early onset childhood obesity and common adult morbid obesity was associated with *BBS4* (rs7178130) and *BBS6* (rs6108572 and rs221667) genes changes [Benzinou et al., 2006].

Most BBS proteins are expressed in the primary cilia, which has so far failed to explain the occurrence of obesityin BBS. There is a hypothesis that BBS proteins are involved in loss of appetite regulation at the central level due to a defect in the ciliary [Büscher et al., 2012]. The BMP-8B protein (bone morphogenetic protein 8B) has been

reported to play an important role in the metabolism through endocrine and paracrine actions, modulating the thermogenesis of the brown adipose tissue [Whittle et al., 2012; Martins et al., 2016]. It seems that BBSome is a critical determinant of the neuronal BMB-8B sensitivity [Rial-Pensado et al., 2022]. The potential mechanism involving *BBS1* gene in inducing obesity is supported by the loss of central BMP-8B responsein mice carrying a single missense mutation in *Bbs1* gene [Rial-Pensado et al., 2022]. Other hypothesis of the development of obesity in BBSis that of impaired membrane expression of the leptin receptor (LepRB) in the hypothalamic cell population. Leptin, a polypeptide hormone, binding to its receptor in the brain, decreases food intake and increases energy expenditure. Genetic alterations of LepRB will promote signaling pathway associated with the development of obesity [Guo et al., 2016; Rouabhi et al., 2021].

Table I.14. Prevalence of Overweight and Obesity

Age	Normal Weight (No/ %)	Overweight (No/ %)	Obesity (No/ %)
<2 years old	53 (44.5%)	39 (32.8%)	27 (22.7%)
2–5 years old	42 (21.4%)	36 (18.4%)	118 (60.2%)
6–11 years old	13 (5.9%)	24 (10.9%)	184 (83.3%)
12–19 years old	17 (8.2%)	31 (14.9%)	160 (76.9%)

Note: Data from Pomeroy et al. [Pomeroy et al., 2021].

Postaxial polydactyly is frequently reported in 79% of patients, with polydactyly of toes being more common than the polydactyly of fingers [Niederlova et al., 2019]. It can affect both hands and feet, only the feet and, less often, only the hands [Reiter, Leroux, 2017; Carey et al., 2020]. The *LZTFL1* gene, which is a negative regulator for the ciliary trafficking mediated by BBSome and the Shh (Sonic hedgehog) signaling, was associated with mesoaxial polydactyly [Schaefer et al., 2014]. It should be noted that brachydactyly and/or syndactyly, with or without polydactyly, are considered minor features of BBS [Forsyth, Gunay-Aygun, 2003].

Cognitive impairment is present in 66% cases with BBS [Niederlova et al., 2019]. Kerr et al. showed that most of the patients have difficulties in perceptual intellectual abilities, auditory attentional capacities, adaptive independence, behaviour. Thus, impairments in verbal fluency are present in 22–40% of the patients, abnormal attention capacity in 69% and severe impairment in functional independence in 74% of the patients [Kerr, Bhan, Heon, 2016]. The connection between BBS proteins and cognitive impairments remains unknown [Niederlova et al., 2019].

Hypogonadism and genitourinary malformations have an incidence of 59% [Niederlova et al., 2019]. Hypogonadism may be apparent at puberty. Male anomalies can vary from micropenis, small-volume testes, cryptorchidism to hypogonadotropic hypogonadism [Mujahid et al., 2018]. Female anatomic anomalies are uterine, fallopian, ovarian or vaginal hypoplasia/atresia, hydrocolpos/hydrometrocolpos, persistent urogenital sinus, and vesicovaginal fistula [Niederlova et al., 2019; Deveault et al., 2011]. Polycystic ovary syndrome has been rarely reported [Mujahid et al., 2018]. The rate of fertility is low, but both sexes can have biological children [Forsythe, Beales, 2013].

Hormone testing revealed hypogonadotropic hypogonadism in some patients, while hypogonadism due to testicular origin was reported in others [Guran et al., 2011; Leroith et al., 1980; Mozaffarian, Nakhjavani, Farrahi, 1979; Green et al., 1989].

Some studies describe a 63% frequency of pituitary abnormalities visible by MRI (pituitary hypoplasia, empty sella, Rathke's cyst, enlarged pituitary gland) accompanied by

hormonal imbalances in 45% of the cases (hyperprolactinemia, low FSH/LH and growth hormone deficiency) [Guran et al., 2011].

The control of the GnRH release by kisspeptin signalling is reported to be involved in the gonadotropic axis activity during foetal life period, the pubertal onset and in maintaining fertility in adults [Uenoyama et al., 2016; Silveira, Tusset, Latronico, 2010]. Congenital ciliary cell dysfunction in BBS may explain the decreased hypothalamic kisspeptin receptor KISS1R with secondary hypogonadism [Uenoyama et al., 2016; Koemeter-Cox et al., 2014]. This appears to be reversible in many cases, supporting the hypothesis that, at puberty, GnRH neurons increase the number of expressed cilia and, thus, could lead to normal development of the gonadotrophic axis in adults [Desai A, et al., 2009; Koscinski et al., 2020]. It should not be overlooked that cryptorchidism and obesity can both impact on gonadic function with the decrease of the ratio testosterone/LH [Koscinski et al., 2020].

Mujahid et al. reported low SHBG in most of the patients with hypogonadism [Mujahid et al., 2018]. Decreased SHBG levels are reported in obesity, glucocorticoid use, insulin resistance, type II diabetes mellitus and hypothyroidism and, thus, we consider that low SHBG in BBS patient could be interpreted in connection with obesity status [Le et al., 2012; Dumoulin et al., 1995; Wallace et al., 2013; Giagulli, Kaufman, Vermeulen, 1994].

Other mechanism of infertility in men with BBS appears to be due to inappropriate conditions for spermatogenesis (short scrotum and obesity which disturb scrotal thermoregulation), unilateral agenesis of a seminal vesicle and partial obstruction of the genital duct by cysts [Koscinski et al., 2020; Mieusset et al., 1993]. However, in adult men, sperm structure does not seem to be affected by primary cilia disfunction in BBS [Koscinski et al., 2020].

Kidney disease is present in 52% of BBS patients [Niederlova et al., 2019]. The spectrum of kidney disease varies from urinary tract malformations (vesicoureteral reflux, hydronephrosis, dysplastic cystic disease, absent, duplex, horseshoe or ectopic kidneys, neurogenic bladder) to chronic glomerulonephritis and defective tubular concentrating ability [Beales et al., 1999]. Other reported anomalies are glomerulosclerosis and tubulo-interstitial fibrosis, glomerular basement membrane disease, mesangial proliferation and sclerosis. Urinary concentration defects (polyuria and polydipsia) caused by reduced concentrating ability due to reduced responsiveness to vasopressin with diabetes-like syndrome, kidney failure, renal anemia, hypertension can be detected.

In a foetus/infant with a structural kidney disease or genitourinary malformations and/or other features (postaxial polydactyly) BBS must be suspected. Prenatal ultrasonography is useful in detection of renal cysts, but in 39% of the patients the results of the foetal ultrasonography are normal and the renal cysts are detected after birth [Mary et al., 2019].

The main consequence is chronic kidney disease (CKD), which contributes to morbidity and mortality in patients with BBS. The majority of cases with renal disease is completely diagnosed at the age of five, but some features could be discovered in the first year of life [Forsythe et al., 2016].

Meyer et al. reported some genotype-phenotype correlations in BBS associated with kidney disease. The severe kidney disease is more often associated with pathogenesis variants in *BBS10* gene (predominantly truncating variants) and *BBS1* gene (predominantly missense variants). The patients with BBS determined by truncating variants in other genes (not only *BBS10*) have an increased risk for kidney failure. The author showed that truncating variants in *SDCCAG8* gene has a highly predictive risk for early onset of kidney failure. Other genes associated with early onset of kidney failure were *IFT172, SDCCAG8* [Meyer et al., 2022].

However, some patients with structural renal anomalies do not develop functional renal disease [Forsythe et al., 2015].

Urological anomalies included urinary incontinence, vesicoureteral reflux, neuropathic bladder and bladder outflow obstructions.

Minor Features

Minor features are represented by various anomalies in different systems and organs: the nervous system, sensorial changes, cardiovascular system, gastrointestinal changes, different endocrine glands, and cutaneous or musculoskeletal changes [Forsythe, Beales, 2013].

Neurodevelopmental abnormalities observed in patients with BBS include ataxia and poor coordination with mild hypertonia of all four extremities, seizures, speech abnormalities, behavioral, and psychiatric abnormalities [Forsythe, Beales, 2013].

The dysmorphism in BBS is not specific, and the most frequent reported features are: a narrow forehead, brachycephaly or macrocephaly, large ears, short, narrow, and downslanted palpebral fissures, deep and widely set eyes, malar flattening, depressed nasal bridge, long and smooth philtrum, retrognathia [Forsythe, Beales, 2013].

Anosmia/hyposmia are related to defects in olfactory cilia or the olfactory bulb [Braun et al., 2016]. Oral/dental abnormalities (50%), such as hypodontia or microdontia, high-arched palate, enamel hypoplasia, posterior crossbite, dental crowding, short roots, and taurodontism are reported [Forsythe, Beales, 2013].

Cardiovascular anomalies are relatively frequent (up to 29% of individuals) and are represented by atrioventricular septal defects, bilateral persistent superior vena cava, interrupted inferior vena cava, and hemiazygos vein continuation. Moreover, there are reports of laterality defects, such as situs inversus totalis, midline abdominal organs, asplenia, or polysplenia [Niederlova et al., 2019]. In BBS, laterality defects have a prevalence 170 times higher than in general population, but the prevalence of dextrocardia and situs inversus totalis in patients with BBS is low (1.6%) [Carey et al., 2020; Forsyth, Gunay-Aygun, 2003; Olson et al., 2019].

Gastrointestinal abnormalities are represented by Hirschsprung disease (2.8%), anatomic anomalies of the gastrointestinal tract (bifid epiglottis, laryngeal, and esophageal webs, bowel atresia, imperforate anus) inflammatory bowel disease (1.1%), celiac disease (1.5%), and liver disease (up to 30% of the patients) [Forsyth, Gunay-Aygun, 2020].

Endocrine/metabolic abnormalities include metabolic syndrome (54.3%), obesity, hyperlipidemia (usually hypertriglyceridemia), insulin resistance, and elevated fasting plasma glucose with or without type 2 diabetes mellitus (15.8%), polycystic ovarian syndrome (14.7%), and subclinical hypothyroidism (19.4%). Subclinical hypothyroidism is a mild form of hypothyroidism characterized by peripheral thyroid hormone levels within normal reference laboratory range, but serum thyroid-stimulating hormone (TSH) levels are mildly elevated. Hashimoto's thyroiditis is the most common form of subclinical hypothyroidism and is more prevalent in BBS patients compared to the general population [Mujahid et al., 2018; Forsyth, Gunay-Aygun, 2020; Tsyklauri et al., 2021].

In addition, other features were reported: *cutaneous dermatoses* (seborrheic dermatitis, keratosis pilaris, striae, hidradenitis suppurativa, acanthosis nigricans), *subclinical sensorineural hearing loss*, *musculoskeletal abnormalities* (scoliosis, leg length discrepancy, club foot, Blount disease, and joint laxity) [Forsythe, Beales, 2013; Forsyth, Gunay-Aygun, 2020].

The clinical diagnosis of BBS is made in the presence of either four major features or three major features and two minor features [Forsythe, Beales, 2013]. To determine the genetic cause in BBS, gene-targeted testing (multigene panel) or comprehensive genomic testing (exome sequencing) is recommended. BBS-specific panels or larger ciliopathy genes panels can be used. In general, single-gene testing by sequence analysis and deletion/duplication analysis is not recommended [Forsyth, Gunay-Aygun, 2020]. This

genetic testing approach is based on the fact that BBS is characterized by locus and allelic heterogeneity, and because of the clinical overlap between ciliopathies [Zaki et al., 2011].

3.2.3. Determinants of Clinical Effects

3.2.3.1. Locus Heterogeneity

In Table I.15., is a summary of data concerning the genes involved in BBS in correlation with tissue specificity [Stenson, 2017; HGNC; Proteinatlas; UniProt].

Table I.15. Genes and proteins in Bardet–Biedl Syndrome: characteristics and locations [Stenson 2017; HGNC; Proteinatlas; UniProt]**.**

No	*Gene symbol*	*Gene name*	*Gene groups*	*Chromosome*	*Protein*	*Localisation*
1	*BBS1*	Bardet-Biedl syndrome 1	BBSome	11q13.2	Bardet-Biedl syndrome 1 protein	Basal Body, Cilium
2	*BBS2*	Bardet-Biedl syndrome 2	BBSome	16q13	Bardet-Biedl syndrome 2 protein	Basal Body, Cilium
3	*ARL6*	ADP ribosylation factor like GTPase 6	ARF GTPase family	3q11.2	ADP-ribosylation factor-like protein 6	Basal Body, Cilium, Cytosol, Transition Zone
4	*BBS4*	Bardet-Biedl syndrome 4	Tetratricopeptide repeat domain containing BBSome	15q24.1	Bardet-Biedl syndrome 4 protein	Basal Body, Cilium
5	*BBS5*	Bardet-Biedl syndrome 5	BBSome	2q31.1	Bardet-Biedl syndrome 5 protein	Basal Body
6	*MKKS*	MKKS centrosomal shuttling protein	Chaperonins	20p12.2	McKusick-Kaufman/Bardet-Biedl syndromes putative chaperonin	Basal Body, Cytosol
7	*BBS7*	Bardet-Biedl syndrome 7	BBSome	4q27	Bardet-Biedl syndrome 7 protein	Basal Body, Cilium
8	*TTC8*	tetratricopeptide repeat domain 8	Tetratricopeptide repeat domain containing BBSome	14q31.3	Tetratricopeptide repeat protein 8	Basal Body, Cilium, IFT
9	*BBS9*	Bardet-Biedl syndrome 9	BBSome	7p14.3	Protein PTHB1	Cilium
10	*BBS10*	Bardet-Biedl syndrome 10	Chaperonins	12q21.2	Bardet-Biedl syndrome 10 protein	Basal body
11	*TRIM32*	tripartite motif containing 32	Tripartite motif containing Ring finger proteins	9q33.1	E3 ubiquitin-protein ligase TRIM32	Intermediate filaments
12	*BBS12*	Bardet-Biedl syndrome 12	Chaperonins	4q27	Bardet-Biedl syndrome 12 protein	Basal body
13	*MKS1*	MKS transition zone complexsubunit 1	B9 domain containing MKS complex	17q22	Meckel syndrome type 1 protein	Basal Body

No	Gene symbol	Gene name	Gene groups	Chromosome	Protein	Localisation
14	*CEP290*	centrosomal protein 290	MKS complex	12q21.32	Centrosomal protein of 290 kDa (Cep290)	Basal Body, Centrosome
15	*WDPCP*	WD repeat containing planar cell polarity effector	Ciliogenesis and planar polarity effector complex subunits	2p15	WD repeat-containing and planar cell polarity effector protein fritz homolog (hFRTZ)	Cytosol, Plasma Membrane, Axoneme
16	*SDCCAG8*	SHH signaling and ciliogenesis regulator SDCCAG8	MicroRNA protein coding host genes	1q43-q44	Serologically defined colon cancer antigen 8	Basal Body, Centriole, Transition Zone
17	*LZTFL1*	leucine zipper transcription factor like 1		3p21.31	Leucine zipper transcription factor-like protein 1	Basal Body, Cilium
18	*BBIP1*	BBSome interacting protein 1	BBSome	10q25.2	BBSome-interacting protein 1	cytoplasm, cytosol
19	*IFT27*	intraflagellar transport 27	IFT-B1 complex RAB, member RAS oncogene GTPases	22q12.3	Intraflagellar transport protein 27 homolog	Basal Body, Cilium, IFT
20	*IFT74*	intraflagellar transport 74	IFT-B1 complex	9p21.2	Intraflagellar transport protein 74 homolog	Basal Body, Cilium, IFT
21	*C8orf37*	chromosome 8 open reading frame 37		8q22.1	Protein C8orf37	Basal Body, Ciliary Root
22	*CCDC28B*	coiled-coil domain containing 28B		1p35.2	Coiled-coil domain-containing protein 28B	centrosome
23	*NPHP1*	nephrocystin 1	NPHP complex	2q13	Nephrocystin-1	Transition zone
24	*SCAPER*	S-phase cyclin A associated protein in the ER	Zinc fingers C2H2-type	15q24.3	S phase cyclin A-associated protein in the endoplasmic reticulum (S phase cyclin A-associated protein in the ER)	Endoplasmic reticulum

There are three categories of gene effects: primary effect (at the molecular level—protein), secondary effect (at the cellular level), and multiple tertiary effects (pleiotropic)—at the phenotypic level of organ/organism (signs and symptoms) [Covic, 2017]. Genes are expressed in all tissues, but there are differences in expression levels.

The majority of genes involved in BBS presents a low tissue specificity. However, the *LZTFL1* gene is mainly expressed in lymphoid tissue while the *BBIP1* gene is specific to testis and the *NPHP1* gene has an expression in skeletal muscle. For single cell type specificity, a cell type enhanced expression has been described in: ciliated cells (*BBS1*, *BBS2*, *ARL6*, *BBS4*, *WDPCP*, *LZTL1*, *IFT27*, *IFT74*, *NPHP1*), rod photoreceptor cells (*BBS1*, *ARL6*, *BBS4*, *BBS5*,

BBS7, TTC8, BBS9, BBS12, CEP290, NPHP1, SCAPER), cone photoreceptor cells (*BBS1, ARL6, BBS4, BBS5, TTC8, BBS9, SCAPER*), spermatocytes (*ARL6, LZTFL1, BBIP1, IFT74, NPHP1*), early spermatids (*BBS5, BBS12, BBIP1, NPHP1*), late spermatids (*BBIP1*), alveolar cells type 1 (*WDPCP*), alveolar cells type 2 (*WDPCP*), club cells (*WDPCP*) [Proteinatlas].
Some BBS forms are considered chaperonopathies because three genes involved in BBS encode chaperon-like proteins. The three chaperonins-like proteins—MKKS, BBS12, BBS7—are involved in assembling BBSome, a multistep process [Alvarez-Satta, Cástro-Sánchez, Valverde, 2017; Billingsley et al., 2010; Deveault et al., 2011]. Chaperonin like BBS proteins are involved in the first stage of assembly of the BBSome and mutations in genes that encode these proteins block the formation of functional complexes [Alvarez-Satta, Cástro-Sánchez, Valverde, 2017]. BBS caused by mutations in chaperonin genes has more severe forms characterized by an earlier onset (especially *BBS10*), higher prevalence of primary diagnostic signs and some borderline signs with other ciliopathies, such as McKusick–Kaufman syndrome (MKKS) and Alström syndrome [Alvarez-Satta, Cástro-Sánchez, Valverde, 2017; Billingsley et al., 2010; Imhoff et al., 2010]. This increased severity could be linked to residual activity/function gain of BBSome genes [Alvarez-Satta, Cástro-Sánchez, Valverde, 2017; Zhang et al., 2012].

Chaperonin-like BBS genes are characterized by a small number of coding exons (one to four) and, thus, a mutational screening of these genes could be applied before a more complex analysis [Alvarez-Satta, Cástro-Sánchez, Valverde, 2017]. Mutations in *MKKS* gene are found in 3–5% of families in which there are disease- causing mutations (Bardet–Biedl Syndrome or McKusick-Kaufman syndrome) [Deveault et al., 2011; Muller et al., 2010]. Mutations in *BBS10* gene represent about 20% of all cases of BBS with certain ethnic variations: 43% in a Danish group and 8.3% in a Spanish cohort [Hjortshøj et al., 2010; Alvarez-Satta et al., 2014].

3.2.3.2. Mutational Heterogeneity

Mutational heterogeneity is important to choose testing strategies. According to the Human Gene Mutation Database Professional (HGMD®) 2020.1 database (accessed in April 2021), 647 pathogenic variants are described. Figures I.25. and I.26. show the implication of different types of genetic modifications in genes of BBSome, respectively the other genes implied in BBS.

The gene of BBSome could present different types of mutations like: missense/nonsense, splicing substitutions, small deletions, gross deletions, small insertions/duplications, small indels, gross insertions/duplications, complex rearrangement (Figure I.26.). For the other genes implied in BBS the most frequent type of mutations is: missense/nonsense, small deletions, and small insertions/duplications (Figure I.27.) [Wheway, Mitchison, 2019; Davis et al., 2011; Lindstrand et al., 2016]. Some BBS forms are produced by mutations with loss-of-function (LOF): nonsense, frameshift, copy number variants, and splicing variants [Pomeroy et al., 2021].

BBSome is a multisubunit complex with eight proteins coding by the genes *BBS1, BBS2, BBS4, BBS5, BBS7, TTC8, BBS9* and *BBIP1* [Khan et al., 2016; HGNC]. The most common mutations are in *BBS1* gene (responsible for 23% of BBS) and *BBS10* gene (identified in 20% of patients with BBS) [Forsythe, Beales, 2013]. Founder mutations have also been described: M390R in *BBS1* gene and C91fsX95 in *BBS10* gene [Khan et al., 2016]. *MKKS* gene is required for BBSome assembly, while *TTC8* gene is required for ciliary trafficking [Nachury et al., 2007; Jin, Nachury, 2009; Seo et al., 2010]. Mutations in the first 18 genes listed in the table I.15. occur in 70–80% of BBS affected families [M'Hamdi et al., 2013]. The *BBS1* and *BBS10* genes are the most common mutated genes in Europe and North America [Forsythe et al., 2014].

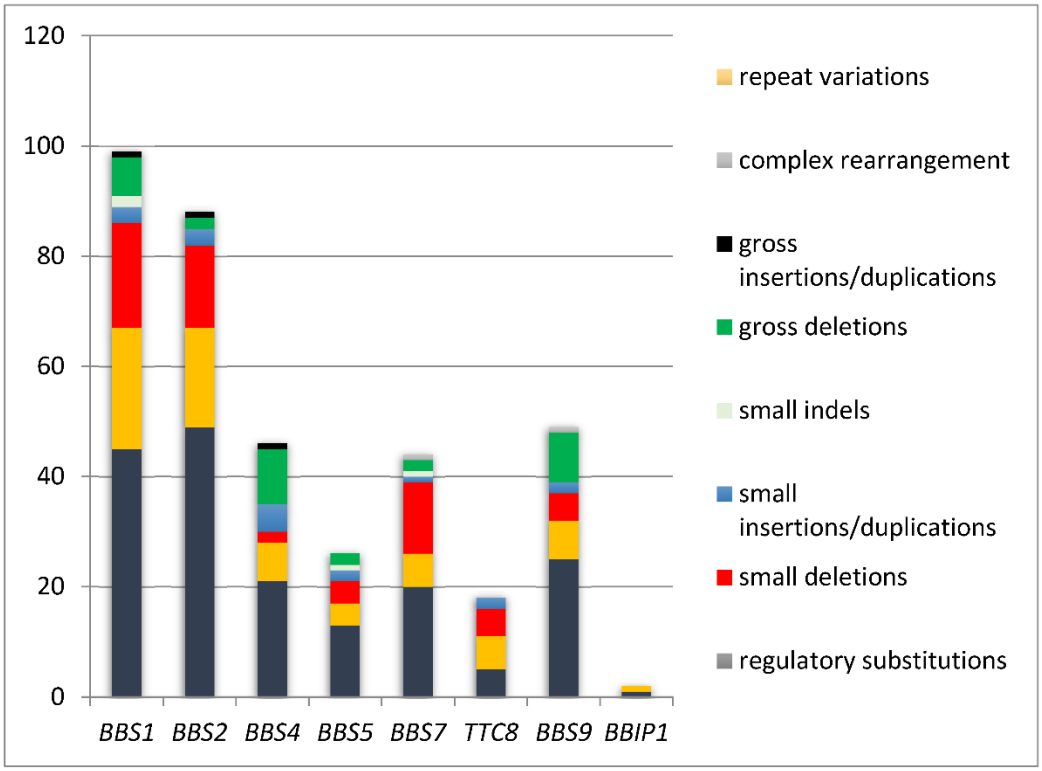


Figure I.25. Pathogenic variants in BBSome [Stenson et al., 2017]**.**
BBS1: Bardet-Biedl syndrome 1; *BBS2*: Bardet-Biedl syndrome 2; *BBS4*: Bardet-Biedl syndrome 4; *BBS5*: Bardet-Biedl syndrome 5; *BBS7*: Bardet-Biedl syndrome 7; *TTC8*: tetratricopeptide repeat domain 8; *BBS9*: Bardet-Biedl syndrome 9; *BBIP1*: BBSome interacting protein 1.

3.2.3.3. Modifiers

The phenotype of BBS can be changed by modifier genes. The PhenoModifier database contains 12 modifying variants described in 6 genes (*TMEM67, MKS1, MKKS, CCDC28B, C8orf37, BBS1*). Modifying genes influence expressivity or pleiotropy [PhenoModifier]. The pleiotropy is modified by some variants of *MKS1* gene: MKS1:c.1112_1114del (p.Phe371del), MKS1:c.1476T>G (p.Cys492Trp), MKS1:c.248A>G (p.Asp83Gly), MKS1:c.368G>A (p.Arg123Gln), MKS1:p.Ile450Thr in association with mutations in *BBS1* and *BBS10* genes [PhenoModifier; Leitch et al., 2008]. All of these patients had seizures, and non-specific sign for BBS or MKS (Meckel–Gruber Syndrome). Therefore, the interaction between the products of the two genes could explain this pleiotropic effect [Leitch et al., 2008].

With an effect on expressivity we mention variants in *TMEM67, MKKS, CCDC28B, C8orf37, BBS1* genes [PhenoModifier; Bölükbaşı et al., 2018; Badano et al., 2006; Badano et al., 2003]. For example, Bölükbaşı et al. found that most severe phenotype of BBS was allowed by following changes: C8orf37:c.533C>T (p.Ala178Val), CCDC28B:c.330C>T (p.Phe110Phe), MKKS:c.1015A>G (p.Ile339Val), and TMEM67:p.Asp799Asp [Bölükbaşı et al., 2018]. Badano et al. showed that CCDC28B: c.430C/T variant (in heterozygote state) determines the introduction of a premature termination codon and the reduction of CCDC28B messenger RNA levels [Badano et al., 2006]. Badano et al. have identified another mutant allele (BBS1: IVS115 + 2T→C) which is correlated with a more severe phenotype in a patient with homozygous mutation in *BBS2* gene [Badano et al., 2003].

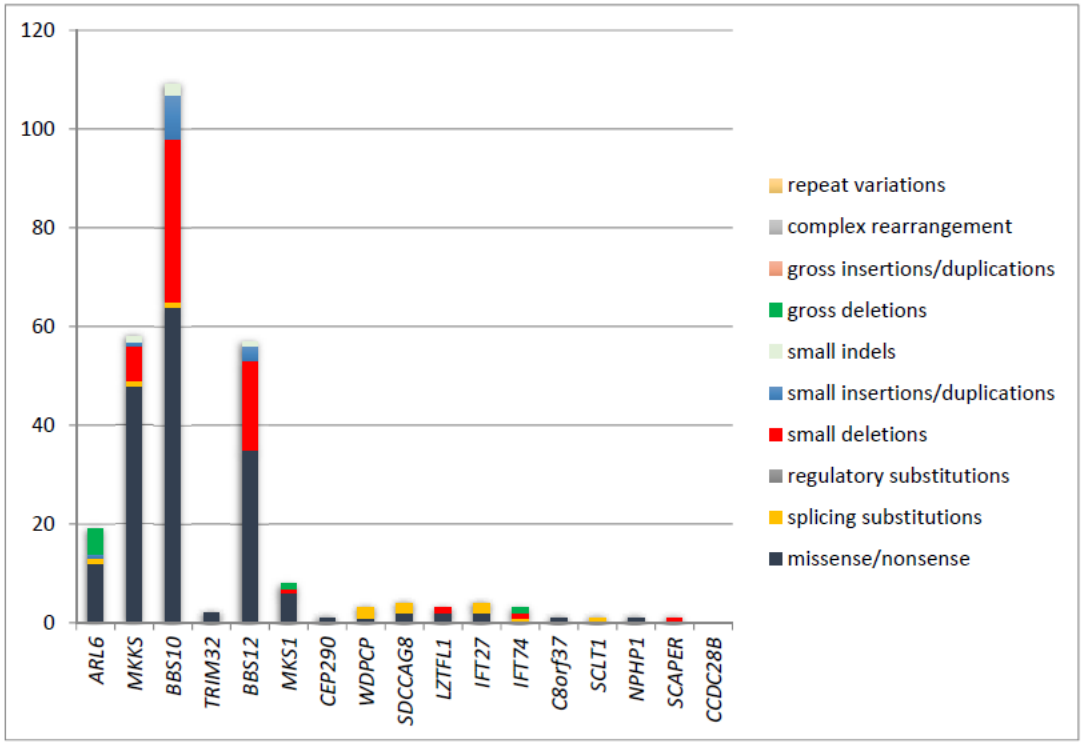


Figure I.26. Pathogenic variants in other genes of BBS [Stenson et al., 2017]**.**
ARL6: ADP ribosylation factor like GTPase 6; *MKKS*: MKKS centrosomal shuttling protein; *BBS10*: Bardet-Biedl syndrome 10; *TRIM32*: tripartite motif containing 32; *BBS12*: Bardet- Biedl syndrome 12; *MKS1*: MKS transition zone complex subunit 1; *CEP290*: centrosomal protein 290; *WDPCP*: WD repeat containing planar cell polarity effector; *SDCCAG8*: SHH signaling and ciliogenesis regulator SDCCAG8; *LZTFL1*: leucine zipper transcription factor like 1; *IFT27*: intraflagellar transport 27; *IFT74*: intraflagellar transport 74; *C8orf37*: chromosome 8 open reading frame 37; *SCLT1*: sodium channel and clathrin linker 1; *NPHP1*: nephrocystin 1; *SCAPER:* S-phase cyclin A associated protein in the ER

3.2.3.4.Disease Protein–Non-Disease Protein Interconnectivity

There is functional interaction between complexes, which explains locus heterogeneity [Mitchison, Valente, 2017; Lee, Chung, 2015]. In an analysis conducted by Keith et al. in 2014, the authors showed the existence of multiple interconnectivity networks [Keith, Robertson, Hentges, 2014]. These relationships involve both the disease protein-disease protein connection, but also the disease protein–non disease protein. In the latter case, it appears that there are proteins which are related to more than one of the proteins encoded by the genes involved in BBS syndrome [Keith, Robertson, Hentges, 2014]. Examples of such proteins are: ALDOB (fructose-bisphosphate aldolase B) (in interaction with products of genes *BBS1*, *BBS2*, *BBS4*, *BBS7*), HSCB (Iron-sulfur cluster cochaperone protein HscB) (in interaction with products of genes *BBS2*, *BBS4*, *BBS1*), ACY1 (Aminoacylase-1) (in interaction with products of genes *BBS2*, *BBS4*, *BBS7*), EXOC7 (Exocyst complex component 7) (in interaction with products of genes *BBS1*, *BBS7*, *BBS4*, *BBS2*), RAB3IP (Rab-3A-interacting protein) (in interaction with products of genes *BBS1*, *BBS4*), FHOD1 (FH1/FH2 domain-containing protein 1) (in interaction with products of genes *BBS1*, *BBS2*, *BBS4*, *BBS7*), PCM1 (Pericentriolar material 1 protein) in interaction with products of genes *BBS1*, *BBS2*, *BBS4*, *BBIP1*), CCDC28B (Coiled-coil domain-containing protein 28B) (in interaction with products of genes *MKKS*, *TTC8*, *BBS2*, *BBS4*, *BBS7*, *BBS1*, *BBS5*) [Keith, Robertson, Hentges, 2014].

Keith postulated that there is an interconnectivity between proteins in the case of locus heterogeneity. Thus, the BBS proteins in the vicinity of proteins involved in locus heterogeneity could be involved in the pathogenesis of similar diseases or may themselves be causes of disease [Keith, Robertson, Hentges, 2014].

The cellular signaling pathways involved in BBS are: hedgehog, Wnt (wingless-related integration site), GPCR (G protein-coupled receptors), mTOR (mammalian target of rapamycin) [Wheway, Nazlamova, Hancock, 2018; Singla, 2006].

The primary cilium is involved in Hedgehog signaling pathway and can act as both positive and negative regulator of this pathway [Wheway, 2018]. Defects in cilia / intraflagellar transport leads to loss of Hh phenotype function in the neural tube and gain of function in the limbs. This explains the common Hh phenotype (especially polydactyly) present in ciliopathies, including BBS [Wheway, Nazlamova, Hancock, 2018; Waters 2011]. Several BBS-associated proteins intervene in the Wnt signaling pathway and play a role in regulating Wnt signaling by degrading Wnt effectors [Wheway, Nazlamova, Hancock, 2018; Wiens et al., 2010]. Disheveled is an essential protein in basal body docking, ciliogenesis and planar cell polarity. Meckelin (TMEM67), TMEM216, MKS1, BBS10, and BBS12 also intervene in the same processes [Wheway, Nazlamova, Hancock, 2018; Jin, Nachury, 2009; Park et al., 2008; Adams et al., 2011]. The lack of these proteins leads to planar cell polarity defects also found in BBS [Wheway, Nazlamova, Hancock, 2018].

G-protein coupled receptors (GPCRs) are important for cilia structures and function. Neuronal cilia integrity is required for brain development and the adequate interaction in the adult brain [Wheway, Nazlamova, Hancock, 2018]. Neuronal cilium formation begins prenatally with pro-cilium formation and continues in the first 8–12 weeks after birth. During postnatal development at the level of the primary neuronal membrane cilium GPCRs appear: somatostatin receptor 3 (SSTR3), melanin-concentrating hormone receptor 1 (MCHR1), serotonin receptor 6 (5HTR6), kisspeptin 1 receptor (KISS1R), dopamine receptors 1, 2, and 5 (D1, D2, and D5), neuropeptide Y receptors, NPY2R and NPY5R. The location of these receptors is different being consistent with the neuronal type. The length of the cilium is important for proper function. For example, the shortening of the cilia of hypothalamic neurons has been correlated with obesity in mice induced by high-fat diet, so that the dysfunction of neural cilia is correlated with childhood obesity in BBS [Wheway, Nazlamova, Hancock, 2018; Han et al., 2014; Mariman et al., 2016]. GPCRs bind to b-arrestin2 and BBS proteins associated with Arl6 and thus trigger ciliary trafficking. When some of these receptors are missing or lacking motifs of recognition by BBSome and b-arrest, an accumulation of receptors and their ectocytosis occurs [Nager et al., 2017]. Rhodopsin and opsin are other examples of GPCRs in rods and cones. Their role is to absorb light and transmit the electrical signal to the brain. If the integrity of the cilia is not adequate, retinal degeneration will occur.

Mammalian target of rapamycin is also involved in BBS. CCDC28B interacts with the SN1 subunit of the mammalian target of rapamycin complex 2 (mTORC2). In this way it regulates the length of the cilia by affecting its assembly/stability [Cardenas-Rodriguez, Badano, 2009].

3.2.3.5. Disease Protein-Disease Protein Interconnectivity

The eight subunits of BBSome are assembled in two parts: head and body. The head is formed by BBS2 which interacts with BBS7. The body is formed by the others proteins (BBS1, BBS4, BBS5, TTC8, BBS9, BBIP1). The BBIP1 is the central part of the BBSome core [Nager et al., 2017; Lorentzen, Kikkawa, 2019]. BBIP1 interacts with BBS4 and TTC8 and thus achieves proper assembly and structural stability of the BBSome complex [Klink et al., 2020]. BBS4 and TTC8 have TPR (tetratricopeptide repeats) subunits. They interact with

the domain β propeller of BBS1 and BBS9, respectively [Klink et al., 2020]. These interactions are essential for a functional BBSome and explain why mutations at the BBS4-BBS1 and BBS8-BBS9 interface leads to disease in patients [Klink et al., 2020].

In a study that analyzed the effects of the proteome of the three modules (BBSome, chaperonin complex, transition zone) and three more genes that are not yet included in a module (*ARL6, TRIM32, WDPCP*) six epistatic interaction effects were identified: *BBS10-BBS5; BBS10-BBS12; BBS10-BBS1; BBS10-MKKS; BBS10-BBS4; BBS12-MKKS*. Moreover, 12 additive interaction effects were found between the main complexes (BBSome, chaperonin and transition zone) and 1 between proteins of transition zone and others gene *BBS1-BBS5, BBS1-BBS2, BBS1-NPHP1, BBS10-NPHP1, BBS2-NPHP1, BBS9- NPHP1, BBS7- NPHP1, BBS2-BBS4, BBS2-MKKS, BBS4-MKKS, BBS4-BBS9, BBS4-BBS7*. On the other hand, no interaction effect was demonstrated in the following genes (nor additive or epistatic effect): *TRIM32, WDPCP, CEP290, MKS1* [Kousi et al., 2020].

3.3. Study of Bardet-Biedl Syndrome

3.3.1. Introduction

Bardet-Biedl Syndrome is characterized by a core of features consisting in: rod-cone dystrophy, postaxial polydactyly, central obesity, urogenital anomalies, learning difficulties and kidney disease. Additional clinical findings: neurodevelopmental and behavioral abnormalities, liver involvement, endocrine and metabolic abnormalities, oral-dental anomalies, craniofacial dysmorphic features, cardiovascular impairment, and hearing loss, complete the clinical pictures [Forsyth, Gunay-Aygun, 2020]. Considering the remarkable heterogeneity, the clinical diagnosis is not easy to establish, however, a clinical diagnostic criterion has been proposed by the presence of four major findings or by combination of tree major features with two minor symptoms (Figure I.25.) [Beales et al., 1999]. Nevertheless, a recent metanalysis suggested that not all patients, molecularly diagnosed with BBS, fulfilled the consensus criteria for clinical diagnosis [Niderlova et al., 2019].

3.3.2. Materials and methods

We are analyzing the clinical aspects of 25 patients diagnosed with BBS. This research is the first study on a cohort of Romanian BBS patients. The main goal was to raise the awareness of medical community regarding the complexity of this disease and to highlight the importance of an early diagnosis that may be essential for the management of the patients.

Our research study focused on clinical aspects of patients with BBS. The study was approved by Institutional Review Board of University of Medicine and Pharmacy "Carol Davila" Bucharest, with respect of Declaration of Helsinki. Written informed consent was obtained from the patients and from their legal guardian.

Diagnosis was made according with consensus criteria established by Beales et al. by the presence of four major features or three major features and two secondary features (Figure I.25.) [Beales et al., 1999]. The patients were fully evaluated by multidisciplinary team, including a pediatrician, child psychiatrist, child neurologist, psychologist, ophthalmologist, endocrinologist, nephrologist and clinical geneticist. Clinical examination, medical history, dysmorphology examination, physical measurements, imagistic studies and blood tests screening, were performed.

3.3.3. Results and discussion

25 patients, 16 females and 9 males were recruited, of which two are siblings. The age at the time of enrolment varied between 2 months and 43 years. 76% of patients were under

18 years old. Most patients had the age between 1 and 10 years, 76% respectively. 8% were under 1 year old, 8% were between 30 and 40 years old and 4% were over 40 years.

The **age of the clinical diagnosis** was, in the majority of cases, in the first year of life, which is lower comparing with other studies. Previous research indicated that the age at diagnosis varied between 5 and 10 years, age at which rod cone dystrophy became symptomatic [Forsythe et al., 2018]. However, suggestion that BBS could be suspicioned even antenatally or immediately after birth were based on the presence of polydactyly, renal or genitourinary malformation [Mary et al., 2019]. According with these reports, the suspicion of BBS diagnosis was raised antenatally in one of our cases, while in two cases the diagnosis was made shortly after birth. Other two cases were diagnosed later at 11, and 12 years, respectively.

Major clinical findings were distributed in our cohort as follows (Figure I.27):

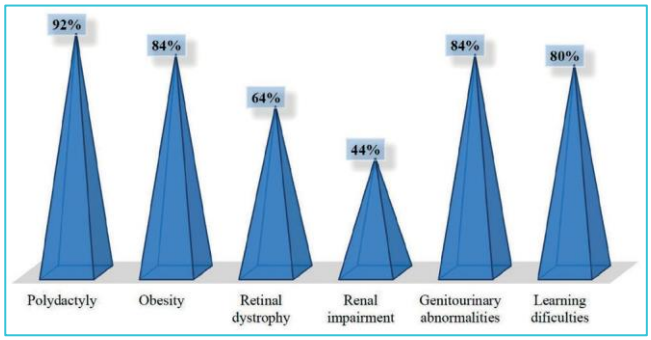


Figure I.27. Distribution of primary clinical findings in our cohort

Polydactyly were observed in 92% of the cases. The accessory digits were present in all four limbs in 52% of patients, only on the feet in 16% or only on the hands in 4%. In 4% the polydactyly was present in both hands and one foot, in 4%, extra digits were present in one hand and both feet, while 12% of cases exhibited polydactyly only in the left limbs. The higher percentage of polydactyly in our cases, comparing with previous reports (63-81%) [Forsythe, Beales, 2013; Forsyth, Gunay-Aygun, 2020; Forsythe et al., 2018] may be explained by a limited number of patients enrolled in the study, however could also suggest a lower rate of diagnosis in BBS patients without polydactyly.

Obesity was recorded in 84% of our patients, in accordance with literature (72-92%) [Beales et al., 1999; Forsythe, Beales, 2013; Forsythe et al., 2018]. Typically, the birth weight is normal and the weight gain commences in the first year of life. In our cohort, the birth weight was towards to the lower percentiles in 40% of the cases, however, within the normal range. Of these, 12% of children was on percentile 1. 28% of children had a normal birth weight. 12% of children had birth weight towards to the upper percentile, while 16% had birth weight above the upper percentile.

Retinal dystrophy was present in a lower percentage in our cohort, 64%, comparing with previous reports (93-94%) [Beales et al., 1999; Forsythe, Beales, 2013; Forsyth, Gunay-Aygun, 2020]. This inconsistency may be due to the young age of some patients in our study, or due to the small number of cases enrolled. Remarkable, the visual deficit was observed in the first year of life in two patients and in the first three years of age in other two cases, earlier than previous reports.

Genitourinary anomalies were noted in 84% of our patients, consistent with literature (59- 98%) [Forsythe, Beales, 2013; Forsyth, Gunay-Aygun, 2020; Forsythe et al., 2018]. All males had small penile size, while 31% of the females had vaginal atresia and other 37% had hypoplastic genitalia.

Renal involvement was identified in 44% of our patients in accordance with previous reports (25-53%) [Beales et al., 1999; Forsythe et al., 2018]. The most frequent anomaly was hydronephrosis seen in 20% of patients, followed by polycystic kidney in 16%. Other structural anomalies, i.e., hypoplastic or atrophic kidney, were detected in 12% of cases. Renal dysfunction affected 28% of cases of which 12% it has progressed to end stage renal disease, and 4% undergone renal transplantation.

Learning difficulties were present in a higher percentage in our patients 80% comparing with previous studies (61-66%) [Forsythe, Beales, 2013; Forsyth, Gunay-Aygun, 2020], which may be due to the small number of patients enrolled, or due to different scale used in evaluation of the patients in our cohort.

Main **secondary features** were present in our patients as following (Figure I.28.): psychomotor delay was noted in 72% of our patients, language delay was observed in 64%, while psychiatric conditions affected 28% of patients. Cardiovascular defects were present in 28% of cases, hepatic involvement in 36%, metabolic syndrome in 32%, while hypothyroidism and type 2 diabetes were remarked in 28%, respectively 24% of cases. Some of these features are consistent with previous reports, including psychomotor delay (50-81%) [Beales et al., 1999; Forsyth, Gunay-Aygun, 2020], language delay (54-81%) [Beales et al., 1999; Forsythe, Beales, 2013], cardiovascular involvement (7-29%) [Forsythe, Beales, 2013; Forsyth, Gunay-Aygun, 2020], type 2 diabetes (6-48%) [Forsythe, Beales, 2013] and hepatic involvement (30%) [Forsyth, Gunay-Aygun, 2020]. Slightly below average reported in previous studies were psychiatric abnormalities (33-35%) [Beales et al., 1999; Forsyth, Gunay-Aygun, 2020] and below average was metabolic syndrome (54%) [Forsyth, Gunay-Aygun, 2020], while hypothyroidism was much frequent in our patients comparing with literature (20%) [Forsyth, Gunay-Aygun, 2020]. These findings could be assigned to the small number of patients enrolled in the research; however, it may also suggest a hallmark of our population modulated by diet or by environmental influence.

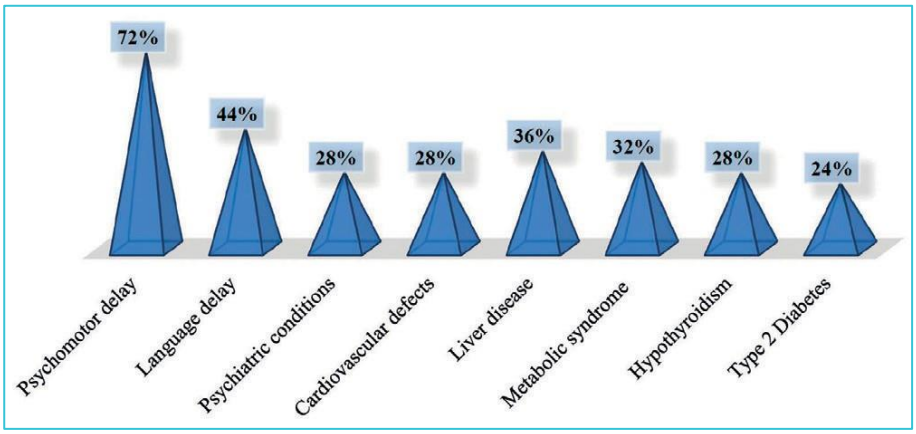


Figure I.28. Distribution of main secondary findings in our cohort

All of our patients had a multisystemic involvement. Many of they were severely affected. Our study confirms the broad clinical presentation of BBS shown in previous reports. Recognition of signs and symptoms of the disease is decisive for an early diagnosis. Thus, the presence of polydactyly in a newborn or even in a fetus, especially in association with other suggestive features, should raise the suspicion of BBS. Enrollment of the patients as soon as possible in a strict monitoring program, accordingly with international guidelines, may prevent some severe complications, which could accelerate or aggravate the most deleterious aspects of the disease such as loss of vision or renal impairment.

3.3.4. Conclusions

Our study was the first analysis of the clinical picture of BBS on a cohort of Romanian patients. BBS is a complex disease which affect the majority of systems and organs (brain, eye, heart, liver, kidney, limbs, genitalia) therefore it has a devastating impact on patients and their families. An early clinical diagnosis of these patients is crucial for anticipation of other phenotypic involvement. Periodic follow up, by a multidisciplinary team, may help in limiting the comorbidities and in improving the life quality of BBS patients.

3.4. Multidisciplinary approach in BBS

The aim of this review was to highlights the need for a multidisciplinary approach.

Both characteristics of BBS – the pleiotropy and the variable onset of the signs and symptoms - request a multidisciplinary approach (Figure I.29.). The multidisciplinary team has a dynamic component correlated with various stages of life and imply different medical specialties: paediatrics, neurology, dentistry, ophthalmology, endocrinology, ENT, genetics, cardiology, psychology, surgery, gastroenterology, nephrology.

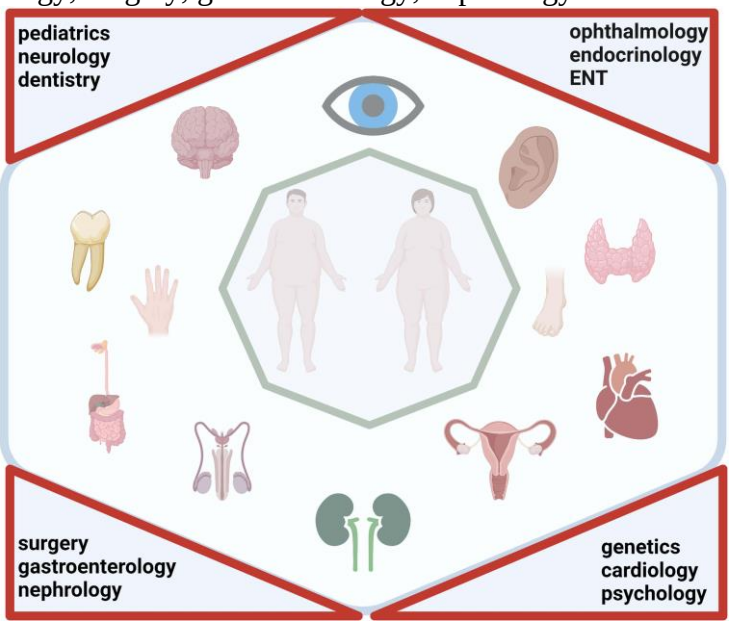


Figure I.29. Pleiotropy and multidisciplinary management in Bardet-Biedl syndrome. Created with BioRender.com.

Ocular Involvement - Rod-Cone Dystrophy

Electroretinography (ERG) is the test of choice [Forsythe et al, 2013]. Thus, early changes can be highlighted in the first two years of life, even before the development of fundus abnormalities [Scheidecker et al., 2015]. The baseline evaluation at the time of diagnosis includes: ophthalmic examination, electroretinogram, visual field testing, fundus examination, ERG and optical coherence tomography (OCT) [Forsyth, Gunay-Aygun, 2003; Bardet -Biedl syndrome guideline development group. Management of Bardet -Biedl syndrome A clinical guideline]. Fundoscopic photographs are recommended for later reference [Carey et al., 2020; Bardet -Biedl syndrome guideline development group.]. Infants and young children must be evaluated for strabismus and nystagmus, while older children and adults must be evaluated also for cataract [Forsyth, Gunay-Aygun, 2003].

The surveillance must be done every year or as directed by ophthalmologist. The follow-up must include: visual acuity, visual field testing, fundus examination, ERG and

screen for cataract, glaucoma and diabetic retinopathy [Bardet -Biedl syndrome guideline development group].

The management aims the correction of refractive errors. The tinted glasses are recommended if photophobia is present [Bardet-Biedl syndrome guideline development group.]. Educational programs must be adapted to progressive visual impairment. The needs of a blind adult in educational programming must be anticipated [Forsythe et al, 2013; Carey et al., 2020; Bardet -Biedl syndrome guideline development group.; Christian, 2007].

Polydactyly

Surgical removal of accessory digits is recommended. It is recommended an early intervention, especially for feet polydactyly.

Obesity

Baseline evaluation includes: measurements of height, weight, head and waist circumference, calculation of BMI and waist-hip ratio, dietary evaluation with caloric intake and dietary components and the assessment of daily physical activity level [Forsyth, Gunay-Aygun, 2003; Carey et al., 2020; Bardet -Biedl syndrome guideline development group]. All these parameters will be evaluated at each medical visit [Forsyth, Gunay-Aygun, 2003].

Obesity treatment is difficult for BBS patients and includes also, as for other obese patients, diet and lifestyle changes. Regular exercise program for weight control and behavioral and family therapy are recommended [Carey et al., 2020]. For those who had a body mass index (BMI)> 30 kg/m² pharmacotherapy is recommended (eg, orlistat, lorcaserin, phentermine-topiramate, naltrexone-bupropion). In patients with a BMI >40 kg/m^2 or those with BMI> 35 kg/m^2 and comorbidities bariatric surgery can be discussed [Hamlington et al., 2015, Daskalakis et al., 2010; Mujahid et al., 2014; Garvey et al., 2016].

Obesity complications are recommended to be treated as in the general population [Forsythe et al, 2013]. To anticipate these complications, there should be made annual measurements of the blood pressure, blood glucose, haemoglobin A1C levels, serum cholesterol and lipid levels.

A novelty in the therapy of the Bardet-Biedl syndrome is the administration of targeted therapies. An example is the use of melanocortin receptor agonists as a potential treatment for obesity [Seo et al., 2009]. Research of the mechanisms leading to aberrant signalling by mutant proteins is ongoing, but there is evidence that there are defects in the leptin-melanocortin hypothalamic axis occurring in the Bardet-Biedl syndrome [Mason, Page, Balikcioglu, 2014]. This leads to resistance to leptin, which generate obesity [Seo et al., 2009]. There are promising results in studies of subcutaneous injection treatment with setmelanotide, a melanocortin 4 receptor agonist, studied now in Phase 3 clinical trials [Haws et al., 2021]. The clinical group evaluated by Haws RM et al included individuals aged >6 years old, with a diagnosis of BBS or Alström syndrome, treated with randomized, double-blind, placebo-controlled therapy. They selected individuals with body mass index ≥30 kg/m^2 (aged ≥16 years old) or weight >97th percentile (aged 6–15 years old). The therapeutic regimen was different depending on the age of the patients. For patients aged >16 years old, the dose of setmelanotide was initially 2 mg/day in the first 2 weeks and 3 mg per day from the 3rd week. For patients aged between 6 and 16 years old, the following regimen was used: first week - 1 mg/ day; second week 2 mg/day; from the 3rd week on 3 mg per day. Weight changes were noted after 52 weeks of treatment. These results include, in BBS patients, weight loss and feelings of early satiety [Haws et al., 2021].

Hypogonadotropic Hypogonadism and/or Genitourinary Anomalies

Some authors recommend annual laboratory assessment for hypogonadism starting at the age of 13 years old. Other authors propose to refrain from initiating testosterone treatment in asymptomatic BBS men but, also, to continue studying this type of treatment that would improve metabolic parameters, including body composition and would increase muscle strength [Mujahid et al., 2018]. The androgen replacement therapy regimen and doses used are those recommended for the paediatric and adult population with hypogonadism [Chioma, Cappa, 2021]. General recommendations for hypogonadotropic hypogonadism treatment, both in women and men, are based on experience and current guidelines [Sanfilippo et al., 2009; Al-Sharefi, Quinton, 2020; Young et al., 2019].

Fertility is reported to be diminished for both sexes but they may have offsprings [Mujahid et al., 2018]. There are few reports of the association of BBS and endometrial carcinoma. It should be considered in women with BBS and risk factors for hyperoestrogenism (obesity, hyperinsulinemia, ovulatory dysfunction) [Grechukhina et al., 2018; Peiker, Boehm, Carol., 1978; Schwab, Kriz, 1980].

Renal Anomalies

Investigation of clinical symptoms as anemia, polyuria, and polydipsia, hypertension, urinary tract infection, renal colic, symptoms of neurogenic bladder or bladder outflow obstruction is important at each evaluation visit.

Baseline investigations and yearly follow-up include basal blood pressure assessment, 24-hour blood pressure monitoring, complete blood cell count (CBC), serum creatinine, urea, cystatin C, electrolytes, glomerular filtration rate (GFR), urinary analysis for glucose, urinary protein (early morning urine analysis for albumin/creatinine ratio), hematuria and osmolarity, bladder and renal ultrasound examination [Forsythe et al, 2013; Carey et al., 2020; Forsyth, Gunay-Aygun, 2003].

For detecting the calyceal anomalies abdominal magnetic resonance imaging can be done.

If structural or functional renal anomalies are present the patient must be referral to a nephrologist and should be monitored as directed by the nephrologist.

Another pathology rarely reported is nephrogenic diabetes insipidus (NDI), explained by the lack of renal response to desmopressin, probable due to vasopressin V2 receptor (V2R) damage [Mujahid et al., 2018]. V2R, normally activated by antidiuretic hormone (ADH), is localized in the basolateral membrane of the principal cells alongside the collecting duct (CD). Marion et al. describe that silencing *BBS10* gene in human CD cell line leads primary to the loss of the cilium, and secondary to diminishing functions of V2R [Marion et al., 2011]. Due to renal resistance to ADH, patients do not have a good response to desmopressin treatment. In patients with NDI, treatment is intended to decrease the polyuria and avoiding hypernatremia and volume depletion (low-sodium diet, drink adequate amounts of water) [Christ-Crain, Winzeler, Refardt, 2021].

In 31% of the children and 42% of the adults with BBS chronic kidney disease is present. End stage renal disease can be detected in early childhood (before the age of 5) or in adulthood related to BBS or to complications as hypertension or type 2 diabetes. 6% of the children and 8% of the adults developed end stage renal disease requiring dialysis or renal transplantation [Forsythe et al., 2017]. After a renal transplant, favorable long-term outcomes have been reported [Haws et al., 2016].

Metabolic Syndrome

Metabolic syndrome has an incidence of 54.3% in patients with BBS [Mujahid et al., 2018]. Early detection of metabolic syndrome followed by appropriate treatment is necessary

in order to prevent later in life complications such as cardiovascular disease and diabetes. The International Diabetes Federation (IDF) consensus definition of metabolic syndrome in children and adolescents (IDF) is being used. In the age group 6–10 years, the metabolic syndrome cannot be diagnosed, but weight reduction is recommended in case of abdominal obesity. In patients aged 10 years or older, metabolic syndrome is defined in the presence of abdominal obesity associated with two or more of the following features (elevated triglyceride, low HDL-cholesterol, high blood pressure, increased plasma glucose). The values used for diagnosis can be found in Table I.16 [International Diabetes federation: the IDF consensus worldwide definition on the metabolic syndrome].

Table I.16. The International Diabetes Federation Consensus Definition of Metabolic Syndrome in Children, Adolescents and Adults

Age Group	Obesity	Triglycerides	HDL-C	Blood Pressure	Glucose (mmol/L) or Known T2DM
10–16 years	≥90th percentile or adult cut-off if lower	≥1.7 mmol/L (≥150 mg/dL)	<1.03 mmol/L (<40 mg/dL)	Sistolic ≥130/ Diastolic ≥85 mmHg	≥5.6 mmol/L (100 mg/dL) (If≥5.6 mmol/L [or known T2DM] recommend an OGTT)
≥16 years	Central obesity*	≥1.7 mmol/L	<1.03mmol/L (<40 mg/dL) in males and <1.29mmol/L (<50mg/dL) in females, or specific treatment for these lipid abnormalities	Systolic >130 or diastolic >85mmHg, or treatment of previously diagnosed hypertension	Impaired fasting glycemia (IFG): fasting plasma glucose (FPG) =5.6 mmol/L (≥100 mg/dL), or previously diagnosed type 2 diabetes

Note: *Central obesity is defined as waist circumference ≥ 94 cm for European men and ≥ 80 cm for European woman; ethnicity specific values for other groups.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; T2DM, Type 2 diabetes mellitus.

Baseline evaluation consists of a lipid panel (triglycerides, HDL-C, LDL-C, total cholesterol) and fasting blood glucose and HgbA1c. These will be repeated annually from the age of 4, if they are normal. In people with metabolic syndrome, the monitoring should be more frequent [Forsyth, Gunay-Aygun, 2003].

Diabetes

Type 2 Diabetes Mellitus has an incidence of 15.8% in patients with BBS [Mujahid et al., 2018]. The diagnostic criteria established by The American Diabetes Association (ADA) are used for diagnosis [American Diabetes Association]. One of the following tests can be used:

1. Fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L) OR
2. 2-h plasma glucose (PG) ≥200 mg/dL (11.1 mmol/L) during OGTT (after age of 12 years old) OR
3. A1C ≥6.5% (48 mmol/mol) OR
4. In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L).

Baseline investigations for insulin resistance/diabetes mellitus include: fasting plasma glucose, OGTT after age of 6 years old. Fasting plasma insulin concentration and hyperinsulinemia may be present from childhood [Bardet -Biedl syndrome guideline development group].

The follow-up of the adolescent patients involves the promotion of healthy eating, physical activity according to visual impairment [Bardet -Biedl syndrome guideline

development group]. Acanthosis nigricans (35.4% of patients with BBS) is a sign of insulin resistance / diabetes mellitus [Haws et al., 2021].

Cognitive Impairment

The baseline assessment should include a full developmental and/or neurocognitive assessment. The specialists involved are: clinical psychologist, developmental paediatrician/specialist in child development, behavioural psychologist and speech therapist [Carey et al., 2020]. The methods used in the evaluation will be adapted to the visual status. Language assessment should be postponed after the age of 2 years old. In children with BBS, the intelligible speech and sentence formation can be delayed up to 4 years. Other speech anomalies are: articulation defects, nasal and/or breathy speech quality. Depending on the clinical situation, the existence of pharyngeal and/or laryngeal muscle incoordination must be considered. In this case, videofluoroscopy and palatal articulation studies must be performed [Bardet -Biedl syndrome guideline development group]. Early speech therapy should be initiated at the first signs of speech impairment.

A mental health assessment is also necessary because patients may have some psychiatric and behavioural conditions, such as: anxiety, mood disorders, depression, psychosomatic manifestations, bipolar disorder and emotional outbursts, hyperactivity, frustration, inflexibility, obsessive/compulsive tendencies, preference for fixed routines, inability to recognize social cues, inappropriate and disinhibited behaviour, shallow affect [Carey et al., 2020; Bardet -Biedl syndrome guideline development group; Barnett et al., 2002; Zelihić, Hjardemaal, von der Lippe, 2020].

Subclinical Hypothyroidism and other hormone imbalances

Subclinical hypothyroidism has been reported in BBS patients more often than expected, with an incidence of 19.4% [Mujahid et al., 2018]. The patient's hypothyroidism was treated with levothyroxine hormone replacement in order to prevent any possible risk of negative effect on growth and development [Mujahid et al., 2018].

The management of hypothyroidism in children is similar to adults, with the particularity that in children the doses are adjusted according to the child's weight (commonly for newborns 10 μg/Kg/day, 1-year-old children 4–6 μg/Kg/day, adolescents 2–4 μg/Kg/day, with conversion to the adult dose of 1.6 μg/Kg/day once endocrine maturation is completely reached [Jonklaas, 2010; Lafranchi, 1992].

According to the guidelines, the target of treatment is to keep the FT4 level in the mid to upper half of the reference range and the TSH level in the mid to lower half of the reference range [American Academy of Pediatrics, 2001; Jonklaas et al., 2014].

Tsyklauri et al. reported autoimmune Hashimoto's thyroiditis as having a higher prevalence in BBS patients than in the overall population, probably due to impaired immunity in patients with ciliopathies [Tsyklauri et al., 2021].

At baseline evaluation, the investigation of the thyroid function (the FT4 and TSH levels corroborated with the ultrasonography of the thyroid) is recommended, and then a yearly evaluation of the thyroid gland function [Forsyth, Gunay-Aygun, 2003].

When pregnancy is possible, preconception level of FT4 and TSH should be evaluated in order to modulate levothyroxine hormone replacement during pregnancy and prevent impairment of foetal brain development [Krassas, Poppe, Glinoer, 2010].

The treatment of the growth hormone deficiency and the hyperprolactinemia is being administered accordingly to the current guidelines.

ENT

Evaluation of the upper airways is important because laryngeal webs and bifid epiglottis can lead to life-threatening complications [Kaur et al., 2021; Poulin, Laframboise, Blouin, 2019]. Another rare abnormality that may be present is the choanal stenosis [Carey et al., 2020].

The literature is poor in assessing anaesthetic risks. In a case study, Smith et al. showed that there were differences between anaesthetic management in paediatric patients (<18 years old) and adults in terms of intubation. Thus, in paediatric patients, intubation was successfully performed by direct laryngoscopy. In adult patients, however, indirect laryngoscopy techniques were required (40% awake fiberoptic technique, 27% video laryngoscopy). The authors estimate that this was due to airway difficulty that worsen with age due to oral and facial anomalies in correlation with progressive morbid obesity [Smith et al., 2016]. Clinical features that are associated with airway difficulty include: dental anomalies, high arched palate, facial dysmorphism and morbid obesity, bifid epiglottis, laryngeal web [Kaur et al., 2021; Poulin, Laframboise, Blouin, 2019; Smith et al., 2016].

There is a special management in terms of both anaesthetic and perioperative risk in patients with BBS. The anaesthesiologist and the multidisciplinary team must follow some steps: evaluation for difficult airways, cardiac anomalies, renal impairment, morbid obesity and intellectual disability [Carey et al., 2020; Smith et al., 2016].

Neurology

The baseline assessment includes careful neurological examination of the coordination and gait. An electroencephalogram should be performed for seizures (found in 9.6% of the patients) [Carey et al., 2020; Forsyth, Gunay-Aygun, 2003; Bardet -Biedl syndrome guideline development group]. Olfactory dysfunction is present with a high incidence and requires a smell identification test. Usually, the olfactory bulb is abnormal on the brain MRI [Forsyth, Gunay-Aygun, 2003; Braun et al., 2016]. MRI is also requested for evaluation of other neurological conditions (ataxia, hypotonia, seizures).

Dentistry

Recommended baseline evaluation includes routine dental care with the assessment for hygiene, dental crowding and hypodontia. In case of malocclusion, extractions or dental crowding, standard interventions are recommended. Antibiotic prophylaxis will be considered in case of dental interventions in individuals with heart disease [Bardet -Biedl syndrome guideline development group]. The follow-up is done every 6 months starting from the age of 1 year old [Carey et al., 2020; Forsyth, Gunay-Aygun, 2003].

Gastroenterology

If gastrointestinal symptoms are present, investigations should be initiated for possible structural and functional abnormalities. For hepatic disease the baseline investigations include: liver ultrasonography to evaluate a possible liver fibrosis and steatosis, measurements of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) level and tests for synthetic function (prothrombin time - PT, partial thromboplastin time - PTT) [Carey et al., 2020; Forsyth, Gunay-Aygun, 2003; Bardet -Biedl syndrome guideline development group.]. If these parameters are normal, they are reassessed annually. People with liver disease should be monitored as directed by the hepatologist [Forsyth, Gunay-Aygun, 2003; Bardet -Biedl syndrome guideline development group].

Cardiology

The baseline evaluation involves auscultation of the heart, electrocardiogram and echocardiogram to assess for congenital heart defects and/or cardiomyopathy. If these conditions are present, the follow-up is recommended at the cardiologist's indication. If the baseline evaluation is normal, re-evaluation is recommended only if symptoms appear [Carey et al., 2020; Forsyth, Gunay-Aygun, 2003; Bardet -Biedl syndrome guideline development

group]. If there are structural cardiac defects, antibiotic prophylaxis is recommended in case of surgical and dental procedures [Carey et al., 2020].

Hypertension is present in 30% of the patients with BBS [Khan et al., 2016]. Guo et al. showed that the neuronal BBSome plays an important role in hemodynamic, sympathetic and vascular regulation. Another cause of hypertension in BBS is the obesity and kidney disease [Guo et al., 2016, Imhoff et al., 2011].

Dermatology

Cutaneous signs are common in BBS, although they are not diagnostic criteria. The mechanisms by which they occur could be: disturbance of keratinisation and keratinocyte function. Encountered dermatosis are: keratosis pilaris, alopecia, nevi, cherry angiomas, acanthosis nigricans, onychodystrophy, striae, ichthyosis vulgaris, pigmentation anomalies, psoriasis, acne vulgaris, confluent and reticulated papillomatosis, seborrheic dermatitis, intertrigo 1, acrochordons, lichen simplex, eczema, asteatotic dermatitis, hidradenitis [Haws, McIntee, Green, 2019]. In the presence of one of these features, a dermatologic examination is requested and the specialist will decide which follow-up path must be applied.

Genetic Testing

Genetic testing is necessary to both certify the diagnosis in those cases in which the clinical diagnostic criteria are not present, and to provide correct genetic counselling to the family and perform a prenatal diagnosis. Testing a single gene is not recommended as there are no genotype-phenotype correlations to justify this approach [Forsyth, Gunay-Aygun, 2003]. Sequencing of the BBS- causing genes by NGS (Next Generation Sequencing) confirms the diagnosis in 80% of the patients [Forsythe et al, 2013].

The percentage of gene mutations in BBS are different. The most involved genes are *BBS1* (23.4%), *BBS10* (14.5%) followed by *BBS2* (9.6%), *BBS12* (6.4%), *MKKS* (6.3%), *CEP290* (6.3%), *BBS4* (5.3%), *ARL6* (5.1%), *SDCCAG8* (4.3%), *BBS7* (4.2%), BBS5 (3.7%), *BBS9* (3.4%), *TTC8* (2.0%), *CFAP418* (1.6%), *MKS1* and *IFT172* (1%), *BBIP1, TRIM32, WDPCP, LZTFL1, IFT27, IFT74* and *SCLT1* (<1%) [Forsyth, Gunay-Aygun, 2003].

For a cost reduction approach, the genetic testing can start by testing recurrent mutations in *BBS1* gene (exon 12, NM_024649: c.1169T> G, p.M390R) and in *BBS10* gene (exon 2, NM_024685: c.271dupT, p.Cys91Leufs * 5). The two mutations are identified in 30% of the patients [Carey et al., 2020]. Currently, testing specific BBS-gene panels or larger ciliopathy genes panels is recommended. This is justified because there is a significant clinical overlap between ciliopathies [Zaki et al., 2011]. If the result is negative, the sequencing of the rarely involved genes is continued [Carey et al., 2020]. Comprehensive genomic testing is another test that can be used. The advantage of genomic testing is that it can identify mutations in genes that have not yet been included in gene panels or mutations in new genes not previously known to be associated with BBS [Forsyth, Gunay-Aygun, 2003]. If genetic testing is not available, it is recommended to initiate the management procedures if the clinical diagnostic criteria are met [Carey et al., 2020].

Final remarks

Bardet - Biedl syndrome is a rare, multisystem, non-motile ciliopathy characterized by a high degree of genetic heterogeneity. The diagnosis and the monitoring and management require a multidisciplinary team. The main features to be evaluated are: ocular involvement, polydactyly, obesity, genitourinary anomalies, renal anomalies, metabolic features, cognitive impairment, hypothyroidism, ENT changes, neurologic modifications, dental particularities, gastrointestinal, cardiologic, and dermatologic aspects. The genetic examination and tests are essential to confirm the clinic diagnosis. The best test to use is the gene panel containing the 26 genes involved in the BBS pathogenic mechanisms

Chapter 4. CHALLENGING DIAGNOSIS AND MULTIDISCIPLINARY APPROACH IN RARE DISEASES

The results of researches related to rare diseases were published in the following papers
ISI ARTICLES
Florea, L., **Caba, L.*,** and Gorduza, E.V. *Genetic Heterogeneity in Bartter Syndrome: Clinical and Practical Importance*. Front Pediatr 2022,10. (**corresponding author**). **IF=3.569**
Pânzaru, M.C., **Caba, L.***, Florea, L., Braha, E.E, Gorduza, E.V. *Epidermolysis Bullosa - a different genetic approach in correlation with genetic heterogeneity*. Diagnostics (Basel) 2022, 12(6): 1325. (**corresponding author**). **IF=3.992**
Ungureanu, M.-C., Hrisca, A., **Caba, L**., Teodoriu, L., Bilha, S., Preda, C., Leustean, L. *SHOX Deletion and Idiopathic Short Stature: What Does the Clinician Need to Know? Case Series Report*. Diagnostics (Basel) 2023, 13, 105. **IF=3.992**
Momanu, A., **Caba, L.***, Gorduza, N.C., Arhire, O.E., Popa, A.D., Ianole, V. and Gorduza, E.V. *Gorham-Stout Disease with Multiple Bone Involvement—Challenging Diagnosis of a Rare Disease and Literature Review*. Medicina (Kaunas) 2021, 57(7):681. **(corresponding author). IF=2.948** • awarded in the UEFISCDI competition "Premierea Rezultatelor cercetarii-Articole PRECISI 2021"
Caba, L., Gug, C., & Gorduza, E. V. *Heterogeneity in combined immunodeficiencies with* associated or syndromic features. Exp Ther Med 2021, 21(1): 1-1. **IF=2.751**.
Gug, C., **Caba, L**.*, Mozos, I., Stoian, D., Atasie, D., Gug, M., & Gorduza, E. V. *Rare splicing mutation in COL1A1 gene identified by whole exomes sequencing in a patient with osteogenesis imperfecta type I followed by prenatal diagnosis: A case report and review of the literature.* Gene 2020, 741: 144565 (**corresponding author**). **IF=3.688** • awarded in the UEFISCDI competition "Premierea Rezultatelor cercetarii-Articole PRECISI 2021"
Panzaru, M. C., Florea, A., **Caba, L.**, & Gorduza, E. V. *Classification of osteogenesis imperfecta: Importance for prophylaxis and genetic counseling*. World J Clin Cases 2023, 11(12), 2604-2620. **IF=1.534**
Ciobanu, C. G., Nucă, I., Popescu, R., Antoci, L. M., **Caba, L.,** Ivanov, A. V., ... & Pânzaru, M. C. *Narrative Review: Update on the Molecular Diagnosis of Fragile X Syndrome*. Int J Mol Sci 2023, 24(11), 9206. **(equal contributions)**. **IF=6.208**
BDI ARTICLES
Bilha, S.C., Teodoriu, L., Velicescu, C. and **Caba, L**. *Pituitary hypoplasia and growth hormone deficiency in a patient with Coffin-Siris syndrome and severe short stature: case report and literature review*. Arch Clin Cases *2022*, 9(3): 121-125.
Spiridon, M. R., Petris, A. O., Gorduza, E. V., Petras, A. S., Popescu, R., & **Caba, L.** *Holt-Oram syndrome with multiple cardiac abnormalities.* Cardiol Res 2018, 9(5): 324.
Caba, L., Ungureanu, C., Grămescu, M., Pânzaru, M., Butnariu, L., Popescu, R., Resmeriţă, I., Saad, H. and Gorduza, E.V. *A new case of hereditary gingival fibromatosis.* Romanian Journal of Functional & Clinical, Macro-& Microscopical Anatomy & of Anthropology/Revista Româna de Anatomie Functionala si Clinica, Macro si Microscopica si de Antropologie 2017, 16(2): 143-147.
Butnariu, L.I., Rusu, C., Pânzaru, M., **Caba, L.,** Popescu, R. and Gorduza, E.V. *Cleidocranial dysplasia: a case report*. Romanian Journal of Functional & Clinical, Macro-& Microscopical Anatomy & of Anthropology/Revista Româna de Anatomie Functionala si Clinica, Macro si Microscopica si de Antropologie 2017, *16*(2): 163-167.
Pânzaru, M., Butnariu, L., Popescu, R., **Caba, L.**, Grămescu, M., Popa, S., Resmeriță, I., Gorduza, E.V. and Rusu, C. *Esophageal atresia and genetic conditions.* Romanian Journal of Functional & Clinical, Macro-& Microscopical Anatomy & of Anthropology/Revista Româna de Anatomie Functionala si Clinica, Macro si Microscopica si de Antropologie 2017, 16(1); 69-73.

4.1. Introduction

Rare diseases (RD) are defined as chronic diseases that affect less than 5 in 10,000 people in the European Union and, according to the US Orphan Drug Act, a rare disease is a condition found in a few individuals out of 200,000. Taken individually they are rare, but in total they affect an estimated number of 27-36 million individuals in the EU and 25-50 million Americans. The true prevalence is difficult to establish accurately because epidemiological reports on rare diseases are not numerous, are not standardized or are difficult to combine in complex summative analyses, specific diagnostic criteria may be missing, there may be differences depending on the geographical area, or the methodology for studies on small populations is different [Austin et al., 2018; Auvin et al., 2018].

Regarding the etiology, over 80% of rare diseases are genetic. These disorders can be classified into: lethal, severe and less severe.

Rare diseases take time, effort and resources to diagnose. It is a reality that there is resistance from the medical staff regarding the term genetic disease, because it induces the idea that it is difficult to test and difficult to treat. It is estimated that it takes an average of 7.6 years for a patient to be diagnosed, during which time they consult an average of 8 specialists for a correct diagnosis, each patient has an average of 2-3 wrong/incomplete diagnoses before receiving the correct diagnosis and only 45% of patients have their costs covered by health insurance. Less than 50% of patients with clinical suspicion receive a molecular diagnosis. Diagnostic difficulties are also due to epimutations that intervene in the transcriptional process by inactivating it. In this way they can mimic an inactivating mutation and cause a phenocopy of a disease. Over 50-75% of RD occur in children and are responsible for 35% of deaths in the first year of life. About 1/3 of children born with a rare disease will not survive to 5 years [Boycott et al., 2019; Katsanis SH, Katsanis N, 2013].

In this context, the global trend of focusing on the topic of rare diseases and the additional knowledge that case presentations or review articles bring is justified.

4.2. Study of Bartter Syndrome

4.2.1. Background

Bartter syndrome (BS) is a rare tubulopathy characterized by polyuria, hypokalemia, hypochloremic metabolic alkalosis, and normotensive hyperreninemic hyperaldosteronism [Konrad et al., 2021]. Incidence is 0.1/100,000 [Orphanet].

The main mechanism is the defective reabsorption of salt, mainly at the thick ascending limb (TAL) of the loop of Henle [Legrand et al., 2018]. TAL intervenes in the homeostasis of extracellular fluid by sodium reabsorption; homeostasis of calcium, magnesium, bicarbonate, and ammonium; and synthesis of uromodulin (Tamm-Horsfall protein) with a role in maintaining the composition of urinary proteins [Vargas-Poussou, 2021].

Hereditary diseases involving proteins of the TAL are classified into three categories according to the TAL function affected: sodium reabsorption, calcium and magnesium reabsorption, and uromodulin synthesis. The first category includes BS types 1–5 and HELIX syndrome (hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerostomia) (produced by mutations in *CLDN10* gene). The second category includes familial hypomagnesemia with hypercalciuria and nephrocalcinosis (produced by mutations in *CLDN16* or *CLDN19* genes), familial hypercalcemia hypocalciuric types 1–3 (produced by mutations in *CASR*, *GNA11*, *AP2S1* genes), and autosomal dominant hypocalcemia types 1–2 (produced by mutations in *CASR*, *GNA11* genes). Hyperuricemic nephropathy, familial juvenile 1 (produced by *UMOD* gene) belongs to the third category [Vargas-Poussou, 2021].

4.2.2. Genetics in Bartter syndrome

Bartter syndrome is caused by mutations in genes encoding K+ channel (*KCNJ1* gene), Cl− channel (*CLCNKA* and *CLCNKB* genes), their cotransporters (*SLC12A1* gene), subunits of these channels (*BSND* gene), or regulators of the expression of certain transport channels (*MAGE-D2* gene).

Table I.17. summarizes the genes implied in the pathogeny of various types of BS. Figure I.30. describes the gene expressions in different segments of the nephron and correspondent type of the BS.

Table I.17. Genes and proteins in Bartter syndrome

[HGNC; UNIPROT; da Silva Cunha, Heilberg, 2018].

Gene (Anterior/ alias symbol)	Approved name	Chromosomal location	Protein—Recommended name	Bartter syndrome type
SLC12A1 (NKCC2)	Solute carrier family 12 member 1	15q21.1	Solute carrier family 12 member 1	**Type 1**
KCNJ1 (Kir1.1 ROMK1)	Potassium inwardly rectifying channel subfamily J member 1	11q24.3	ATP-sensitive inward rectifier potassium channel 1	**Type 2**
CLCNKB (hClC-Kb)	Chloride voltage-gated channel Kb	1p36.13	Chloride channel protein ClC-Kb (ClC-K2)	**Type 3**
BSND (BART DFNB73)	Barttin CLCNK type accessory subunit beta	1p32.3	Barttin	**Type 4a**
CLCNKA (hClC-Ka)	Chloride voltage-gated channel Ka	1p36.13	Chloride channel protein ClC-Ka (ClC-K1)	**Type 4b**
CLCNKB **(hClC-Kb)**	Chloride voltage-gated channel Kb	1p36.13	Chloride channel protein ClC-Kb (ClC-K2)	**Type 4b**
MAGE-D2 (JCL-1, BCG1, 11B6, MAGE-D2, HCA10, MAGED, MGC8386)	MAGE family member D2	Xp11.21	Melanoma-associated antigen D2	**Type 5 (Transient BS)**
CASR	Calcium sensing receptor	3q13.33-q21.1	Extracellular calcium-sensing receptor	AD hypocalcemic hypercalciuria

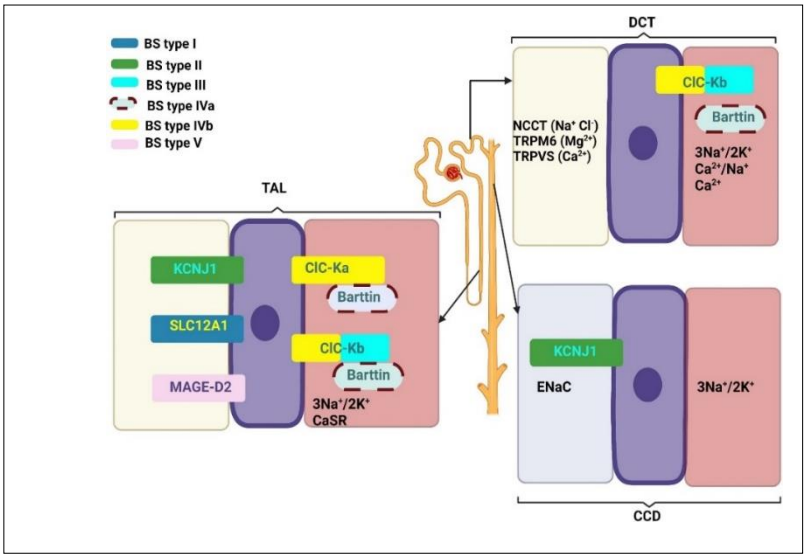


Figure I.30. Gene expressions in different segments of the nephron and correspondent type of the Bartter syndrome. Created with BioRender.com.

4.2.3. Clinical signs

The typical symptoms in BS in prenatal period are polyhydramnios, and premature delivery, while after the birth could be identified polyuria, polydipsia, signs of hypovolemia, failure to thrive, and poor growth [Walsh et al., 2018].

The age of presentation is different according to the type of mutation. For the BS types 4 and 5, polyhydramnios is earlier compared to BS types 1 and 2. The onset of BS type 3 is later in life and only rarely in the antenatal period [Konrad et al., 2020]. There are rare cases of asymptomatic children diagnosed with BS. These cases presented hypokalemia metabolic alkalosis, normal blood pressure, and nephrocalcinosis, and usually such patients are discovered after a screening applied in a family with positive history of BS [Seys et al., 2017; Bettinelli et al., 2007; Konrad et al., 2000; Brochard et al., 2009].

Antenatal Symptomatology

The most frequent feature is the polyhydramnios detected during pregnancy, starting at 22 weeks of gestation [Legrand et al., 2018]. The onset of polyhydramnios is earlier in fetuses with BS types 4 and 5 (*BSND* or *MAGED2* variants) and represent the most severe forms. In BS type 1 (*SLC12A1*) and BS type 2 (*KCNJ1*), the onset of polyhydramnios is much tardive, concordant with a lower severity. In BS type 3 (*CLCNKB*), polyhydramnios is either absent or mild [Legrand et al., 2018; Laghmani et al., 2016].

Antenatal genetic testing and biochemical analysis of amniotic fluid, the concentration of total protein and alpha-fetoprotein (decreased in BS), the Bartter index (which is defined corresponding to the multiplication of total protein and of AFP, both expressed in multiple of median—MoM) with 86% sensitivity and 84% specificity, can be used to confirm the diagnosis [Allaf et al., 2015; Garnier et al., 2010].

Neonatal Symptomatology

All BS types, except BS type 3, have a neonatal onset with preterm birth (median gestational age between 29 and 33 weeks) and massive polyuria which lead to dehydration and rapid weight loss. Infants with BS type 4 have supplementary sensorineural hearing loss [Konrad et al., 2020; Birkenhäger et al., 2001].

The diagnosis can be confirmed using a next-generation sequencing (NGS) testing with gene panels that contain *SLC12A1*, *KCNJ1*, *BSND*, CLCNKA, *CLCNKB*, *MAGED2*, and *SLC12A3* genes. These tests have 75% sensitivity and 90–100% specificity [Ashton et al., 2018].

Childhood Symptomatology

Older infants and young children with BS type 3 present fail to thrive, growth retardation, and polyuria/polydipsia that lead to hypovolemia, persistent thirst, salt craving, constipation, unexplained fever, hypotonia, and recurrent vomiting [Seys et al., 2017; Bettinelli et al., 2007; Konrad et al., 2000].

Teenage Symptomatology

Older children and adolescents with BS type 3 can present thirst, salt craving, fatigue, muscle weakness, cramps, nocturia, constipation, poor growth, and pubertal delay.
In BS type 4, there is a risk for chronic kidney disease (CKD) and end stage renal disease (ESRD) [Konrad et al., 2020].

Bartter syndrome type 5 (transient type) has some extrarenal clinical features: frontal bone cyst, dysmorphic facies, hydrocephalus and Chiari malformation, Marfanoid habitus

with arachnodactyly and mitral insufficiency, pyloric stenosis, high blood pressure, interauricular communication, left ventricle hypertrophy, right aortic arch, retroesophageal left subclavian artery, moderate pulmonary stenosis, enteropathic acrodermatitis zinc deficiency type, angioma, thrombocytopenia, and deafness after the age of 2 [Legrand et al., 2018; Vargas-Poussou, 2021; Arthuis et al., 2018].

The laboratory findings constant in all forms of BS are hypochloremic metabolic alkalosis, elevated renin and aldosterone levels, low to normal blood pressure due to chronic hypovolemia, low urine osmolality due to impaired concentrating ability, and hypokalemia (potassium levels that are less than 3 mmol/L). Transient neonatal hyperkalemia may be present only in patients with BS type 2. Hypercalciuria is present in patients with BS types 1 and 2 (associated with nephrocalcinosis) and also in BS type 5 (however, the nephrocalcinosis is rare and mild in this type). The normocalciuria is present in patients with BS types 3 and 4, and the hypocalciuria in some patients with BS type 3. The mild hypomagnesemia may be present in some patients with BS type 3. Plasma Cl/Na ratio can be normal in BS types 1 and 2, decreased in BS types 3 and 4, and increased in BS type 5 [Konrad et al., 2021; Walsh et al., 2018; Peters et al., 2002; Castrop, Schießl, 2014; Oppermann et al., 2007; Oppermann et al., 2006].

4.2.4. Genetic testing

Genetic testing is recommended in any clinical suspicion of BS. The European Rare Kidney Disease Reference Network Working Group for Tubular Disorders recommends the use of a gene panel testing. This panel must contain not only all genes that cause BS and Gitelman syndrome but also genes that overlap phenotypically with BS and should be considered in differential diagnosis with BS: *SLC12A1*, *KCNJ1*, *CLCNKB*, *CLCNKA*, *BSND*, *MAGED2*, *SLC12A3*, *CASR*, *KCNJ10*, *SLC26A3*, *CLDN10*, *SCNN1A*, *SCNN1B*, *SCNN1G*, *NR3C2*, *HSD11B2*, *CYP11B1*, *CLCN2*, *KCNJ5*, and *CACNA1H* [Konrad et al., 2021]. Genetic testing is required for several reasons: confirmation of clinical diagnosis, adequate genetic counseling, adequate management of diseases with overlapping phenotypes, screening for deafness in BS type 4, and avoidance of aggressive treatment in transient BS type 5 [Konrad et al., 2021]. Mutational heterogeneity is important in BS. According to the Human Gene Mutation Database Professional HGMD Professional 2021.4 (accessed in February 2022), 350 pathogenic variants were described. Figure I.31. shows the implication in BS of different types of genetic modifications. The most common was missense/non-sense mutation, splicing substitution, small deletions, and gross deletion especially in *SLC12A1*, *CLCNKB*, and *KCNJ1* genes.

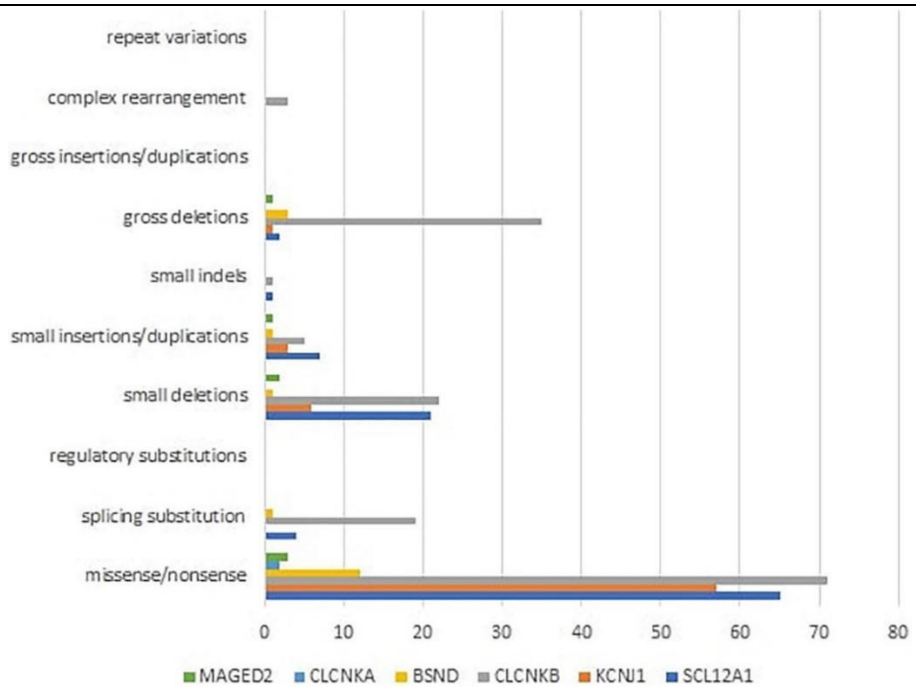


Figure I.31. Different types of pathogenic variants in genes implied in Bartter syndrome [Stenson et al., 2017].

4.2.5. Differential diagnosis

The differential diagnosis of BS should be made with conditions where the main signs are poyhydramnios, salt loss, salt loss with hypokalemic alkalosis, hypokalemic alkalosis without salt loss, and nephrocalcinosis [Konrad et al., 2021; Konrad et al., 2020].

The polyhydramnios may be a sign present in aneuploidia, different gastrointestinal malformations, and congenital chloride diarrhea (dilated intestinal loops present).

The salt loss can be a manifestation in pseudohypoaldosteronisme type I, but in this case is associated with metabolic acidosis and hyperkalemia.

The salt loss with hypokalemic alkalosis can be a sign in congenital chloride diarrhea, pseudo-BS (in cystic fibrosis, for example), surreptitious vomiting, surreptitious laxative use (in these entities, the additional finding is low urinary chloride), Gitelman syndrome (associated hypocalciuria and hypomagnesemia), HNF1B (hepatocyte nuclear factor 1 beta), nephropathy (associates renal malformation, MODY5—maturity onset diabetes of the young type 5, hypomagnesemia), HELIX syndrome (hypercalcemia is present), autosomal dominant hypocalcemia, EAST/SeSAME syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance), and surreptitious diuretic use [Konrad et al., 2021; Konrad et al., 2020].

Hypokalemic alkalosis without salt loss associated hypertension with low renin/aldosterone and can be found in primary hyperaldosteronism, apparent mineralocorticoid excess, and Liddle syndrome [Konrad et al., 2021; Konrad et al., 2020].

The nephrocalcinosis can also be found in distal renal tubular acidosis (metabolic acidosis), medical conditions without metabolic alkalosis (proximal tubular defects, familial hypomagnesemia/hypercalciuria), and apparent mineralocorticoid excess [Konrad et al., 2021; Konrad et al., 2020].

4.2.6. Management in Bartter syndrome

The antenatal therapy consists of repeated amniocentesis and/or maternal administration of NSAID (non-steroidal anti-inflammatory drug) in order to reduce the amniotic fluid volume. There are potential risks for the fetus (such as necrotizing

enterocolitis or premature closure of the ductus arteriosus). The evidence is not sufficient to show that the benefit outweighs the potential adverse effects [Konrad et al., 2021]. The main therapeutic interventions are: Na Cl supplementation, KCl supplementation, NSAID, nutrition, Mg supplementation, potassium-sparing diuretics, angiotensin-converting enzyme inhibitors, and angiotensin receptor.

The postnatal therapy consists of maximal caloric intake in order to facilitate optimal growth [Konrad et al., 2021], fluid repletion, sodium chloride (NaCl) supplementation of at least 5–10 mEq/kg/d, and potassium chloride (KCl) 1–2 mEq/d, spread out in frequent doses throughout the day, in order to compensate the urinary losses [Konrad et al., 2021; Kömhoff et al., 2004].

Non-steroidal anti-inflammatory drugs that inhibit prostaglandin E2, which contributes to high urinary NaCl losses, are recommended in case of inadequate response in symptomatic patients with BS, especially in early childhood [Ranade, Somberg, 2001]. Oral magnesium supplementation is recommended in case of hypomagnesemia [<1.70 mg/dl (0.7 mmol/L)] [Kömhoff et al., 2004; Blanchard et al., 2017]. In case of symptomatic hypokalemia despite the supplements, potassium-sparing diuretics (such as spironolactone, eplerenone, or amiloride), angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers can be used [Konrad et al., 2021; Kömhoff et al., 2004].

The patients with BS should be followed in pediatric or adult centers experienced in tubular disorders. The recommended frequency of hospital visits is 3–6 months for infants and young children and 6–12 months for adults. All the recommendations are summarized in Table I.18. A clinical work on hydration status, degree of polyuria, muscular weakness, growth, and psychomotor development is recommended at each follow-up visit [Konrad et al., 2021; Konrad et al., 2020].

Table I.18. Follow-up in Bartter syndrome [Konrad et al., 2021; Konrad et al., 2020].

	Frequency of visits in centers	Clinical work up	Biochemical work up	Cardiac work up	Renal ultrasound
Infants	3–6 months	At each follow up visit	At each follow up visit		12–24 months
Young children	3–6 months	At each follow up visit	At each follow up visit		12–24 months
Older children	6–12 months	At each follow up visit	At each follow up visit		12–24 months
Adult patients	6–12 months	At each follow up visit	At each follow up visit	In case of palpitations or syncope	12–24 months
Level of recommendation	Grade C	Grade C	Grade C	Grade C	Grade C

A biochemical work focused on metabolic acid-base status (either blood gas or by measurement of venous total CO_2), serum electrolytes (natremia, potassium, chloride, magnesium, and bicarbonate levels), renal function, microalbuminuria, urinary calcium excretion, PTH, and urine osmolality for the detection of nephrogenic diabetes insipidus is recommended at each follow- up visit [Konrad et al., 2021; Konrad et al., 2020].

Growth hormone deficiency may be considered for children with growth retardation despite treatment.

Renal ultrasound is recommended to be performed at least every 12–24 months to monitor the occurrence of kidney stones, nephrocalcinosis, and obstructive uropathy.

Cardiac work out is recommended for patients (particularly adults) with palpitations or syncope. The physician can use the drugs that slow the sinus rhythm (beta-blockers, calcium channel blockers as verapamil, diltiazem, digoxin) or drugs that influence the QT interval (proton pump inhibitors, fluoroquinolones, macrolides, gentamicin, or antiviral drugs). Electrocardiography, Holter, and stress electrocardiography are recommended to detect cardiac arrhythmias which are determined by a prolonged QT interval in the context of hypokalemia and hypomagnesemia [Konrad et al., 2021; Konrad et al., 2020].

The quality of life is important to be assessed by using age-specific standardized questionnaires. The quality-of-life scores are related by biochemical parameters (potassium and aldosterone) [Konrad et al., 2021; Eder et al., 2020].

Sports can be performed with a good hydration and additional salt and electrolytes.

Anesthesia for patients with BS must be performed after improvement of hypokalemia and hypomagnesemia, which in addition with anesthetic agents can lead to neuromuscular blockage. Potassium levels > 3.0 mmol/L and magnesium > 0.5 mmol/L are suggested by guidelines [Gallagher, Soar, Tomson, 2016].

Kidney Disease

Renal disease is characterized by urolithiasis, obstructive uropathy, and nephrocalcinosis. Nephrotic-range proteinuria has also been reported in patients with BS [Walsh et al., 2018; Bettinelli et al., 2007; Puricelli et al., 2010]. Renal biopsy can show diffuse glomerular and tubule-interstitial lesions with enlarged glomeruli and focal segmental glomerulosclerosis [Seys et al., 2017].

Only some patients have an evolution to end-stage kidney disease. CKD can occur as a late manifestation in patients with BS, particularly types 1, 4a, and 4b [Walsh et al., 2018; Seys et al., 2017; Jeck et al., 2001]. The CKD in BS seems to have several mechanisms: chronic stimulation of the renin–angiotensin system, periodic dehydration, prematurity, nephrocalcinosis, long-term NSAID drug treatment, and chronic hypokalemia [Vargas-Poussou, 2021].

There are few reported cases of kidney transplants [Seys et al., 2017; Blethen, van Wyk, Lorentz, Jennette, 1985; Takahashi et al., 1996; Kim et al., 2000; Ueki et al., 2000; Kitanaka et al., 2006; Lee et al., 2011]. The electrolytes and urinary concentrating abnormalities were corrected. No evidence of recurrent disease was observed.

Few studies reported growth failure with growth hormone (GH) deficiency in patients with BS, particularly type 3 who have severe metabolic abnormalities. The poor growth explanation is unclear: acid-base or electrolyte disturbances in BS or whether it is an intrinsic part of the disorder. After metabolic control, recombinant human GH supplementation can be performed [Walsh et al., 2018; Bettinelli et al., 2007; Akil, Ozen, Kandiloglu, Ersoy, 2010; Gil-Peña et al, 2010; Buyukcelik et al, 2012].

4.2.7. Conclusions

Knowledge of clinical heterogeneity in BS is important for initiating genetic investigations and proper management. Early molecular diagnosis is desirable for personalized therapy.

4.3. Study of Epidermolysis Bullosa

4.3.1. Background

Epidermolysis bullosa (EB) is a heterogeneous group of rare genetic disorders characterized by mucocutaneous fragility and blister formation after minimal trauma [Has et

al., 2020]. EB presents a variable expression with a wide phenotypic spectrum ranging from localized, mild, acral blistering, and normal life expectancy, to generalized, severe blistering and extracutaneous involvement, which could lead to infections, electrolyte imbalances, or respiratory distress, as well as poor prognosis. Nail dystrophy, keratoderma, and atrophic scarring are common features. Major extracutaneous complications may develop in some subtypes of EB: laryngeal or esophageal stenosis, ectropion, corneal opacification, pseudosyndactyly, and microstomia. Pyloric atresia, nephropathy, muscular dystrophy, cardiomyopathy, and interstitial lung disease, are encountered in rare forms of EB. Some forms of EB (e.g., severe and intermediate recessive dystrophic EB) are associated with an increased risk of developing cutaneous squamous cell carcinoma [Has et al., 2020; Rimoin, Pyeritz, Kor, 2013].

Four major EB types are described based on the level of skin cleavage: EB simplex (EBS) [with changes at intraepidermal (epidermolytic) level], junctional EB (JEB) [with changes at the intra-lamina lucida (lamina lucidolytic) level], dystrophic EB (DEB) [with changes at the sub-lamina densa (dermolytic) level], and Kindler EB [with multiple changes at the cutaneous level] [Has et al., 2020]. Precise diagnosis requires the correlation of clinical data with immunofluorescence antigen mapping (IAM), transmission electron microscopy (TEM), and mutational analysis [Rimoin, Pyeritz, Kor, 2013]. In EBS the structure and function of keratin intermediate filaments are altered and the intracellular components of the hemidesmosomes are mutated or missing [Rimoin, Pyeritz, Kor, 2013]. In JEB, the transmembrane and extracellular proteins of the hemidesmosomes and the anchoring filaments are modified [Rimoin, Pyeritz, Kor, 2013]. In DEB, the anchoring fibrils can be absent, reduced in number, or abnormal. In KS, there are multiple cleavage planes (intraepidermal, junctional, or dense sub-lamina) determined by abnormal fermitin family homolog 1, which is a component in the focal adhesion complexes [Rimoin, Pyeritz, Kor, 2013].

Researches in recent decades have deciphered some of the pathogenic changes in EB, mainly through histological and genetic studies. These researches proved an important genetic heterogeneity and changed the classification of disorders. Our paper is trying to present synthetically the gene mutations and their implications on the cellular level in connection with their clinical features.

4.3.2. Genes and Proteins Involved in Epidermolysis Bullosa

In EB, cell–matrix interactions are mainly altered. Normally cell–matrix interactions are achieved through two elements: hemidesmosomes and focal adhesion.

Hemidesmosomes (HD) are specialized structures that stably anchor the keratinocytes of the epidermis to the basement membranes. This is done by assembly between the intracellular and transmembrane proteins [Rimoin, Pyeritz, Kor, 2013]. HD type I (the classic one) is present in the pseudo-stratified epithelium where interactions between intracellular proteins (plectin, dystonin) and transmembrane protein integrin (α6β4, collagen XVII) and CD151 antigen normally occur. HD type II is found in simple epithelial tissue and consists only of α6β4 integrin and plectin [Wang et al., 2020].

The focal adhesion is allowed by different proteins: integrin α3β1, transmembrane collagen XIII, and fermitin family homolog 1 (FFH1) [Rimoin, Pyeritz, Kor, 2013; Sawamura, Nakan, Matsuzaki, 2010].

Table I.19. summarizes the main genes involved in the pathogenesis of EB and the encoded proteins.

Table I.19. Genes and proteins involved in epidermolysis bullosa [Has et al., 2020; HGNC; UniProt].

Gene Symbol (Previous)	Approved Name	Chromosomal Location	Protein—Recommended Name	Epidermolysis Bullosa Type
KRT5	Keratin 5	12q13.13	Keratin, type II cytoskeletal 5	EB simplex, AD EB simplex, AR
KRT14	Keratin 14	17q21.2	Keratin, type I cytoskeletal 14	EB simplex, AD EB simplex, AR
PLEC	Plectin	8q24.3	Plectin	EB simplex, AD EB simplex, AR
KLHL24	Kelch-like family member 24	3q27.1	Kelch-like protein 24	EB simplex, AD
DST	Dystonin	6p12.1	Dystonin	EB simplex, AR
EXPH5	Exophilin 5	11q22.3	Exophilin-5	EB simplex, AR
CD151	CD151 molecule (Raph blood group)	11p15.5	CD151 antigen	EB simplex, AR
LAMA3	Laminin subunit alpha 3	18q11.2	Laminin subunit alpha-3	Junctional EB, AR
LAMB3	Laminin subunit beta 3	1q32.2	Laminin subunit beta-3	Junctional EB, AR
LAMC2	Laminin subunit gamma 2	1q25.3	Laminin subunit gamma-2	Junctional EB, AR
COL17A1	Collagen type XVII alpha 1 chain	10q25.1	Collagen alpha-1(XVII) chain	Junctional EB, AR
ITGA6	Integrin subunit alpha 6	2q31.1	Integrin alpha-6	Junctional EB, AR
ITGB4	Integrin subunit beta 4	17q25.1	Integrin beta-4	Junctional EB, AR
ITGA3	Integrin subunit alpha 3	17q21.33	Integrin alpha-3	Junctional EB, AR
COL7A1	Collagen type VII alpha 1 chain	3p21.31	Collagen alpha-1(VII) chain	Dystrophic EB, AD Dystrophic EB, AR
FERMT1	FERM domain containing kindlin 1	20p12.3	Fermitin family homolog 1	Kindler EB, AR

The main interactions between proteins are shown in Figure I.32.

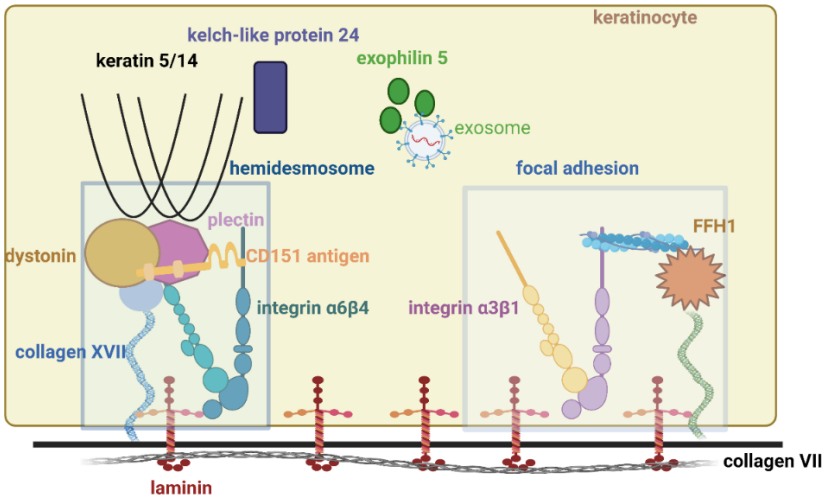


Figure I.32. Main proteins involved in epidermolysis bullosa. Created with BioRender.com (accessed 6 April 2022).

4.3.3. Correlations Genotype–Phenotype in EB

4.3.3.1. EB Simplex (EBS)

EBS is the most common type of EB accounting for ~70% of all EB [Bardhan et al., 2020]. EBS has a prevalence of 6/1,000,000 individuals and an incidence of 7.87 per one million live births [Fine, 2016]. EBS is characterized by skin blistering due to intraepidermal cleavage (within the basal layer of keratinocytes) [Bardhan et al., 2020]. In general, blistering is caused by trauma, rarely occurs spontaneously, and tends to heal without scarring. EBS has a variable spectrum of severity ranging from mild blistering of the hands and feet to generalized forms with extracutaneous involvement and is sometimes fatal. Onset varies by subtype and occurs, usually, at birth or during infancy, although patients with localized EBS may not develop their first blisters until adolescence or early adulthood [Sánchez-Jimeno et al., 2018; Fine, 2010]. Mutations in the *KRT5* and *KRT14* genes occur in 75% of cases with EBS [Lane et al., 1992]. The most recent EB classification includes 14 EBS subtypes based on the distribution and severity of the blisters, specific cutaneous lesions, mode of inheritance, affected gene/protein, and extracutaneous manifestations [Has et al., 2020].

EBS, Localized

The most common and mildest subtype of EBS is localized EBS, previously known as Weber–Cockayne disease, with a reported incidence of 3.67 per one million live births [Fine, 2016], but probably a significant percentage of mild cases remain undiagnosed. Localized EBS is characterized by the formation of blisters usually limited to the palms and soles of the feet. The lesions can also appear in other regions of recurrent trauma, such as the knees and shins of a crawling toddler or flexures during hot weather. The age of onset is variable, and the lesions frequently develop in infancy/early childhood and are rarely present at birth or appear in adolescence/adulthood. Nail involvement is uncommon. Common complications are secondary infections, especially foot blisters. Lesions worsen in the warmer months and some patients develop focal palmoplantar keratoderma during adulthood [Fine, 2010; Mariath, Santin, Schuler-Faccini, Kiszewski, 2020]. Intraoral blisters or ulcerations are seen during infancy and usually are asymptomatic [Fine, 2010; Wright, 2010]. The disorder has an autosomal dominant inheritance and is produced by missense mutations in the *KRT14* and *KRT5* genes [Pfendner, Sadowski, Uitto, 2005]. The mutations are located outside the highly conserved boundary motifs of the rod domain, usually in the head, tail, or non-helical portions, including the linker area of keratin. They are most frequently found in clusters including in the non-helical L12 linker motif, in the amino-terminal homologous domain (H1) of keratin-5, or in the 2B segment of keratin-14 [Szeverenyi et al., 2008; Chamcheu et al., 2011]. Hut et al. proposed a genomic mutation detection system for exons 1, 4, and 6 of *KRT14* that encode the 1A, L1-2, and 2B domains containing the mutation hotspots [Hut et al., 2000]. Jiang et al. suggest that in localized EB sequences, coding for the head and the non-helical linker regions of *KRT5* should have propriety for the mutation screening [Jiang, Zhu, Sun, Gu, 2021]. However, mutations were also discovered in the conserved 1A and 2B helix hotspots, but with conservative amino acid changes [Chamcheu et al., 2011]. This is consistent with a report by Cho et al. regarding the influence of polarity on the severity of EBS [Cho et al., 2014].

EBS, Severe, AD, KRT14/5

Severe EBS, with a reported incidence of 1.16 per one million live births, is characterized by generalized and severe blistering and birth-onset. A suggestive feature is the presence of multiple small blisters in a grouped or arcuate configuration, which explains the previous name “EB herpetiformis”. Hemorrhagic blisters are also present. The involvement of the oral mucosa and nail dystrophy are common. Mucosal involvement may interfere with feeding, especially in neonates and infants. Inflammation can occur in hemorrhagic blisters

followed by milia and hypo- and hyperpigmentation of the skin. The lesions tend to improve with age or paradoxically, in some cases, during periods of heat or fever. Progressive confluent palmoplantar keratoderma is common, being precocious (childhood-onset), and more severe than in other subtypes. This subtype is frequently associated with marked morbidity and in a minority of cases with neonatal/infancy mortality [Fine, 2010; Mariath, Santin, Schuler-Faccini, Kiszewski, 2020; Fine et al.,2008; Pfendner, Bruckner, 1998].

Dominant-negative mutations in *KRT14* and *KRT5* have been clustered in regions involved in the highly conserved ends of the rod domains or the helix boundary motifs of keratin. Substitutions are frequently reported and involve highly conserved amino acids within the helix initiation or termination motifs blocking the heterodimerization of keratin polypeptides. Common mutations change the glutamic acid from position 477 of keratin-5 (*KRT5* E477) or the arginine from position 125 of keratin-14 (*KRT14* R125). Both mutations cause the extensive formation of cytoplasmic protein aggregates, a hallmark of severe EBS [Fine, 2010]. Monoallelic in-frame deletion, splice-site, or nonsense mutations were also reported, leading to abnormal proteins with dominant-negative effects. Major changes in polarity or acidity are associated with this subtype [Has, Fischer, 2019; Sathishkumar et al., 2016; Coulombe]. Around 70% of cases with severe EBS are generated by one mutation in the *KRT5* gene (c.1429G > A; *p*.Glu477Lys or E477K) and three other in the KRT14 gene (c.373C > T [*p*.Arg125Cys or R125C]; c.374G > A [*p*.Arg125His or R125H]; c.368A > G [*p*.Asn123Ser or N123S]) [Pfendner, Sadowski, Uitto, 2005]. Vahidnezhad et al. reported a case with digenic inheritance, an association of a mutation in the *KRT5* gene and others in the *KRT14* gene [Vahidnezhad et al., 2016].

EBS, Intermediate, AD, KRT14/5

This subtype, previously known as Koebner EBS, has an intermediate phenotype, between localized EBS and severe EBS. Blisters appear at birth or in the first few months with generalized distribution, which are milder than those in severe EBS but without a "herpetiform" configuration. The frequency of milia, scarring, nail dystrophy, and oral lesions is intermediate between that of localized EBS and severe EBS. Focal palmoplantar keratoderma can be observed. Lesions worsen in the warmer months. Lesions tend to improve in adolescence when they may become localized to the hands and feet [Bardhan et al., 2020; Fine, 2010; Mariath, Santin, Schuler-Faccini, Kiszewski, 2020]. Pathogenic variants in the 1A or 2B segments (except the beginning of 1A, 1B, and the end of 1B, which associate severe phenotype) of the rod domain of *KRT5* and *KRT14* are common in intermediate EBS cases. These mutations do not interfere with the elongation process during filament assembly, so filaments essentially appear normal upon ultrastructural examination but are structurally weakened [Arin, 2009; Müller et al., 2006].

EBS, Intermediate or Severe, AR, KRT14/5

EBS due to *KRT14* or *KRT5* pathogenic variants is frequently inherited in an autosomal dominant mode but autosomal recessive cases were also reported. Most recessive cases are produced by *KRT14* variants and have a phenotype similar to previously described subtypes, but an improvement in blistering with age is not expected. Focal dyskeratotic skin lesions were also reported. Homozygous mutations in *KRT5* lead to severe phenotype, extracutaneous manifestations, and early mortality. Nonsense, missense, splice site, and deletions in *KRT14* have been associated with recessive inheritance. The unaffected parents of each patient were heterozygous for the respective mutations [Has et al., 2006]. Rugg et al. consider that these mutations are likely to be associated with a nonsense-mediated messenger RNA decay leading to a functional "knockout" of keratin-14 [Rugg et al., 1994]. Jonkman et al. suggested that increased expression of keratin-5 has a compensatory effect because keratin-14 knockout mice die within the first few weeks after birth [Jonkman et al., 1996].

EBS with Mottled Pigmentation

EBS with mottled pigmentation (EBS-MP) with a reported incidence of 0.07 per one million live births, presents generalized blistering from birth but the severity of the lesions is intermediate. The hallmark feature is mottled or reticulate macular pigmentation typically of the neck, upper trunk, and acral skin. Small hyperpigmented macules appear in early childhood, progress over time, and coalesce into a reticulate pattern. Hypopigmented macules are interspersed. The pigmentation does not occur in areas of blistering and often disappears in adult life. Punctate palmar and plantar keratoderma and nail dystrophy may occur. The majority of cases (more than 90%) presented a missense mutation (c.74C > T [*p*.Pro25Leu or P25L]) in the *KRT5* gene [Moog et al., 1999]. The pigmentary anomalies observed in this EB form could be correlated with the modification of melanosome transport where the non-helical head domain of keratin-5 is involved [Irvine et al., 2001, Uitto, Richard, McGrath, 2007]. However, the pathogenic mechanism is incompletely deciphered and some modifiers could interfere with the function of keratin -5. For example, in some cases the pathogenic mutation c.356T > C (*p*.Met119Thr or M119T) in the *KRT14* gene was identified [Harel, Bergman R, Indelman M, Sprecher, 2006].

EBS, Migratory Circinate

EBS, migratory circinate is a rare subtype, previously known as EBS with migratory circinate erythema. It is characterized by generalized blistering from birth with a background of inflammatory migratory circinate erythema that fades and heals with hyperpigmentation (sometimes with a mottled pattern) but without scarring. Nail dystrophy may occur. Some mutations were reported in this form of EB. For example, Gu et al. reported a heterozygous deletion c.1649delG (*p*.Gly550fs) in exon 9 of the *KRT5* gene which leads to a frameshift and delayed termination codon in two unrelated families with EBS, migratory circinate. Lee et al. identified a de novo in-frame 12-bp deletion in exon 7 of the *KRT5* gene, which alters the 2B domain of keratin-5 [Lee, Choi, Kim, Kim, 2018]. Mutations in the keratin-5 tail domain have been related to EBS with unusual features, such as mottled pigmentation and pigmentary disorders, suggesting a possible role of this domain in the regulation of inflammation and pigmentation [Gu et al., 2003; Kumagai et al., 2017].

EBS, AD, with PLEC Mutations

Previously known as Ogna EBS, this subtype presents birth-onset and mild skin blistering, mainly acral and occasionally widespread. The characteristic features are easy skin bruising with the formation of violaceous and hypopigmented macules. Koss-Harnes et al. found the same mutation in exon 31 of the *PLEC* gene (c.6328C > T [*p*.Arg2110Trp or R2110W]) in two unrelated families with Ogna EBS. This mutation changes the plectin polypeptide, which connects the basal keratins to the hemidesmosomal plaque, and generates an aberrant ultrastructure of hemidesmosomes attachment plates and a frequent fragmentation of hemidesmosomes [Koss-Harnes et al., 2002]. Bolling et al. identified mutations in *PLEC* in 6/16 of individuals with biopsy-proven EBS who lack identifiable pathogenic variants in *KRT5* or *KRT14* genes. They suggest that *PLEC* mutations may be more common than previously realized [Bolling et al., 2014].

EBS, AR, with PLEC Mutations

This rare subtype with recessive inheritance has a more severe phenotype than the dominant form. EBS with AR *PLEC* mutations is characterized by generalized skin blistering that heals with scarring and hyperpigmentation. Nail dystrophy is severe. Mucous membranes and the heart and muscles are spared [Bardhan et al., 2020]. Gostynska et al. identified homozygosity for a nonsense mutation c.46C > T [*p*.Arg16X] in the first exon of the gene encoding plectin isoform 1a, in two sisters from a consanguineous family. Plectin has eight

tissue-specific isoforms in humans, arising from the alternate splicing of the first exon. The isoform 1a is not expressed in striated or cardiac muscle tissue, so muscular dystrophy or cardiomyopathy are not expected to develop in these cases [Gostyńska et al., 2015].

EBS, Intermediate with Muscular Dystrophy

EBS, intermediate with muscular dystrophy (EBS-MD) is an autosomal recessive disorder characterized by early generalized blistering and variable (usually during childhood) onset of progressive limb-girdle type, muscular dystrophy. Considerable variability in the severity of the muscle weakness, sometimes not noticeable until the fourth decade of the patient's life, is reported. Onychodystrophy, focal plantar keratoderma, and mucosal involvement are common. Abnormal dentition (decay teeth), upper respiratory tract stenosis, urethral strictures, dilated cardiomyopathy, ventricular hypertrophy, and alopecia have been reported [Vahidnezhad, Youssefian, Saeidian, Uitto, 2019]. The majority of EBS-MD patients present compound heterozygous or homozygous truncation mutations in exon 31 of the *PLEC* gene, which encodes the rod domain of plectin. Natsuga et al. examined plectin expression in the skin of patients with *PLEC* mutations. In EBS-MD, the expression of the N- and C-terminal domains of plectin remained detectable, although the expression of rod domains was absent or markedly reduced. The alternative splicing of exon 31, resulting in a rodless but still partially functional plectin, was suggested to account for the milder phenotype. Few EBS-MD cases have in-frame mutations in the N-terminal domain of plectin, where the actin-binding domain (ABD) and spectrin repeats are conserved. It is possible that the binding deficits with integrin beta-4 and the collagen alpha-1(XVII) chain, which may explain the phenotype [Pfendner, Rouan, Uitto, 2005; Sawamura et al., 2007; Natsuga et al., 2010; Has et al., 2018].

EBS, Severe with Pyloric Atresia

EBS, severe with pyloric atresia (EBS-PA) is characterized widespread generalized blistering or an absence of skin at birth and pyloric atresia. Antenatally, pyloric atresia can manifest with polyhydramnios. Additional features include failure to thrive, aplasia cutis, anemia, sepsis, intraoral blistering, urethral stenosis, and urologic complications. Death usually occurs in infancy [Vahidnezhad, Youssefian, Saeidian, Uitto, 2019]. Immunohistochemical studies showed an absent expression of plectin. EBS-PA patients typically have nonsense or frameshift mutations, outside of exon 31 in *PLEC* gene, which leads to loss of both full-length and rodless plectin. Inheritance is autosomal recessive [Natsug, 2015].

EBS, Intermediate with Cardiomyopathy

This subtype is characterized by marked erosions in the limbs at birth, healing with dyspigmentation and cribriform atrophic scars, follicular atrophoderma, and late-onset dilated cardiomyopathy. Keratoderma, milia, nail and oral involvement, and progressive diffuse alopecia are reported [Bardhan et al., 2020; Has, Fischer, 2019; Has, 2017]. All cases had a heterozygous gain-of-function in *KLHL24* gene start codon mutation, with c.1A-G being the most prevalent. This mutation produces a truncated KLHL24 protein lacking the initial 28 amino acids (KLHL24-ΔN28). The substrate of the KLHL24 protein is keratin-14 and the more stable KLHL24-ΔN28 due to gain-of-function variants inducing the excessive ubiquitination and degradation of keratin-14. Hee et al. consider that *KLHL24* gene mutations disturb the turnover and degradation of intermediate filaments [He et al., 2016]. Schwieger-Briel et al. showed that KHL24 is expressed at similar levels in keratinocytes and cardiomyocytes and may disrupt the degradation of the structural cytoskeletal proteins involved in mechanical resilience [Schwieger-Briel et al., 2019]. Hedberg-Oldfords et al. reported familial cases with cardiomyopathy due to *KHLH24* gene mutation with polyglucosan accumulation in some cardiomyocytes and with an accumulation of glycogen,

desmin, and tubular structures in the cardiomyocytes and in skeletal muscle fibers. They suggest a pivotal role for KLHL24 during cardiogenesis, based on strong *KLHL24* gene expression in early ventricular myocytes and later in the established heart ventricle. As desmin is the cardiac homologue of keratin-14, Vermeer et al. hypothesized that KLHL24-ΔN28 leads to the excessive degradation of desmin, affecting tissue morphology and function. Also, dominant mutations in desmin are associated with a severe form of cardiomyopathy [Hedberg-Oldfors et al., 2019; Yenamandra et al., 2018; Vermeer et al., 2021].

EBS, Localized or Intermediate with Dystonin Deficiency

EBS, localized or intermediate with dystonin (BP230) deficiency presents an early-onset with predominantly acral blistering, larger (several centimeters) than in localized EBS. The blisters appear in areas of mechanical trauma but also in non-pressure-prone sites. Blistering could heal without scarring or with post-inflammatory hypo- or hyperpigmentation. Asymptomatic plantar keratoderma was reported [McGrath, 2015]. Loss-of-function mutations in the *DST* gene lead to a complete absence of hemidesmosomal plaques, a loss of adhesion, and increased cell spreading and migration. Reduced integrin beta-4 at the cell surface and increased levels of keratin-14 and integrin beta-1 were detected in abnormal cells. Mild phenotype, in contrast to autoimmune bullous pemphigoid who have autoantibodies to dystonin, could be explained by an upregulation of keratin-14 expression. The inheritance is autosomal recessive, but a semi-dominant transmission mode is also plausible because some heterozygous recall some blistering in childhood [Has, Fischer, 2019; McGrath, 2015; Ganani et al., 2021].

EBS, Localized or Intermediate with Exophilin-5 Deficiency

EBS, localized or intermediate with exophilin-5 deficiency is characterized by localized or generalized intermittent blistering with onset at birth or in early childhood. Skin fragility improves with age, but lesions could heal with hypopigmentation or mottled pigmentation, especially on the trunk and proximal limbs. Skin atrophy and acral blistering with hemorrhagic crusts are cited. Diociaiuti et al. consider that the lack of extracutaneous and adnexal involvement, together with the modest phenotype, differentiates this subtype from the common dominant EBS-MP due to keratin mutations [Bardhan et al., 2020; Diociaiuti et al., 2020]. McGrath et al. reported disruption of the keratin filament network, more cortically distributed F-actin, and significantly reduced cell adhesion in keratinocytes from patients with truncating mutations in *EXPH5*. Monteleon et al. demonstrated that exophilin-5 is involved in the delivery of lysosome-related organelles (LROs) to the plasma membrane and is essential for the differentiation of human keratinocytes. LROs are also involved in the packaging and trafficking of melanin, which may explain the pigmentation anomalies. Nonsense and frameshift mutations with autosomal recessive inheritance were reported [Has; McGrath et al., 2012; Turcan et al., 2016; Monteleon Lee, Ridky, 2019].

EBS, Localized with Nephropathy

EBS, localized with nephropathy presents early-onset blistering, particularly on pretibial areas associated with nephropathy. Early alopecia, poikiloderma, and nail dystrophy may occur. Involvement of the ocular, oral, gastrointestinal (including esophageal webbing), and urogenital mucosal membranes is reported. Nephropathy manifests with proteinuria and progression to end-stage renal disease [Bardhan et al., 2020; Has, Fischer, 2019; Vahidnezhad et al., 2018]. Homozygous frameshift and splice-site mutations in exon 5 of the *CD151* gene leading to truncated proteins without an integrin-binding domain, were reported [Crew et al., 2004; Vahidnezhad, Youssefian, Saeidian, Uitto, 2019; Vahidnezhad et al., 2018; Naylor et al., 2022].

4.3.3.2. Junctional EB

Junctional EB (JEB) is a disease with different prevalence in different geographic areas. The USA National EB Registry reported a prevalence of 0.49 per one million population, whereas the Dystrophic Epidermolysis Bullosa Research Association of America showed a prevalence of 3.59 per million per one million population. In Germany, the prevalence of disease was estimated at 6.7 per one million population [Fine, 2016; Kelly-Mancuso, Kopelan, Azizkhan, Lucky, 2014; Hammersen et al., 2016]. Early lethality of severe forms could explain the differences. The incidence is higher in the Middle East, due to the high inbreeding coefficient. The inheritance is autosomal recessive, but germline mosaicism and uniparental isodisomy were reported [Fassihi et al., 2005; Cserhalmi-Friedman, Anyane-Yeboa, Christiano, 2002; Abu Sa'D et al., 2006]. In JEB skin cleavage occurs within the lamina lucida of the basement membrane zone. The severity of cutaneous and mucosal fragility varies considerably ranging from forms with early lethality to milder phenotypes. A characteristic feature is represented by mature dental enamel anomalies ranging from small pits in the enamel surface to generalized hypoplasia. Impaired adhesion of the odontogenic epithelium from which ameloblasts are derived is involved in abnormal enamel formation [Bardhan et al., 2020; Fine, 2010; Wright, 2010]. On a clinical basis, JEB was divided into several categories.

JEB, Severe

In severe JEB, previously known as Herlitz JEB, extensive mucocutaneous blistering with early-onset (at birth or in the neonatal period) may lead to large erosions with extensive loss of proteins, fluids, and iron, which increases susceptibility to infection and electrolyte imbalance. Sometimes, at birth, blisters may be mild and localized to periungual, buttock, or elbow regions [Bardhan et al., 2020; Fine, 2010]. The pathognomonic feature is an exuberant granulation tissue located in orofacial (which produces microstomia), periungual, or friction regions, Accumulation of subglottic granulation tissue may lead to a weak, hoarse cry, stridor, and respiratory distress. Alopecia and mature dental enamel defects are common. Involvement of the mucous membranes of the upper respiratory tract, esophagus, bladder, urethra, rectum, and cornea has been reported. Scarring pseudosyndactyly of the hands and feet with severe loss of function has been cited. JEB has the highest risk of infant mortality among the EB subtypes, and the major causes are sepsis, failure to thrive, or tracheolaryngeal obstruction [Bardhan et al., 2020; Fine, 2010; Mariath, Santin, Schuler-Faccini, Kiszewski, 2020; Wright, 2010; Kelly-Mancuso, Kopelan, Azizkhan, Lucky, 2014]. Biallelic mutations in the *LAMA3*, *LAMB3*, and *LAMC2* genes were identified in severe JEB. Varki et al. reported a high proportion of *LAMB3* mutations. The majority lead to premature stop codons, mRNA decay, and synthesis of no protein or to truncated unstable polypeptides. The most frequent mutation in the *LAMB3* gene (45–63%) is c.1903C > T (*p*.Arg635Ter or *p*.R635X) [Rousselle, Beck, 2013]. The distribution of laminin 332 in multiple epithelial basement membranes, including those of the cornea, kidney, lung, thymus, brain, gastrointestinal tract, and lung explains the extracutaneous features [Varki, Sadowski, Pfendner, Uitto, 2006; Kiritsi, Has, Bruckner-Tuderman, 2013]. However, Abu Sa'd et al. reported a case of severe lethal JEB caused by a homozygous mutation in the *COL17A1* gene [Abu Sa'D et al., 2006].

JEB, Intermediate

JEB, intermediate previously called JEB non-Herlitz, presents a less severe clinical phenotype than JEB severe, with a reduced tendency to develop exuberant granulation tissue. Generalized blisters (that predominate in sites exposed to friction, trauma, or heat), heal with atrophy and pigmentation anomalies. Alopecia, enamel defects, and dystrophy or absence of nails are common. Also, a milder involvement of the mucous membranes of the upper

respiratory tract (with a lower risk of upper airway occlusion), bladder, and urethra was reported, and adult patients have an increased risk of developing squamous cell carcinoma on their lower extremities in areas of chronic blistering, long-standing erosions, or atrophic scarring [Fine, 2010; Mariath, Santin, Schuler-Faccini, Kiszewski, 2020; Yuen, Jonkman, 2011]. Specific mutations in the *LAMA3*, *LAMB3*, and *LAMC2* genes (missense or splice-site or compound heterozygosity) that lead to partially functional laminin 332 are reported in this subtype. The intermediate JEB phenotype is also associated with mutations in the *COL17A1* gene. The hallmark of these phenotypes was the total lack of collagen XVII in the skin due to different mutation mechanisms (nonsense/insertions and deletions predicted to result in premature termination/splice site with the production of truncated unstable molecules). The majority of mutations were located in exons 51 and 52. Notably, splice-site mutations occurred preferentially in intron 51 [Kiritsi et al., 2011]. In this subtype, Jonhman and Pasmooji reported the first cases with revertant mosaicism, both in collagen and laminin deficiency, sustained by the reexpression of the deficient protein on skin specimens. Revertant mosaicism has since been documented in EB forms, implicating the *COL17A1*, *KRT14*, *LAMB3*, *COL7A1*, and *FERMT1* genes [Jonkman, Pasmooij, 2009; Meyer-Mueller et al., 2022]. Cases with self-improving JEB and milder than expected phenotypes were also reported. The possible underlying molecular mechanisms are an alternative modulation of splicing, a spontaneous readthrough of premature termination codons, or a skipping of exons containing stop codons [Has et al., 2020; Chavanas et al., 1999; McGrath et al., 1999; Pacho et al., 2011].

JEB, with Pyloric Atresia

JEB, with pyloric atresia, presents an association between generalized blistering at birth and pyloric atresia. Other gastrointestinal anomalies, such as duodenal and anal atresia, are rarely reported. Aplasia cutis congenita, atrophic scarring, enamel anomalies, oral involvement, and nail dystrophy with patulous nail folds are common. Exuberant granulation tissue in the perioral, neck, and upper back regions may occur. This disorder is associated with a significant risk of genitourinary anomalies (polypoid bladder lesions, urethral stricture, dysplastic kidney, hydronephrosis, ureterocele) and infantile or neonatal death [Varki, Sadowski, Pfendner, Uitto, 2006; Pfendner, Lucky, 2008; Chung, Uitto, 2010]. JEB with pyloric atresia is associated with mutations in the *ITGA6* or *ITGB4* genes. The majority of the mutations reside in the *ITGB4* gene, being nonsense or missense mutations in the amino-terminal extracellular domain that facilitate the association of the alpha-6 or beta-4 subunits. Loss of function mutations in the *ITGA6* gene have been identified in some cases [Varki, Sadowski, Pfendner, Uitto, 2006; Vahidnezhad, Youssefian, Saeidian, Uitto, 2019; Lestringant et al., 2003]. The absence of alpha-6 integrins modifies the adhesion of the collecting duct cells to the basal membrane and makes the kidney-collecting system susceptible to degeneration and injury, which explains renourinary features [Viquez et al., 2016]. DeArcangelis et al. demonstrated by alfa-6 integrin ablation in mice that loss of intestinal epithelial cells/basal membrane interactions initiates the development of inflammatory lesions that progress into high-grade dysplasia and carcinoma [De Arcangelis et al., 2017].

JEB, Localized

Localized JEB is characterized by mild blistering, often acral, variable nail dystrophy, enamel defects, and a tendency to develop cavities. In contrast to the other JEB subtypes, alopecia, extensive atrophic scars, and extracutaneous findings are rarely reported [Mariath, Santin, Schuler-Faccini, Kiszewski, 2020; Kiritsi et al., 2011]. Mutations in the *LAMA3*, *LAMB3*, *LAMC2*, *COL17A1*, *ITGB4*, and *ITGA3* genes were reported. Mutations allowing the expression of a residual protein (usually missense or splicing) lead to this mild phenotype. Condrat and Has stated that as little as 5–10% of residual protein, even if truncated and

putatively partially functional, significantly alleviates the phenotype. Some mutations in *COL17A1* that are predicted to lead to a premature stop codon (associated with severe phenotype) escape this outcome because of alternative splicing. Out of the 56 exons of *COL17A1*, 54 are in-frame and can be skipped without shifting the reading frame [Has et al., 2018; Kowalewski et al., 2016; Condrat, He, Cosgarea, Has, 2019].

JEB, Inversa

Congenital blistering and erosions confined to flexural areas are suggestive of this rare form of JEB. Blistering is usually severe and may heal with atrophic scarring and milia formation. Nail dystrophy, enamel anomalies and dental caries, oral, esophageal, and vaginal involvement are common. Reduced expression of laminin 332 due to biallelic mutations in the *LAMA3, LAMB3,* and *LAMC2* genes was reported [Fine et al., 2008; Gedde-Dahl et al., 1994].

JEB, Late-Onset

In JEB with late-onset (JEB-lo) the blistering starts in childhood and affects the hands and feet and, to a lesser extent, the elbows, knees, and oral mucosa. Other clinical features are palmoplantar hyperhidrosis, enamel defects, and progressive skin atrophy. The disappearance of dermatoglyphs because of scarring is reported [Bardhan et al., 2020]. Yuen et al. reported cases with mutations located in the fourth noncollagenous domain (NC4) of the alpha-1(XVII)-chain gene (c.3908G > A, [*p*.R1303Q or *p*.Arg1303Gln]), which are predicted to affect protein folding and laminin 332 binding. They suggested that missense mutations located in the NC4 domain may be specific for JEB-lo [Yuen, Jonkman, 2011].

Laryngo–Onycho–Cutaneous Syndrome

Laryngo–onycho–cutaneous syndrome (LOC), previously called Shabbir syndrome, is characterized by a hoarse cry in the neonatal period, by marked exuberant granulation tissue, in particular affecting the larynx, conjunctiva, and periungual/subungual sites, and by skin blistering and erosions. In contrast to the excessive blistering and erosions described in severe JEB, patients with LOC have minimal blistering but more extensive granulation tissue. Ocular granulation tissue may extend leading to symblepharon and corneal opacification (suggestive features of LOC). Progressive laryngeal granulation can lead to severe respiratory compromise and premature death. Aberrant granulation tissue could also develop on the face, neck, epiglottis, trachea, and main bronchi. Nail dystrophy and enamel anomalies are common [Bardhan et al., 2020; Fine et al., 2008; McLean et al., 2003; Siañez-González Pezoa-Jares, Salas-Alanis, 2009]. In the affected members of 15 families, McLean et al. identified a homozygous single nucleotide insertion in the *LAMA3* gene (c.151dup; [V51fs]), predicting a stop codon in exon 39 that is specific to laminin alpha-3A, a protein secreted only by the basal keratinocytes of stratified epithelia. They suggested that LOC may be caused by the dysfunction of keratinocyte–mesenchymal communication and hypothesized that the laminin alpha-3A N-terminal domain may be a key regulator of the granulation tissue response. All cases reported are Punjabi origin, suggesting a possible founder effect. Prodinger et al. reported 3 new mutations in the *LAMA3* gene, outside exon 39 and underscores that molecular diagnostics can be challenging [McLean et al., 2003; Prodinger et al., 2021].

JEB, with Interstitial Lung Disease and Nephrotic Syndrome

The association of congenital nephrotic syndrome, interstitial lung disease, and skin fragility is suggestive of JEB with interstitial lung disease and nephrotic syndrome (ILNEB). The respiratory and renal features predominate and rapid progression usually leads to death in early infancy. The renal anomalies occurring in patients with ILNEB include congenital nephrotic syndrome, focal-segmental glomerulosclerosis, bilateral renal cysts, unilateral

kidney hypoplasia, and ectopic conjoint kidney. Patients present variable degrees of cutaneous involvement, nail dystrophy, and sparse hair. Cases with mild phenotypes (without renal anomalies or without lung disease) were reported [Has, Fischer, 2011; Colombo et al., 2016; Kinyó et al., 2021]. Biallelic mutations (missense, frameshift, or in splice sites) in the *ITGA3* gene were identified. Has et al. reported cases with functionally null mutations and a severe course of disease. Mutations allowing expression of a residual, truncated, or dysfunctional protein may lead to a milder phenotype and improved survival. Liu et al. stated that the phenotype of the *ITGA3* gene mutation may be determined by the residual function of the mutant integrin alpha-3 strain [Has et al., 2012; Liu et al., 2021].

4.3.3.3. Dystrophic EB

In dystrophic EB (DEB) the plane of skin cleavage is below the lamina densa in the most superficial portion of the dermis. DEB may be inherited in a dominant (DDEB) or recessive (RDEB) pattern. The prevalence of DDEB and RDEB is quite similar: 1.49 and 1.35 per one million live births respectively [Fine, 2016]. In DEB, blisters, and ulcerations heal with significant scarring and milia formation. Generally, the recessive form is more severe than DDEB; however, there is significant phenotypic overlap between subtypes. All subtypes of DEB are caused by mutations in the *COL7A1* gene, the gene coding collagen VII, the main constituent of the anchoring fibrils at the cutaneous basement membrane zone [Has et al., 2020; Mariath, Santin, Schuler-Faccini, Kiszewski, 2020; Has et al., 2018]. Hovnanian et al. stated that the nature and location of these mutations are important determinants of the phenotype [Hovnanian et al., 1997]. Mariath et al. suggested that the DEB phenotype is determined by the expression and residual function of collagen VII [Mariath, Santin, Schuler-Faccini, Kiszewski, 2020].

DDEB

The majority of DDEB cases result from dominant-negative mutations. Missense substitutions that replace glycine in the collagenous triple-helical domain (frequently in exons 73-75) are reported in over 75% of cases. The most common DDEB-causing mutations are c.6100G > A (*p*.Gly2034Arg or G2034R) and c.6127G > C (*p*.Gly2043Arg or G2043R) [Varki, Sadowski, Uitto, Pfendner, 2007; Hammami-Hauasli, Raghunath, Bruckner-Tuderman, Küster, 1998]. The conservation of glycine residues in every third position of the amino acid sequence is required for the tied packing of the triple helix and these substitutions highly destabilize the triple helix [Persikov et al., 2004]. Other substitutions, insertions, deletions, and splice-site variants have also been described. These mutations involve amino acids essential for the structure of the triple helix and the stability of the anchoring fibrils. However, an inter- and intrafamilial phenotypic variability is reported [Chung, Uitto, 2010; Nakamura et al.,2004; Dang, Murrell, 2008].

Intermediate DDEB

This subtype presents with generalized blisters from birth or early infancy, milia, albopapuloid lesions, atrophic scarring, and nail dystrophy. Acral sites, elbows, and knees are commonly affected. Mucous membranes may also be involved leading to microstomia, ankyloglossia, and esophageal stenosis, although less commonly than in severe RDEB [Has et al., 2020; Mariath, Santin, Schuler-Faccini, Kiszewski, 2020].

Localized DDEB

Blistering is confined to the hands, feet, and milia and atrophic scars can also occur. There is no extracutaneous involvement. Rare cases with progressive nail dystrophy and without any other sign of skin fragility are reported [Yang et al., 2020]. A pretibial form with

the development of lesions predominantly in the anterior lower legs is described [Christiano et al., 1995].

RDEB - Severe RDEB

The most severe subtype of DEB, formerly known as Hallopeau-Siemens RDEB, is associated with generalized blistering at birth, progressive extensive scarring, and development of microstomia, ankyloglossia, esophageal stenosis, flexion contractures of limbs, and pseudosyndactyly. Alopecia, milia, and permanent loss of nail plates are common. Eye involvement with corneal erosions, symblepharon, ectropion, and loss of vision is also observed [Fine et al, 2008; Fine et al., 2005; Tong et al., 1999]. The lifetime risk of aggressive squamous cell carcinoma is greater than 90% [Fine et al., 2009]. Biallelic nonsense or frameshift *COL7*A1 gene mutations (insertions/deletions, substitutions, or splice sites) that result in premature termination codons were reported. The consequences for the protein are severe: the absence of or a markedly reduced collagen VII [Varki, Sadowski, Uitto, Pfendner, 2007; Dang, Murrell, 2008; Fine et al., 2009; Gardella et al., 2002].

RDEB - Intermediate RDEB

Phenotype is similar to intermediate DDEB, but with greater severity of joint contractures and pseudosyndactyly in some cases. Extracutaneous involvement is milder than in severe RDEB. The risk of developing squamous cell carcinomas is also increased (47.5% by age 65) but less common than in severe RDEB and neoplasia occurs later in adulthood [Bardhan et al., 2020; Fine et al., 2005; Fine et al., 2009]. Many patients are compound heterozygous for a premature stop codon and a glycine substitution within the collagenous domain. The mutations may affect the association of polypeptides and the stability of the triple helix or may cause conformational change [Varki, Sadowski, Pfendner, Uitto, 2006; Chung, Uitto, 2010; Dang, Murrell, 2008].

RDEB, Inversa

This rare subtype is characterized by a peculiar course. Generalized blistering of intermediate severity occurs in the neonatal period, improves with age, and tends to localize to flexure sites in adults. Mucosal involvement (oral, esophageal, anal, genitourinary) is similar but milder than in severe RDEB [Fine et al. 2010; Akker et al., 2011; Chiaverini et al., 2010]. Van den Akker et al. reported specific glycine or arginine substitutions in the carboxyl portion of the triple-helical domain caused by a missense mutation in the *COL7A1* gene. Patients were homozygotes or compound heterozygotes (missense mutation/loss of function mutation). The localization of the amino acid substitutions in specific domains correlates with the synthesis of a thermolabile collagen VII that is specifically less stable in the warm flexural regions [Akker et al., 2011; Chiaverini et al., 2010].

RDEB, Localized

The phenotype is similar to localized DDEB. Splice-site mutations and other amino acid (non-glycine) substitutions were reported. In localized RDEB, splice-site mutations result in exon skipping, without altering the remaining protein sequence. This abnormal collagen VII allows the assembly of the anchoring fibrils with small functional defects, which explains the phenotype [Schwieger-Briel et al., 2019; Hovnanian et al., 1997; Gardella et al., 2002].

DEB, Pruriginosa

DEB, pruriginosa *(DEB-Pr)* is an unusual subtype that presents blistering in infancy and late-onset (adolescence/adulthood) of intense pruritus and linear cords of lesions (papules, nodules), especially on the extensor surfaces of the limbs (initially on the lower legs). Nail dystrophy, milia, and atrophic scarring are common [Bardhan et al., 2020]. Cases with

autosomal dominant and autosomal recessive inheritance have been described and glycine substitutions in the collagenous domain, splice-site mutations, and small deletions have been reported. Some of these mutations have been reported in cases with other subtypes of DEB, without pruritus. No specific correlation of the genotype–phenotype has been established. Patients were shown to synthesize a normal or variably reduced amount of type VII collagen, which was correctly deposited at the dermal–epidermal junction [Nakamura et al., 2004; McGrath Schofield, Eady, 1994; Mellerio et al., 1999; Murata et al., 2000; Drera et al., 2006]. Studies have excluded other triggering factors, including atopy, elevated IgE levels, matrix metalloproteinase 1 gene polymorphisms, filaggrin gene mutations, and interleukin 31 gene haplotypes [Drera et al., 2006; Almaani et al., 2009; Schumann, Has, Kohlhase, Bruckner-Tuderman, 2008; Nagy, Tanaka, Techanukul, McGrath, 2010].

DEB, Self-Improving

Previously known as transient bullous dermolysis in a newborn, this rare subtype is characterized by generalized blistering at birth followed by significant improvement within the first 2 years of life [Fine, 2010]. Both dominant and recessive inheritance have been reported in cases of self-improving DEB. The most frequently reported mutations are glycine substitutions and splice-site variants resulting in the skipping of exons (e.g., exon 36). The immunofluorescence shows the accumulation of granular intraepidermal deposits of collagen VII, which regresses with time [Fine et al. 1990; Hatta, Takata, Shimizu, 1995]. Christiano et al. suggested that with advancing age, the abnormal polypeptides become degraded at an increasing rate, thus diminishing their dominant-negative effects. The genotype–phenotype relationship remains unclear because of the limited number of cases [Christiano, Fine, Uitto,1997; Fassihi et al., 2005; Shi et al., 2015].

DEB, Severe, Dominant, and Recessive

The phenotype is indistinguishable from severe RDEB, with severe mucocutaneous involvement from birth. Compound heterozygosity for dominant *COL7A1* glycine substitution mutation and recessive mutation (frameshift leading to a premature termination codon) on the second allele has been reported [Varki, Sadowski, Pfendner, Uitto, 2006; Christiano et al., 1996; Weinel et al., 2008; Turczynski et al., 2016].

4.3.3.4. Kindler EB

In contrast to other types of EB, Kindler EB (KEB) presents a blister formation at different levels of the dermal–epidermal junction: below the lamina densa, within the lamina lucida, or within basal keratinocytes. A single or multiple cleavage planes may be seen within the same sample of skin. KEB manifests with generalized blistering (more prominent on extremities) at birth followed by the development of photosensitivity and progressive poikiloderma. Palmoplantar keratoderma and skin atrophy may occur. Extracutaneous findings include chronic gingivitis, periodontitis, esophageal strictures, ectropion, anal stenosis, and colitis. Pseudosyndactyly has been reported. Patients with KEB have an increased risk of developing cutaneous squamous cell carcinoma (66.7% in those >60 years of age), usually occurring in the fourth to fifth decade of life [Fine, 2010; Guerrero-Aspizua et al., 2019]. KEB is caused by a homozygous mutation in the *FERMT1* gene. Zhang et al. suggested that fermitin family homolog 1 is also important for the suppression of UV-induced inflammation and DNA repair [Zhang et al., 2017]. The protein is predominantly expressed in the epithelial cells in the skin, oral mucosa, and the gastrointestinal tract, explaining the distribution of manifestations [Jobard et al., 2003; Meves, Stremmel, Gottschalk, Fässler, 2009]. Deletions, insertions, nonsense, splice-site, and missense mutations (majority loss-of-function) have been reported. Has et al. suggested that mutations compatible with the expression of an abnormal protein (e.g., in-frame) will translate into mild phenotypes, whereas null mutations cause severe forms [Has et al., 2011].

4.3.4. Conclusions

Epidermolysis bullosa is characterized by high-clinical, allelic, and locus heterogeneity. These features could be explained by the multitude of proteins that are involved in communication and signaling at the basal layers of the skin. In addition, the phenotypes are overlapping and different mutations in the same genes produce different forms of the disease. The deciphering of pathogenic mechanisms, corroborated with the discovery of the genotype–phenotype correlations, will offer the basis of personalized management and the prevention of complications.

4.4. Study of *SHOX* deletion

4.4.1. Introduction

Idiopathic short stature (ISS) is usually defined in children as GH-sufficient (normal response at provocative tests), with height < −2 SD for age, sex, and population group, in the absence of chromosomal, systemic, endocrine, or nutritional abnormalities, normal birth weight and length (birth weight and length > −2 SD), harmonious small stature and absence of major dysmorphic features, without psychosocial problems, and with proper nutrition [Rapaport, Wit, Savage, 2021]. Idiopathic short stature or small for gestational age (SGA) are not a definitive diagnose. Labelling children only as having idiopathic low stature or SGA after initial clinical, endocrine, and radiological evaluation explains the highly variable response to growth hormone treatment in these cases. However, in some rare situations, growth hormone treatment can be harmful, as in Bloom syndrome or Nijmegen syndrome.
Short stature is caused by variants with polygenic inheritance. Recent studies have proposed that many children classified as ISS could have a monogenic defect with the mildest spectrum of syndromic conditions. In these cases, growth impairment may be caused by defects of the GHRH-GH-IGF1 cascade or factors involved in growth plate development. The latest are gene mutations of different local factors associated with short stature and phenotypes varying from severe, disharmonious dwarfism to subclinical skeletal dysplasia [Dauber, Rosenfeld, Hirschhorn, 2014; Karimian, Chagin, Sävendahl, 2012; Vasques, Andrade, Jorge, 2019].

The longitudinal bone growth is a coordinated process of chondrocyte proliferation, maturation, and hypertrophy; matrix synthesis with vascular invasion at the end; migration of osteoblasts/osteocytes, and other bone marrow cell types. Critical regulators of cartilage and bone formation are: hormones (GH, IGFs, T3/T4, vitamin D, glucocorticoids, oestrogens, and androgens), paracrine factors (retinoic acid, Indian hedgehog protein, and PTHrP (PTH related peptide), BMPs (bone morphogenetic proteins), FGFs (fibroblast growth factors), and their receptors, CNP (C-type natriuretic peptide), and its receptor, proinflammatory cytokines (TNF, IL-1β, and IL-6), extracellular matrix molecules and intracellular proteins.

Short Stature Homeobox (SHOX) protein, encoded by *SHOX* gene, is such of intracellular proteins. The SHOX protein contains a homeodomain, a structure frequently seen in transcription factors involved in body patterning. As a transcription activator, it stimulates and coordinates the proliferation and differentiation of chondrocytes, increases natriuretic peptide B (NPPB) transcription, inhibits *FGFR3* gene expression, and interacts with the SOX trio (*SOX9, SOX5,* and *SOX6* genes), which function as the significant chondrogenic factors activating the enhancer of the *ACAN* gene [Marchini, Ogata, Rappold, 2016; Faienza et al., 2021; Fukami, Seki, Ogata, 2016; Spurna et al., 2022].

SHOX deletion

SHOX deletion has become the main recognized monogenic cause, responsible for 2.6% of the nonsyndromic cases of short stature [Rapaport, Wit, Savage, 2021; Child, Rappold, Blum, 2012].

The *SHOX* gene is located on the tip of the short arms of both sex chromosomes X and Y, inside the telomeric part of pseudoautosomal region 1 (PAR1) of the X chromosome at Xp22.3 and the Y chromosome at Yp11.3. Genes in this region of the sex chromosomes do not undergo X-inactivation; therefore, two active copies are available for normal skeletal growth and development in both males (46, XY) and females (46, XX) [Rapaport, Wit, Savage, 2021; Binder, 2011]. There is no difference between the *SHOX* gene in female and male subjects. The *SHOX* region is a hotspot for frequent recombination events between the X and Y chromosomes [Binder, 2011]. A spatiotemporally restricted expression pattern of *SHOX* exists in the middle portion of the arm, elbow, radius, ulna, and a few wrist bones; on the lower limbs, the expression resembles the upper limb development. *SHOX* is also present in the first and second pharyngeal arches (development of the maxilla, mandible, and some ear bones). Another gene *SHOX2* is also expressed in limbs and the developing heart [Marchini, Ogata, Rappold, 2016; Fukami, Seki, Ogata, 2016; Spurna et al., 2022].

Clinical signs that are more or less specific for *SHOX* deletion: low arm span/height ratio; height sitting height/height ratio; extremity-to-trunk ratio < 1.95 + 0.5× height (metric); BMI > 50th percentile; muscular hypertrophy appearance**,** especially in the calves, has been reported in about one-third of *SHOX*-deficient patients [Gürsoy et al., 2020]; Madelung deformity; cubitus valgus with increased carrying angle of the elbow; bowing and shortening of the forearm; shortening of the fourth and fifth metacarpals; high arched palate; scoliosis; micrognathia [Fukami, Seki, Ogata, 2016; Binder, Ranke, Martin, 2003; Rappold *et al.*, 2007].

Some clarifications are necessary regarding these elements:

- the arm span is significantly reduced compared to the standing height;
- the leg length is considerably shorter than expected from the sitting height due to the mesomelia associated with SHOX deficiency;
- the extremities-to-trunk ratio is not applicable to children younger than six years old when this body disproportion is normal;
- longitudinal follow-up studies of female patients with *SHOX* haploinsufficiency showed that body disproportion often deteriorates during puberty. Hence, a normal ratio in a schoolgirl excludes *SHOX* deficiency with a high negative predictive value of almost 100% [Fukami, Seki, Ogata, 2016; Marstrand-Joergensen et al., 2017];
- BMI above the 50th percentile for age/sex is a significant predictor, reflecting disproportion due to decreased leg length and muscular hypertrophy rather than increased fat mass. Notably, children with idiopathic short stature without *SHOX* deficiency typically have a decreased BMI [Gürsoy et al., 2020].

Rappold et al. developed a scoring system that includes the body segment ratio and other indices of SHOX deficiency. At a cutoff score of 7 (of a total score of 24), the positive prediction rate for identifying a *SHOX* gene point mutation or deletion was 19%. However, downstream or upstream SHOX enhancers are unknown at the moment [Rappold *et al.*, 2007]. In addition, another diagnostic algorithm was elaborated in 2020 by Vannelli et al. [Vannelli et al., 2020].

Phenotypic variation exists among individuals with SHOX deficiency because a high-arched palate is more prevalent in Turner syndrome, whereas Madelung deformity, a short forearm and lower leg, bowing of the forearm and tibia, and muscular hypertrophy are more prevalent in Léri–Weill syndrome [Child, Rappold, Blum, 2012].

In Turner syndrome, there are probably other factors (haploinsufficiency of other genes on the X chromosome, chromosomal imbalance) that contribute to the short stature. The SHOX deficiency in Turner syndrome may explain cubitus valgus, Madelung deformity, mesomelia, disproportionate skeletal sizes, a high-arched palate, and micrognathia [Child, Rappold, Blum, 2012].

Radiological characteristics in SHOX deficiency are: triangulation of the distal radial epiphysis; lucency of the ulnar border of the distal radius; the enlarged diaphysis of the radius and bowing of the radius; short fourth and fifth metacarpal; pyramidalization of the carpal row; convexity of the distal radial metaphysis [Vannelli et al., 2020].

SHOX-deficient short stature should be suspected in children with a first-degree relative with SHOX deficiency and one of the following: disproportionate short stature (young school age); Madelung deformity (older school age); short stature and specific minor abnormalities [Binder, 2011; Binder, Rappold, 2005; Rappold *et al.*, 2007; Vannelli et al., 2020; Ezquieta et al., 2002; Ogata, 2002].

Binders et al. and Vanneli et al. have been proposed several testing algorithms for children with idiopathic short stature [Fukami, Seki, Ogata, 2016; Binder, Rappold, 2005; Vannelli et al., 2020]. A diagnostic algorithm for SHOX mutation screening is presented in Figure I.33.

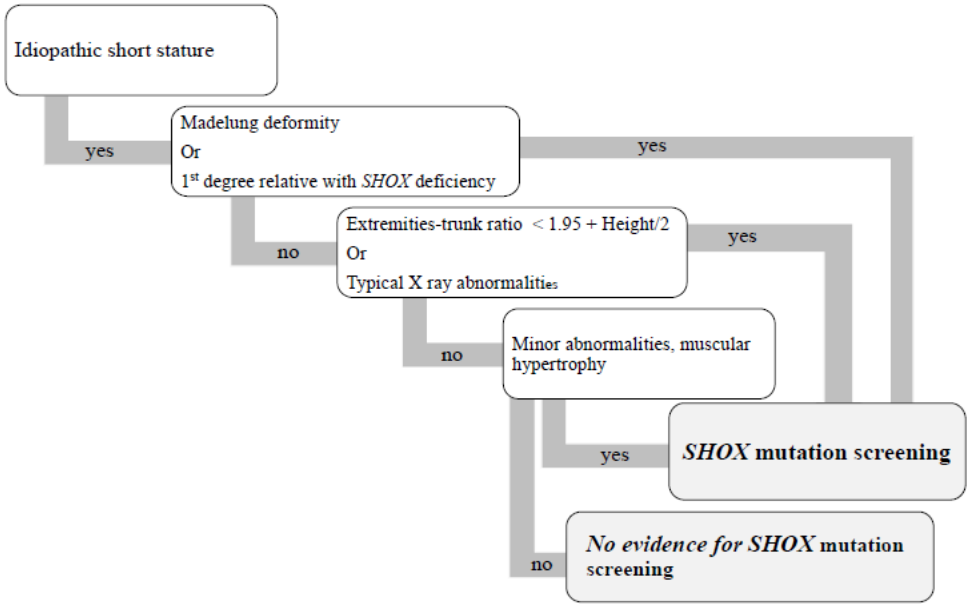


Figure I.33. Diagnostic algorithm for SHOX mutation screening
(adapted from Vanelli 2020, Binder 2011, 2018 and Fukami 2016).

The phenotype is not correlated with the size and type (missense or frameshift) of mutations, with identical *SHOX* abnormalities being detected in patients with ISS and LWD individuals with normal stature. Hormonal status seems to influence the phenotype expression of *SHOX* haploinsufficiency (more severe in postpubertal female patients). The mutations involving only the downstream enhancer regions lead to slightly milder phenotypes with more harmonious short stature than mutations or deletions in the exons [Fukami, Seki, Ogata, 2016; Binder, 2011].

4.4.2. Materials and methods

The study group is kindred of five children (three boys and two girls), one of the boys having another father, raised in different foster homes, with their mother having short stature (145 cm, meaning −3.42 SD). For clinical evaluation, growth charts for the Romanian population were used [Pascanu et al., 2016]. This study was approved by the local Ethical Committee; informed consent was signed by the guardians.

Genetic Testing

DNA was extracted from peripheral blood samples stored with an EDTA agent using Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA) or Invitrogen PureLink® Genomic DNA Kit (Invitrogen, Waltham, MA, USA). The MLPA analysis was performed according to the manufacturer's protocol. We used a commercially available P018 SALSA® MLPA® kit (MRC-Holland, Amsterdam, The Netherlands). Data analysis was performed using the Coffalyser.NET software (MRC-Holland, Amsterdam, The Netherlands).

4.4.3. Results

Case 1:

The first case is a thirteen-year-old boy, a full-term birth weighing 2800 g. He is a stepbrother in the other four cases. The first evaluation, at the age of eight, showed short stature (H = 117 cm, −2.56 SD), a BMI of 19 Kg/m^2 (56th percentile), and a mid-parental height (MPH) of 163.1 cm (−2.14 SD). The clinical exam revealed an abnormal development with mezomelic short stature: height 137 cm (−2.64 SD), a BMI of 20.1 Kg/m^2 (77th percentile), puberty P3G3, arm span = 136 cm, sitting height = 76 cm, subischial leg length = 62 cm, extremities/trunk ratio = 2.6 (<2.64), sitting height/height = 0.55 (>2.5 SD). The hormonal assessment revealed normal thyroid status, GH sufficient. Finally, the genetic test showed the *SHOX* gene mutation: heterozygous deletion of approximately 1.21 MB in the Xp22PAR region between genomic positions 500.427 and 1.712.090 (hg19), using the MLPA technique.

Case 2:

The second case is a nine-year-old boy born small for gestational age (SGA): weight 2400 g and length 46 cm at 40 weeks of pregnancy (−2.45 SD). At the age of four, the physical examination revealed harmonious short stature: height = 94 cm (−3.6 SD) and weight = 12 kg and a BMI in the 2nd percentile. All hormonal parameters were within normal limits. Considering the diagnosis of SGA, treatment with rhGH was initiated. After five years of treatment, the height is 123 cm (−2.66 DS) and the extremities-to-trunk ratio was 2.63, excluding a mezomelic short stature at this moment. Considering the kinship with Case No. 1, the genetic test was performed. The result confirms a *SHOX* gene mutation: a heterozygous deletion of approximately 1.21 MB in the Xp22PAR region between genomic positions 500.427 and 1.712.090 (hg19).

Case 3:

The third case is an eight-and-a-half-year-old boy, born prematurely with 1700 g at 34 weeks of gestation (>−2 SD). He was diagnosed at seven and a half with short stature: height 110 cm (−3.2 SD), weight 16 kg, BMI 3rd percentile, and MPH 170.4 cm (−1.03 SD). Treatment with rGH was initiated. At 12 months of treatment, the height was 118 cm (−2.68 SD). He has not mezomelic short stature. On the hand X-ray, shortening of the fourth metacarpal bone and discrete convexity of the distal radial metaphysis was noted. The genetic test was done and showed a heterozygous deletion of approximately 1.21 MB in the Xp22PAR region between genomic positions 500.427 and 1.712.090 (hg19).

Case 4:

The fourth case is a five-year-old girl with a low normal birth weight (2300 g at 36 weeks). Clinical exam revealed: height = 103 cm (−1.6 SD), weight = 16.5 kg, BMI = 29th percentile, and MPH = 170.4 cm (−1.03 SD). She tested positive for *SHOX* deletion due to her family history—a heterozygous deletion of approximately 1.21 MB in the Xp22PAR

region between genomic positions 500.427 and 1.712.090 (hg19). Treatment with rGH was postponed due to our country's guidelines for GH treatment for short stature.

Case 5:

The fifth case is an 11-year-old girl without short stature: height 141 cm (−1.41 SD), weight 32.5 kg, BMI 16 kg/m^2, and MPH 170.4 cm (−1.03 SD). She tested negative for *SHOX* deletion.

4.4.4. Discussion

The nonspecific and variable phenotype of SHOX deficiency frequently leads to diagnostics such as skeletal dysplasia with disharmonious short stature, ISS, or SGA. Rapaport et al. stated [Rapaport, Wit, Savage, 2021] that short children born SGA should be classified in the same group as ISS. Complex investigations are requested to identify precise aetiology, leading to optimal clinical management. The genetic assessment also permits to exclude conditions associated with short stature for which rhGH treatment is contraindicated, such as chromosomal instability and DNA repair defects syndromes due to the risk of neoplasia (e.g., Bloom syndrome, Nijmegen syndrome), debatable (e.g., neurofibromatosis type1), or where higher doses are needed for an appropriate growth response (e.g., IGF1R haploinsufficiency, SHOX haploinsufficiency). In cases with SHOX deletion, the phenotype is highly variable; the moment of diagnosis may be from birth in Langer Mesomelic Dysplasia, children with SGA, or adult life when the diagnosis is established due to family gene analysis. The generalist, paediatrician, and endocrinologist may notice the short stature from childhood; ggstill, the diagnosis of SHOX deletion is particularly challenging in preschool age when skeletal mesomelic shortening and Madelung deformity are not apparent [Ogata, 2002]. Identifying patients with SHOX deficiency as early as possible is essential because GH treatment is most effective when started at a young age.

Short Stature Treatment in SHOX Deficiency

Recombinant human growth hormone (rhGH) therapy for short stature due to *SHOX* deficiency is approved. There are no concerns regarding treatment safety [Cianfarani, 2021]. In a prospective, open-label, randomized study, including patients with *SHOX* deficiency treated with recombinant GH, the final adult height was more than −2 SD in 57% of the patients [Blum et al., 2013]. GH treatment in subjects with varying forms of *SHOX* deficiency increases height velocity. Height gains from treatment initiation to adult height were about 1.1–1.2 SDS (∼7 cm)—as cited in Turner syndrome.

The treatment response is better in patients with deletions in the *SHOX* gene enhancer area than in those with deletions in the *SHOX* gene. The maxim catch-up growth occurs in the first 12 months of treatment.

There are also psychosocial factors such as self-image, social integration, and school performance that may be influenced by height [Child, Rappold, Blum, 2012]. Adverse events in GH treatment patients with *SHOX* are like those reported in other GH-treated paediatric populations [Benabbad et al., 2017]. However, the period of study for GH administration is relatively short. Therefore, long-term follow-up of a significant number of patients treated with GH for *SHOX* deficiency is required. There is no systematic effect on the skeletal anomalies of this disorder induced by growth hormone treatment. The combination therapy of GH and GnRHa may effectively promote stature growth in patients with *SHOX* deficiency by delaying premature fusion of the growth plates. Still, the clinical experience with combined therapy is poor. The combination therapy of GH and aromatase inhibitors for decreasing bone age maturation cannot be applied to girls. The long-term safety and efficacity are still uncertain [Marchini, Ogata, Rappold, 2016].

In our kindred, Case no. 1 had typical clinical aspects for SHOX deletion: low height,

BMI > percentile 50%, appearance of muscular hypertrophy, mezomelic short stature with extremities/trunk ratio < 2.64 and sitting height/height > 2.5 SD. Children with ISS and a low mean parental height do not exclude rGH treatment because it may be *SHOX* haploinsufficiency. The risk of a child inheriting the SHOX gene deficiency is 50% when one of the parents is affected; if both parents are SHOX gene-deficient, there is a 50% risk of moderate-to-mild hypostature by SHOX deficiency, a 25% chance of severe Langer dwarfism, and a 25% chance of having none of the conditions [Vannelli et al., 2020].

Case no. 2 has SGA, a low BMI, and no clinical aspects suggestive of *SHOX* deletion, but we may explain the modest rGH treatment response with the genetic diagnostic. He has a first-degree relative with *SHOX* deficiency.

Case no. 3 has short stature, a low BMI, a normal birth weight for gestational age, and discrete radiological signs of skeletal dysplasia; he was tested positive for *SHOX* deletion. Children with SGA who do not have catch-up growth until the age of four and probably it has an unspecified growth disorder with prenatal onset, and the cause is generally as "idiopathic" as the ISS.

Cases 4 and 5 do not have short stature (height > −2 SD).

Genetic testing is always recommended for short stature if it is severe or associated with microcephaly/relative macrocephaly, dysmorphic traits, disproportions of body segments highlighted by auxological determinations, intellectual disability, and a positive family history [Rapaport, Wit, Savage, 2021; Sireteanu et al., 2014].

4.4.5. Conclusions

Considering the importance of an early diagnosis and treatment, special attention should be paid to the family history of short stature, growth parameters at birth, and the clinical and radiological examination. Our cases emphasize this aspect, especially in patients with a family history. The clinician must be aware of the genetic causes of idiopathic short stature to properly select patients for genetic testing and interpret the genetic tests in the clinical and therapeutic context.

4.5. Study of Coffin Siris Syndrome

4.5.1. Introduction

Coffin-Siris syndrome (CSS) is a rare genetic multisystemic disease caused by heterozygous mutations in a large panel of genes that are part of the Brahma/BRG1-associated factor (BAF) complex [Vasko et al., 2021]. The BAF complex plays an essential role in chromatin remodeling, and pathogenic variants in several of its components, including *ARID*, *SMARC* and *SOX* family genes, have been associated with CSS [Lopez, Wood, 2015]. Although other BAFopathies have also been described, CSS is the most well-known; it is also acknowledged as the "fifth digit syndrome", as it was initially described by Coffin and Siris [Coffin, Siris, 1970] in three unrelated probands that associated severe mental disability and 5^{th} finger/nail hypoplasia. The clinical phenotype is highly variable, with many features which are nonspecific and can also be encountered in other genetic disorders, thus rendering the diagnosis a real challenge in clinical practice. Fleck et al. proposed minimal criteria for the diagnosis of CSS, which is classically described as the association of typical coarse facial features, cognitive disability and developmental delay, 5^{th} finger/nail hypoplasia and hypertrichosis/hirsutism [Fleck et al., 2001]. However, the clinical spectrum of manifestations is very wide, with various degrees of cognitive delay and miscellaneous cardiac, gastrointestinal, genitourinary and central nervous system (CNS) malformations [Vergano, Sluijs, Santen, 2019]. Around 300 subjects with known mutations were enrolled in the CSS/BAF complex registry in 2021 [Vasko et al., 2021; Vasko, Schrier Vergano, 2022].

Part of the constitutional features, short stature is common in CSS (66% in the cohort described by Schrier et al [Schrier et al., 2012]). While approximately half of the patients are reported to have intrauterine growth restriction at birth, most of them are further on presented as "failure to thrive" [Schrier et al., 2012]. Baban et al. reported in 2008 a case of pituitary hypoplasia, with growth hormone deficiency (GHD) [Baban et al., 2008].

4.5.2. Case report

We present the case of a 4-year-old girl with personal history of iron deficiency anemia, vitamin D deficiency rickets, ID and hyperkinetic disorder, admitted to the Pediatric Endocrinology Department for the investigation of severe short stature. Family history was unavailable, as she was in foster care. On clinical examination, her height was 91 cm (-3.2 standard deviations (SD) according to national nomograms [Pascanu et al, 2016], <3rd percentile according to the World Health Organization (WHO) [World Health Organisation. WHO Child growth standards: Height-for-age girls 2 to 5 years (percentiles)], she was underweight (body mass index= 12.1 kg/m^2, <1st percentile) and had microcephaly (head circumference= 43 cm, < 3rd percentile), cognitive disability and developmental delay (predominantly in the linguistic area). She had coarse facial features: bushy eyebrows, bulbous nose, flat nasal bridge, dental anomalies, thick lips, dental anomalies, bilateral epicanthal fold. She associated pectus excavatum and bilateral hypoplastic nails of the 5th finger and toe. Other anomalies included scoliosis and pilonidal sinus.

Hormonal profile showed normal thyroid and adrenal function, a low basal growth hormone concentration and insulin growth factor-1 (IGF-1) levels towards the lower limit of normal (Table I.20). Since basal GH may also be encountered in normal children (due to its neural control with intermittent release) [Yau, Rapaport, 2022], further dynamic testing for GHD was performed, according to the national protocol. Arginine (inhibits somatostatin release from the hypothalamus) and glucagon (generates fluctuation in blood glucose) stimulate GH secretion and are frequently used for GHD testing, with minimal side effects (nausea, vomiting, abdominal pain in both, late hypoglycemia for glucagon) [Caputo et al., 2021; Hawkes et al., 2016]. In our patient, GH concentrations failed to increase above the cut-off of 7 ng/ml at arginine and glucagon stimulation tests, respectively. No side effects occurred.

Blood chemistry was otherwise normal, as well as vitamin D levels (Table I.20.). Screening for celiac disease was negative. Bone-age was delayed by 2 years compared to the chronological age and the hand and foot X-rays confirmed hypoplastic distal phalanx of the 5th digit in the hands and feet (Figure I.34.). Therefore, GHD was confirmed, and brain MRI was performed, revealing agenesis of the corpus callosum, bilateral hippocampal atrophy and pituitary hypoplasia (Figure I.35.). Echocardiography and abdominal ultrasound were normal. Ophthalmological examination revealed convergent strabismus in the left eye. Audiogram was normal. Karyotype was 46,XX.

Table I.20. Results of the hormonal assessment.

Parameter	Value	Normal range
TSH (uUI/ml)	2.4	0.33-6.3
FT4 (ng/dl)	1.02	0.89-1.76
Cortisol (8 AM) (µg/dl)	18	5-25
GH (basal) (ng/ml)	0.7	0-8
IGF1 (ng/ml)	50.4	49-289
25(OH)D3	31	>30

TSH= thyroid-stimulating hormone, FT4= free T4, GH= growth hormone, IGF1= insulin-like growth factor 1

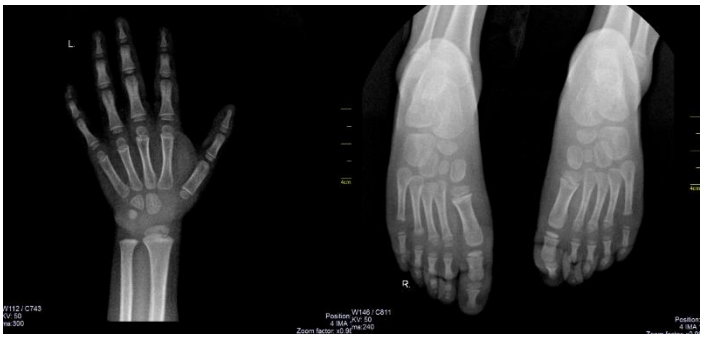


Figure I.34. X-rays of the hands and feet showing delayed bone age and hypoplastic distal phalanx of the 5th digit in the hands and feet.

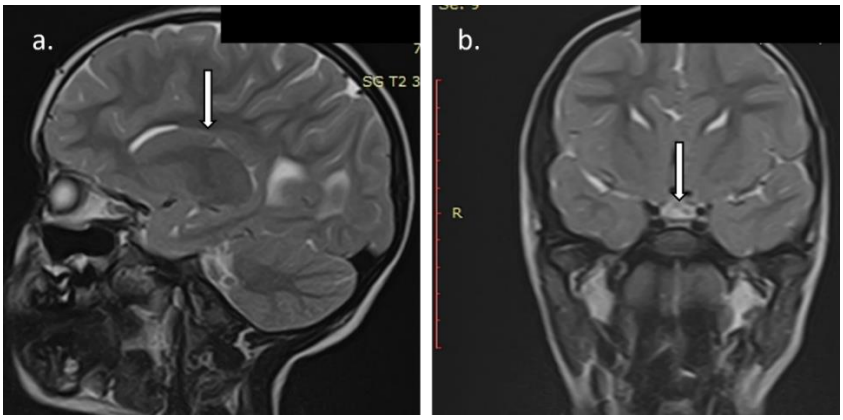


Figure I.35. Brain MRI revealing agenesis of the corpus callosum (a.) and pituitary hypoplasia (b.).

Taking into account the clinical and radiological findings, the patient met the clinical criteria proposed in 2012 by Schrier et al. [Schrier et al., 2012], and thus genetic examination established the diagnosis of CSS on the basis of: ID associated with hypoplastic 5th finger/nail, coarse facial features and systemic ectodermal (dental anomalies), constitutional (microcephaly, short stature) and organ-related (brain malformations) features. Molecular testing for gene defects associated with CSS was, unfortunately, not available.

The child was started on recombinant human GH (rhGH) replacement therapy 0.035 mg/kg/day, with excellent response: she gained 12 cm in height in the first 12 months of treatment, entering the normal growth curve for age (103 cm, -2SD according to the national nomograms [Pascanu et al, 2016]. She continued pediatric, neuropsychiatric and endocrinological monitoring.

4.5.3. Discussion

Short stature in children is a common referral reason in pediatric endocrinology, and standard investigations comprise pituitary function along with GHD testing, thyroid function assessment and laboratory work-up for organic causes of growth failure (e,g, renal, hepatic, gastrointestinal disorders, celiac disease, inflammatory disorders) [Seaver, Irons, 2009; Preda et al., 2013]. Genetic evaluation for short stature usually comprises evaluation for Turner syndrome in girls, *SHOX* gene deficits or Silver-Russel syndrome. However, the need for genetic evaluation of various monogenic causes of short stature is undervalued, especially in the absence of definitive criteria for selecting patients who would truly benefit from genetic examination and testing [Seaver, Irons, 2009; Preda et al., 2013; Dauber, Rosenfeld, Hirschhorn, 2014]. Dauber et al. proposed that children exhibiting a height below -3 SD or a

height < -2.5 SD with at least one more additional feature, such as the presence of microcephaly, ID, severe GHD and/or multiple pituitary hormone deficiency, additional dysmorphic features or malformations, GH insensitivity, evidence of skeletal dysplasia, being born short-for-gestational-age (SGA) without catch-up growth or having a single parent with severe short stature, should be genetically evaluated, as they have a high degree of suspicion for underlying genetic mutations explaining their short stature. This would not only provide an explanation for their family but would also help diagnose other congenital anomalies that could be present [Dauber, Rosenfeld, Hirschhorn, 2014].

Chromatin remodeling via nucleosome alteration and repositioning underlies gene transcriptional regulation. The BAF complex is one of the ATP-dependent chromatin remodeling complexes, involved in embryonic and neural development, via the regulation of neuron-specific gene expression up to adulthood. Recent genome wide-association studies led to the discovery of several neuron-specific BAF subunit gene mutations, mainly associated with neurodevelopmental disorders, among which the most well-known is CSS [Alfert, Moreno, Kerl, 2019]. Up to the writing of this manuscript, identified BAF subunit mutations associated with CSS include *ARID1A*, *ARID1B*, *ARID2*, *SMARCA4*, *SMARCB1*, *SMARCE1*, *SMARCC2*, *DPF2*, *SOX4* and *SOX11* [Vasko et al., 2021; Vasko, Schrier Vergano, 2022; Miyake, Tsurusaki, Matsumoto, 2014] (Table I.21.). Most cases appear *de novo* and are inherited in an autosomal dominant manner [Vergano, Sluijs, Santen, 2019]. Two thirds of the mutations in CSS are caused by *ARID1B* mutations. *ARID1B* haploinsufficiency is associated with syndromic short stature, thus explaining growth retardation in CSS, while *de novo* missense mutations are associated with idiopathic short stature, without developmental delay [Vasko et al., 2021; Sim, White, Lockhart, 2015]. The clinical phenotype associated with reduced AT-rich interactive domain-containing protein 1B (ARID1B) levels in CSS is highly variable, probably due to the expression variability of other subunits of the BAF complex. Nevertheless, ARID1B is likely to play an important role in brain development, as ID is consistently reported [Yu et al., 2015]. Large-scale exome sequencing studies found *ARID1B* variants in unspecified cohorts with ID [Wright et al., 2015; Hoyer et al., 2012]. Recent research draws the attention towards the presence of only minor differences between *ARID1B* pathogenic variants causing CSS and *ARID1B* mutations causing ID, thus recommending similar management in both conditions [van der Sluijs et al., 2019].

Table I.21. Various BAF mutations and associated distinct phenotype trait in CSS.

BAF subunit mutation	Phenotype trait in CSS
ARID1A	Important delay in walking and crawling
ARID1B	2/3 of CSS cases; ID consistently reported; prominent hypertrichosis
ARID2	Shorter birth length
SMARCA4	Frequent anatomic anomalies; milder ID
SMARCB1	Severe CSS phenotype; important speech delay; frequent kidney malformations
SMARCE1	Frequent kidney malformations; Scoliosis
SMARCC2	Cardiac abnormalities
Other variants reported: *DPF2* *SOX4* *SOX 11*	 Possible craniosynostosis Mild facial dysmorphism Syndactyly of toes 2-3 Ocular anomalies

CSS – Coffin-Siris Syndrome; ID – intellectual disability

A recent large genotype-phenotype correlation revealed similar phenotypes across all genetic variants in CSS: the most common phenotypes reported were the classical fifth

digit/nail hypoplasia (41%), sparse scalp hair (47%), hypertrichosis (52%) and hypotonia (43%). However, distinct phenotype traits may be encountered with different genetic variants (Table I.21) [Vasko et al., 2021; Bögershausen, Wollnik, 2018; Machol et al., 2019; Zawerton et al., 2019].

The most noticeable differential diagnosis to consider is Nicolaides-Baraitser syndrome, also part of the BAFopathies, characterized by mutations in the *SMARCA2* gene. It overlaps with CSS with regards to the presence of characteristic coarse facial features, sparse scalp hair and ID, but usually the 5th digit nail/distal phalanx hypoplasia/aplasia is absent, with other digital anomalies being present (prominence of interphalangeal joints or of distal phalanges) [Vergano, Sluijs, Santen, 2019].

Fetal alcohol syndrome should be considered due to the association of hypoplastic nails, growth restriction and multiple congenital organ anomalies, especially concerning the CNS. However, the typical facial features are clearly distinct: short palpebral fissure, smooth philtrum and thin upper vermillion [Riley, Infante, Warren, 2011].

Other overlapping syndromes include (1) Brachymorphism-Onychodysplasia-Dysphalangism (BOD) syndrome which shares the short 5th finger, dysplastic nails, wide mouth, broad nose, but typically milder ID, (2) DOOR syndrome (deafness, onychodystrophy, osteodystrophy and mental "retardation") which is distinguished by the association of osteodystrophy and profound hearing loss, (3) Cornelia de Lange syndrome which may associate 5th finger hypoplasia, ID and multiple cardiac, gastrointestinal and genitourinary malformations, but has distinctive craniofacial features (arched eyebrows, upturned nose, small teeth and microcephaly), (4) Mabry syndrome which overlaps CSS due to the presence of coarse facial features, hypoplastic 5th digits and ID but presents itself with very high levels of serum alkaline phosphatase and (5) 4q21 deletion syndrome presenting with curbed 5th digit nail and ID, but with characteristic facial appearance (broad forehead, widely spaced eyes and frontal bossing) [Vasko et al., 2021; Schrier et al., 2012].

In the absence of standardized criteria, Schrier et al. [Schrier et al., 2012] proposed an algorithm that might help clinicians to evaluate the likelihood of CSS in a suspected individual. Thus, ID and/or developmental delay together with 5th digit/nail hypoplasia must be present in order to further consider the diagnosis of CSS. Afterwards, at least one feature in each of the following three categories of anomalies must be present: (1) ectodermal (hirsutism/hypertrichosis, sparse scalp hair or dental anomalies), (2) constitutional (microcephaly, intrauterine growth restriction, failure to thrive, short stature or frequent infections) and (3) organ-related (cardiac, gastrointestinal, renal, brain/cranial malformations, vision problems or hearing loss). Further on, the combination of specific facial features at the level of eyebrows and lips – both thick in type A CSS or thin in type B CSS- must be present. Differential diagnoses must be made with the above-mentioned syndromes [Schrier et al., 2012]. This algorithm is highly valuable for clinicians in the absence of standardized criteria for CSS diagnosis, especially if genetic testing is not possible.

Our patient met all the criteria in the algorithm of Schrier et al.[Schrier et al., 2012] (ID and hypoplastic 5th finger and nail, ectodermal -dental anomalies, constitutional-microcephaly and short stature, organ-related- brain malformations and vision problems) with facial features matching type A CSS. Genetic testing for *ARID1B* was, unfortunately, not possible. At this time point, the diagnosis of CSS is mainly clinical, due to the high variability of genotype and phenotype expression [Vergano, Deardorff, 2014]. However, as genetic knowledge is growing, one would expect genetic testing to fit the criteria for CSS diagnosis in the future.

Endocrinological evaluation and growth monitorization is recommended in CSS caused by *ARID1B* gene mutation. In a recent cohort, 7 out of 51 patients were diagnosed with GH deficiency, similar to our patient [van der Sluijs et al., 2019]. Also, around 30% of the

patients were appreciated to have short stature. Thus, GH deficiency may be under recognized in *ARID1B* genetic variant, while GH therapy is reported in a limited number of CSS cases [van der Sluijs et al., 2019; McCague et al., 2020]. Recently, growth charts for individuals with CSS gave been published [McCague et al., 2020].

4.5.4. Conclusions

The association between ID/developmental delay and 5th finger/nail hypoplasia should raise the suspicion of CSS. Growth should be assessed and monitored if CSS is confirmed. CNS anomalies, including pituitary hypoplasia, should be assessed. If GHD is confirmed, GH therapy should be started.

4.6. Study of Hereditary gingival fibromatosis

4.6.1. Introduction

Gingival fibromatosis is a rare oral disorder characterized by pathological, diffuse or local growth of gingiva, usually involving both arches. The clinical aspects of the gingiva are: normal in color, fibrous consistency and in some cases can interfere with tooth eruption [Kather 2008, Gawron 2016]. The gingival enlargement has aesthetic and functional consequences because can interfere with speech, lip continence, mastication, occlusion and facial appearance [Kather 2008]. It may cause also periodontal problems (bone loss and bleeding). Regarding the etiology, the gingival fibromatosis could be classified in: drugs induced fibromatosis, hereditary gingival fibromatosis, systemic disorders and genetic syndromes with fibromatosis [Kather 2008].

Hereditary gingival fibromatosis (HGF) (OMIM 135300) is a nonsyndromic form characterized by genetic locus heterogeneity (candidate chromosomal regions are: 2p2122; 5q1322; 2p23.322.3; 11p15) and autosomal dominant inheritance [Hart 1998, Xiao 2001, Zhu 2007, Kather 2008]. The causative gene is Son of Sevenless1 (*SOS1*). *SOS* gene discovered in Drosophila melanogaster has two human homologues *SOS1* and *SOS2*. *SOS1* gene contains 7 different domains: two histone domains, Rho GEF homology, PH/Rem helical linker, Eps8 binding, regulatory domain, catalytic domain and Grb2/E3b1 binding. The C terminal segment has a proline-rich domain which interacts with proteins such Grb2 for Ras and E3b1 for Rac pathways [Pierrre 2011]. The Ras pathway is involved in aberrant regulation of malignant transformation (cell proliferation, transformation, survival and mobility) [Buday 2008]. Protein SOS1 is a nucleotide exchange factor which stimulates the reorganization of the actin cytoskeleton, cell invasion and migration. HGF is associated with a mutation in proline-rich C terminal domain of SOS1 gene which produced cell proliferation by constitutive SOS1 membrane recruitment and activation of the Ras/MAPK pathway. This activation has high intensity and duration and produces a faster transition from G1 to S phase in cell cycle of gingival fibroblasts [Kather 2008, Jang 2007].

The histopathological analysis reveals: epithelial acanthosis, collagen deposits, the extent of fibrosis and inflammatory infiltrates [Pêgo 2016]. Usually, the onset of the disease is in childhood during permanent tooth eruption and the recurrences are common. The management includes surgical removal of the hypertrophic tissue, good oral hygiene, local drug delivery, full mouth disinfection and host immune response modulation [Gawron 2016, Shetty 2010].

4.6.2. Case report

A 31 years old Caucasian female was referred to the medical genetic consultation due to pronounced fibrous gingival overgrowth. Her medical history revealed that the onset was in childhood during the eruption of permanent dentition and the patient was treated by conventional gingivectomy three times (16, 17, 30 years old) with poor long-term results.

Clinical examination showed generalized gingival hyperplasia involving both the maxillary and mandibular arches, with morphologically normal teeth. The gingiva was firm on palpation (fibrous consistency), with normal pink colour and covering some dental crowns (Figure I.36.). The speech and mastication are impaired. Family history is positive: her mother and her brother have some degree of gingival overgrowth (not require surgical removal) and this information is an argument for dominant inheritance.

In order to make the differential diagnosis, we made some supplementary exams: Xray examinations of skull, foot, hand and spine, cardiac and abdominal ultrasound, oto-rhinolaryngology exam, ophthalmologic exam. The patient was surgically treated by excision of the excessive tissue. Gingivectomy improves, even temporary, the speech, mastication, aesthetics and self-esteem. Recurrence is relatively characteristic in gingival fibromatosis.

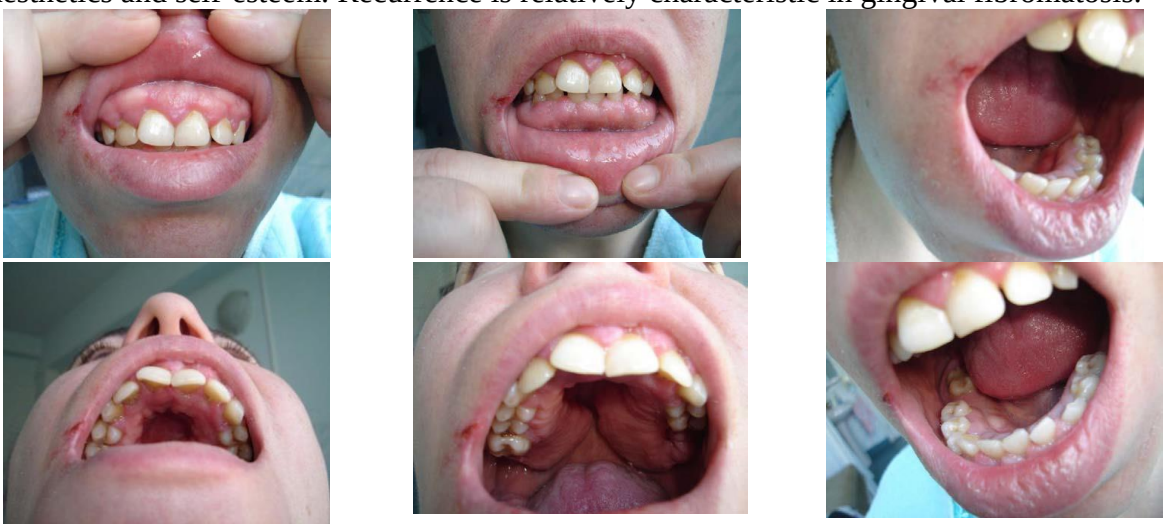
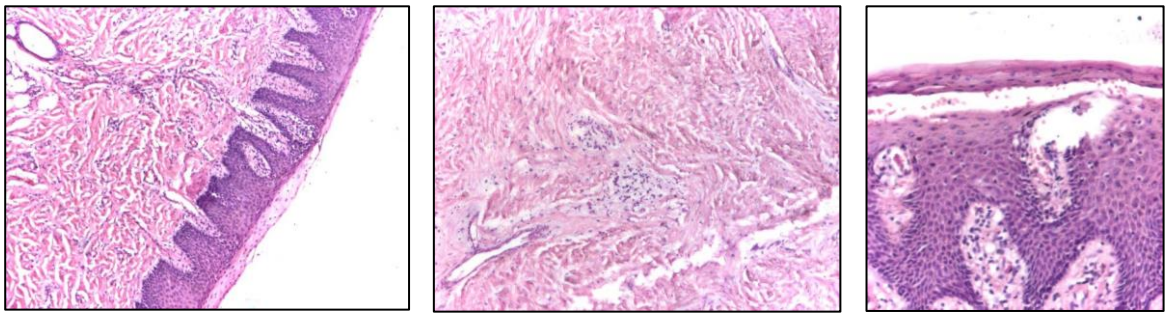


Figure I.36. Clinical features: moderate gingival enlargement with normal colour (both mandible and maxilla are involved)

The histopathological analysis is consistent with gingival fibromatosis: acanthosis and parakeratosis, fibrous tissue with collagen deposits and low-plasma cell lymphocyte inflammatory infiltrate (Figure I.37.).

The molecular testing for the gene *SOS1* is useful in diagnosis and for the family management, but in our patient was impossible to done.



A B C

Figure I.37. Histologic morphology from gingival tissue. A. HEx4 Stratified squamous epithelium with parakeratosis B. HEx4 Stratified squamous epithelium with acanthosis and parakeratosis C. HEx10 Fibrous tissue with collagen deposits and low-plasma cell lymphocyte inflammatory infiltrate

4.6.3. Discussion

HGF is a benign condition and the correct management imposes to check some signs specific for genetic syndrome and to take a good personal and family history. Differential diagnosis was made with three categories of gingival enlargement: associated with some drugs consumption, systemic disease and genetic syndromes with gingival fibromatosis.
Gingival enlargement could appear after *drugs consumption* like: Phenytoin, Calcium Channel Blockers (Nifedipine, Verapamil), immunosuppressive agents (Cyclosporine) [Gawron 2016, Kataoka 2005]. In our case this etiology was ruled out because the patient do not take such medication.

The systemic diseases like leukemia or sarcoidosis produce gingival fibromatosis, but were excluded in our case by haematological analysis, Xray exam and pneumology consultation. Genetic syndromes associated with gingival fibromatosis are: Oculodental syndrome, Gingival fibromatosis with sensorineural hearing loss, Oculocerebral syndrome with hypopigmentation, Zimmermann–Laband syndrome, Hyaline fibromatosis syndrome,

Oculodental syndrome (Rutherford syndrome, OMIM 180900) is a rare genetic disorder characterized by corneal dystrophy, gingival fibromatosis and non-eruption of tooth [Gawron 2016]. The ophthalmologic examination in our patient was normal and there were no problems with tooth eruption.

Gingival fibromatosis with sensorineural hearing loss (Jones syndrome, OMIM 135550) is an autosomal dominant disorder which associate progressive sensorineural hearing loss [Gawron 2016]. ENT exam was normal, and our patient did not present deafness.

Oculocerebral syndrome with hypopigmentation (Cross syndrome, OMIM 257800) is an extremely rare autosomal recessive condition characterized by ocular anomalies, intellectual disability, athetoid movements and skin hypopigmentation, silver gray hair color [Gawron 2016]. Because our patient presented only gingival fibromatosis, we excluded this syndrome.

Zimmermann–Laband syndrome (OMIM 135500) is an autosomal dominant rare disorder characterized by gingival fibromatosis, coarse facial appearance, defects of the nose, ear and absence or hypoplasia of nails or terminal phalanges of hands and feet, hepatosplenomegaly, hirsutism [Gawron 2016]. In our case Xray examinations of skull, foot, hand and spine were normal. Similar results were confirmed by cardiac and abdominal ultrasounds. The patient has no facial dysmorphia and hirsutism. Thus, we excluded also this disease.

Hyaline fibromatosis syndrome (Murray– Puretic Drescher syndrome, OMIM 228600) is a rare autosomal recessive disorder with gingival fibromatosis, osteolysis, osteoporosis, osteopenia, recurring subcutaneous tumors, recurrent infections, painful joint contractures, diarrhea in severe forms [Gawron 2016]. This syndrome was ruled out because has just gingival fibromatosis from all signs.

4.6.4. Conclusions

HGF is a benign condition characterized by gingival fibromatosis that affects both maxillary arches. This disease presents an autosomal dominant transmission and is characterized by variable expression. For this reason, the discovery of such case imposes clinical examinations for close relatives. To make a correct differential diagnosis the management of patients imposes multiple clinical examinations (in different specialties) and diverse types of laboratory or imagistic examinations.

II. FUTURE DIRECTIONS OF CAREER DEVELOPMENT

II.1. PERSPECTIVES ON THE ACADEMIC ACTIVITY

The activities of the university teaching staff are complex and aim at: educational and teaching activities, activities of the academic community both at the research and administrative level, personal and professional development activities. Thus, the teaching staff must be an expert in the act of teaching-learning, a transmitter of knowledge, but also of educational methods and styles; a mentor and leader of students (undergraduate, master's, doctoral students); a manager who directs and organizes the activities carried out in the respective group; social and moral model for students, a transmitter of values and attitudes; professional who analyse, study and interpret psychosocial phenomena in the group [Anita Woolfolk, 2017]. Therefore, I will follow the development of the didactic career by participating in the medical genetics discipline team in teaching, evaluation, and research activities. The target group is represented by students, master's students, doctoral students, and residents. The modalities are represented by courses, practical lessons, case presentations, works at congresses, bachelor's theses, and of course, after obtaining the habilitation certificate, doctoral theses. I will constantly be concerned with the enrichment of learning support materials: renewed courses every 5 years, monographs, richly illustrated and representative educational materials for various thematic areas of research, the development and extension of courses for various categories of residents who study medical genetics. I will participate together with colleagues from other university centers in the realization of a common medical genetics course for both students and residents in order to standardize training in the field of genetics in our country. I intend to co-write a monograph on nutrigenomics and a monograph on hereditary cancers.

II.2. PERSPECTIVES ON THE MEDICAL ACTIVITY

The field of genetics is a medical field that knows a continuous expansion as well as the characterization of genetic diseases, the emergence and development of laboratory techniques, as well as the treatment of genetic diseases. Thus, in order to be up to date with all this news, I will participate in national courses or organized by international societies in clinical or laboratory medical genetics, but also in other related specialties. To ensure the transfer of information, I will participate as an organizer or lecturer at congresses/scientific events related to medical genetics or other specialties. I also propose learning new techniques for the diagnosis of genetic diseases and their introduction into clinical practice. In order to clarify the diagnosis in complex cases, I will collaborate with reference specialists from the country or abroad.

II.3. PROJECTS IN THE SCIENTIFIC ACTIVITY

The research activity involves three indispensable poles: the human resource, the material resource, and the research directions.

The strategies aim at the development of multidisciplinary research teams with already formed/new research cores from the university center where I carry out my activity, as well as from other centers according to the field of interest and competences of the partners. A particularly important aspect that I will promote is the involvement of young researchers (master's students, PhD students) but also students in these teams.

The research directions are based on the fields of interest of the last years or derived from them and are in line with the research directions at the world level: oncogenetics, rare

diseases, malformative pathology and chromosomal diseases, nutrigenomics, genetic heterogeneity in genetic diseases.

- **Oncogenetics**

In this field, I will consider the evaluation of the genetic causes of cancer and the validation of investigation protocols in the case of hereditary and familial cancers. This is mandatory because it allows the identification of people at risk from the family and in this way a prevention of the occurrence of cancer through oncological follow-up programs. I will focus on the use in the respective protocols of the family history that can highlight family aggregation, the age of appearance of tumors, but especially the presence of multiple congenital anomalies or other non-oncological symptoms or the presence of precursor lesions or benign lesions. In this case, the multidisciplinary team must also be made up of the specialists involved in the detection of these non-oncological manifestations. This is feasible because I carry out my activity in a hospital classified as having level I competence and covering most medical and surgical specialties. At the same time, the activity in the Iasi University Center allows collaboration with the oncology sections from the other hospitals.

- **Rare diseases**

Rare diseases represent a major public health problem due to the large number of entities, the enormous costs, and the challenges involved in the diagnosis and management of patients. As a number of distinct entities, there are estimated to be around 5,000-8,000 rare diseases in the European Union. The number differences derive from the lack of a universally valid definition for rare diseases, the difference in terminology and disease hierarchies in various major knowledge sources on rare diseases – Orphanet, OMIM, GARD, DOID, and NCI Thesaurus. Thus, it was quantified that the majority - 6270 rare diseases - are present in at least three such sources, and 4023 diseases appear only in one source [Haendel et al., 2020]. 80% of rare diseases have a genetic etiology. It represents a challenge for the health system for several reasons: the risk of not responding to the patient's needs, the risk of not guaranteeing equal access to treatment (for example, orphan drugs are expensive and often unavailable). They are often diseases ignored by the medical community and neglected by pharmaceutical companies because making drugs is complicated, time-consuming, and often expensive. Orphan drugs are a separate category as they are expensive and often address serious conditions with a poor prognosis in the absence/delay of treatment, in some cases they require as an additional measure a personalized treatment according to the patient's pharmacogenetic characteristics in order not to waste precious time with a treatment with reduced efficacy. I will try to establish a partnership with other medical genetics centers that would attract European funding in order to enable us to investigate genetic disorders at the molecular level with the best tools available. I will participate in the establishment of multidisciplinary teams with competences in the management of rare diseases. Valid premises for the feasibility of these multidisciplinary teams are previous scientific collaborations with other colleagues in the field of nephrology, endocrinology, medical imaging, gastroenterology, ophthalmology, etc.

- **Malformative pathology and chromosomal disorders**

The World Health Organization (WHO) estimates that 240,000 newborns die worldwide within 28 days of birth every year due to birth defects. Birth defects cause 170,000 deaths of children between the ages of 1 month and 5 years [WHO]. Since birth defects are associated with long-term disability and represent a burden for the health system, society, and families, it is necessary to identify the causes and subsequent preventive measures. I was a member of the research project PN-II-PT-PCCA-133/2014 on the theme of "Implementing a diagnostic

algorithm based on complex analysis of the genomic profile for patients with congenital and developmental abnormalities", which is a premise for the continuation of research to identify the causes and mechanisms causing congenital anomalies. The integration of advanced methods for the analysis of genomic anomalies (Array CGH, Next Generation Sequencing, PCR, MLPA, MS-MLPA) in the management protocols of congenital anomalies will be desirable in the future.

- **Nutrigenomics**

Nutrigenomics identifies correlations between the genetic status of the patient and the nutritional profile or the use of certain nutrients as drugs/foods.

Current medicine has the attributes of genomic medicine, which must be personalized, predictive, preventive, proactive or prospective, and participatory. This is also valid in multifactorial diseases. Since the metabolic pathology related to dietary imbalances is an important component in the medical act nowadays, a direction of research aimed at the genetic characterization of patients with obesity, diabetes, and the complications of metabolic syndrome is justified. Thus, the identification of certain genetic polymorphisms would contribute to a personalized, much more efficient approach to these patients.

The validation of the results of further research will be done by publishing them in ISI rated journals (at least 2-3 ISI articles/year) or BDI indexed journals (2 articles/year) as main author.

I will support PhD students in the development of research projects and in obtaining mobility grants abroad, in the publication of research results in established journals, and in the completion of doctoral studies.

In conclusion, the approach of some current research topics in world research, the formation of multidisciplinary teams of experts and young researchers, the participation in grant competitions are prerequisites for the development of both the personal career and the Genetics research core from Iasi.

III. REFERENCES

1. Abbas B, Abbas S, Malik SM, Rahim M, Umair M, Khurshid Z. Consanguineous marriages and dental anomalies: a cross-sectional analytical study. *Int J Dent*. 2022, 2022:9750460.
2. Abu Sa'D J, Indelman M, Pfendner E, Falik-Zaccai TC, Mizrachi-Koren M, Shalev S, Ben Amitai D, Raas-Rothshild A, Adir-Shani A, Borochowitz Z-U, et al. Molecular Epidemiology of Hereditary Epidermolysis Bullosa in a Middle Eastern Population. *J. Investig. Dermatol.* 2006, 126: 777–781.
1. Adamiok-Ostrowska A, Piekiełko-Witkowska A. Ciliary genes in renal cystic diseases. *Cells* 2020, 9, 907.
2. Adams M, Simms RJ, Abdelhamed Z, Dawe HR, Szymanska K, Logan C, Wheway G, Pitt E, Gull K, Knowles MA, et al. A meckelin–filamin A interaction mediates ciliogenesis. *Hum. Mol. Genet.* 2011, 21, 1272–1286.
3. Adams NA, Awadein A, Toma HS. The retinal ciliopathies. *Ophthalmic Genet*. 2007, 28(3):113–125.
4. Aitchison A, Hakkaart C, Day RC, Morrin HR, Frizelle FA, Keenan JI. APC Mutations Are Not Confined to Hotspot Regions in Early-Onset Colorectal Cancer. *Cancers* 2020, 12: 3829.
5. Ajmal M, Khan MI, Neveling K, Tayyab A, Jaffar S, Sadeque A, Ayub H, Abbasi NM, Riaz M, Micheal S, et al. Exome sequencing identifies a novel and a recurrent BBS1 mutation in Pakistani families with Bardet–Biedl syn-drome. *Mol. Vis.* 2013, 19, 644–653.
6. Akbani R, Akdemir KC, Aksoy BA, et al. Genomic classification of cutaneous melanoma. *Cell* 2015, 161: 1681–1696.
7. Akil I, Ozen S, Kandiloglu AR, Ersoy B. A patient with Bartter syndrome accompanying severe growth hormone deficiency and focal segmental glomerulosclerosis. *Clin Exp Nephrol*. 2010, 14:278–82.
8. Akimoto N, Ugai T, Zhong R, Hamada T, Fujiyoshi K, Giannakis M, Wu K, Cao Y, Ng K, Ogino S. Rising incidence of early-onset colorectal cancer—A call to action. *Nat. Rev. Clin. Oncol.* 2020, 1–14.
9. Akker PCVD, Mellerio JE, Martinez AE, Liu L, Meijer R, Dopping-Hepenstal PJC, Van Essen AJ, Scheffer H, Hofstra R, McGrath J, et al. The inversa type of recessive dystrophic epidermolysis bullosa is caused by specific arginine and glycine substitutions in type VII collagen. *J. Med. Genet.* 2011, 48: 160–167.
10. Al Mahi A, Ablain J. RAS pathway regulation in melanoma. *Dis. Models Mech.* 2022, 15: dmm049229.
11. Albino AP, Le Strange R, Oliff AI, et al. Transforming Ras Genes from Human Melanoma: A Manifestation of Tumor Heterogeneity? *Nature* 1984, 308: 69–72.
12. Alfert A, Moreno N, Kerl K. The BAF complex in development and disease. Epigenetics Chromatin 2019. 2019; 12:1–15.
13. Allaf B, Dreux S, Schmitz T, Czerkiewicz I, Le Vaillant C, Benachi A, et al. Amniotic fluid biochemistry in isolated polyhydramnios: a series of 464 cases. *Prenat Diagn.* 2015, 35: 1331–5.
14. Almaani N, Liu L, Harrison N, Tanaka A, Lai-Cheong JE, Mellerio JE, McGrath JA. New Glycine Substitution Mutations in Type VII Collagen Underlying Epidermolysis Bullosa Pruriginosa but the Phenotype is not Explained by a Common Polymorphism in the Matrix Metalloproteinase-1 Gene Promoter. *Acta Dermatol. Venereol.* 2009, 89: 6–11.
15. Almeida FT, Pachêco-Pereira C, Porporatti AL, Flores-Mir C, Leite AF, De Luca Canto G, Guerra ENS. Oral manifestations in patients with familial adenomatous polyposis: A systematic review and meta-analysis. *J. Gastroenterol. Hepatol.* 2016, 31: 527–540.
16. Al-Sharefi A, Quinton R. Current national and international guidelines for the management of male hypogonadism: helping clinicians to navigate variation in diagnostic criteria and treatment recommendations. Endocrinol Metab. 2020, 35(3):526–540.
17. Altesha MA, Ni T, Khan A, Liu K, Zheng X. Circular RNA in cardiovascular disease. *J. Cell. Physiol.* 2019, 234: 5588–5600.
18. Álvarez-Satta M, Castro-Sánchez S, Pereiro I, Piñeiro-Gallego T, Baiget M, Ayuso C, Valverde D. Overview of Bardet-Biedl syndrome in Spain: Identification of novel mutations inBBS1, BBS10 and BBS12genes. *Clin. Genet.* 2014, 86, 601–602.

19. Álvarez-Satta M, Cástro-Sánchez S, Valverde D. Bardet-Biedl syndrome as a chaperonopathy: Dissecting the major role of chaperonin-like BBS proteins (BBS6-BBS10-BBS12). *Front. Mol. Biosci.* 2017, 4, 55.
20. Amatori S, Tavolaro S, Gambardella S, Fanelli M. The dark side of histones: Genomic organization and role of oncohistones in cancer. *Clin. Epigenet.* 2021, 13: 71.
21. American Academy of Pediatrics. Committee on Genetics. American Academy of Pediatrics: health supervision for children with Down syndrome. *Pediatrics.* 2001, 107(2):442–449.
22. American Diabetes Association. Diabetes Overview. Diagnosis. Available from: https://www.diabetes.org/diabetes/a1c/diagnosis. Accessed June 1, 2022.
23. Amirlak I, Dawson KP. Bartter syndrome: an overview. *QJM.* 2000, 93: 207–15.
24. Angulo MA, Castro-Magana M, Sherman J, Collipp PJ, Milson J, Trunca C, Derenoncourt AN. Endocrine abnormalities in a patient with partial trisomy 4q. *J. Med. Genet.* 1984, 21: 303–307.
25. Annerén G, Lübeck P-O. Trisomy 4q31 →qter due to a maternal 4/8 translocation. *Hereditas* 2008, 100: 45–49.
26. Arin MJ. The molecular basis of human keratin disorders. *Qual. Life Res.* 2009, 125: 355–357.
27. Armakola M, Higgins MJ, Figley MD, Barmada SJ, Scarborough EA, Diaz Z, Fang X, Shorter J, Krogan NJ, Finkbeiner S, et al. Inhibition of RNA lariat debranching enzyme suppresses TDP-43 toxicity in ALS disease models. *Nat. Genet.* 2012, 44: 1302–1309.
28. Arthuis CJ, Nizon M, Komhoff M, Beck BB, Riehmer V, Bihouee T, et al. A step towards precision medicine in management of severe transient polyhydramnios: MAGED2 variant. *J Obstet Gynaecol.* 2018, 39: 395–7.
29. Ashoor G, Poon LC, Syngelaki A, Mosimann B, Nicolaides KH. Fetal Fraction in Maternal Plasma Cell-Free DNA at 11–13 Weeks' Gestation: Effect of Maternal and Fetal Factors. *Fetal Diagn. Ther.* 2012, 31: 237–243.
30. Ashton EJ, Legrand A, Benoit V, Roncelin I, Venisse A, Zennaro MC, et al. Simultaneous sequencing of 37 genes identified causative mutations in the majority of children with renal tubulopathies. *Kidney Int.* 2018, 93: 961–7.
31. Ashton GH, McLean WHI, South AP, Oyama N, Smith FJ, Al-Suwaid R, Al Ismaily A, Atherton DJ, Harwood C, Leigh IM, et al. Recurrent Mutations in Kindlin-1, a Novel Keratinocyte Focal Contact Protein, in the Autosomal Recessive Skin Fragility and Photosensitivity Disorder, Kindler Syndrome. *J. Investig. Dermatol.* 2004, 122: 78–83.
32. Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evantal N, Memczak S, Rajewsky N, Kadener S. circRNA biogenesis competes with pre-mRNA splicing. *Mol. Cell.* 2014, 5: 55–66.
33. Assawamakin A, Wattanasirichaigoon D, Tocharoentanaphol C, Waeteekul S, Tansatit M, Thongnoppakhun W, Limwongse C. A novel maternally-derived insertional translocation resulting in partial trisomy 4q13.2-q22.1 with complex translocation t(8;20) in a family with intellectual disability. *Am. J. Med. Genet. Part A* 2012, 158: 901–908.
34. Attard TM, Giglio P, Koppula S, Snyder C, Lynch HT. Brain tumors in individuals with familial adenomatous polyposis: A cancer registry experience and pooled case report analysis. *Cancer* 2007; 109: 761–766.
35. Aufiero S, Van Den Hoogenhof MMG, Reckman YJ, Beqqali A, Van Der Made I, Kluin J, Khan MA, Pinto YM, Creemers EE. Cardiac circRNAs arise mainly from constitutive exons rather than alternatively spliced exons. *RNA* 2018; 24: 815–827.
36. Austin C P, Cutillo C M, Lau L P et al. Future of rare diseases research 2017–2027: an IRDiRC perspective. Clinical and translational science 2018; 11(1): 21-27.
37. Auvin S, Irwin J, Abi-Aad P, Battersby A. The problem of rarity: estimation of prevalence in rare disease. Value in Health 2018; 21(5): 501-507.
38. Ávila-López PA, Guerrero G, Nuñez-Martínez HN, et al. H2A. Z overexpression suppresses senescence and chemosensitivity in pancreatic ductal adenocarcinoma. *Oncogene* 2021, 40: 2065–2080.
39. Baban A, Moresco L, Divizia MT, et al. Pituitary hypoplasia and growth hormone deficiency in Coffin-Siris syndrome. Am J Med Genet A. 2008; 146A:384–8.
40. Babochkina T, Mergenthaler S, De Napoli G, et al. Numerous erythroblasts in maternal blood are impervious to fluorescent in situ hybridization analysis, a feature related to a dense compact

nucleus with apoptotic character. *Haematologica*. 2005, 90(6): 740-745.
41. Baccichetti C, Tenconi R, Anglani F, Zacchello F. Trisomy 4q32 leads to 4qter due to a maternal 4/21 translocation. *J. Med. Genet.* 1975, 12: 425–427.
42. Bach DH, Lee SK, Sood AK. Circular RNAs in cancer. *Mol. Ther. Nucleic Acids* 2019, 16: 118–129.
43. Badano JL, Kim JC, Hoskins BE, Lewis RA, Ansley SJ, Cutler DJ, Castellan C, Beales PL, Leroux MR, Katsanis N. Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. *Hum. Mol. Genet.* 2003, 12, 1651–1659.
44. Badano JL, Leitch CC, Ansley SJ, May-Simera H, Lawson S, Lewis RA, Beales PL, Dietz HC, Fisher SJ, Katsanis N. Dissection of epistasis in oligogenic Bardet–Biedl syndrome. *Nature* 2006, 439, 326–330.
45. Baker K and Beales PL. Making sense of cilia in disease: the human ciliopathies. Am *J Med Genet C Semin Med Genet* 2009, 151C(4): 281-95.
46. Bankir L, Figueres L, Prot-Bertoye C, Bouby N, Crambert G, Pratt JH, et al. Medullary and cortical thick ascending limb: similarities and differences. *Am J Physiol Renal Physiol.* 2020, 318: F422–42.
47. Bardet -Biedl syndrome guideline development group. Management of Bardet -Biedl syndrome A clinical guideline, Version 14; April 28, 2014. Available from: http://www.orpha.net/national/data/IE-EN/www/uploads/BardetBiedl2014.pdf. Accessed September 19, 2022.
48. Bardhan A, Bruckner-Tuderman L, Chapple ILC, Fine J-D, Harper N, Has C, Magin TM, Marinkovich MP, Marshall JF, McGrath JA, et al. Epidermolysis bullosa. *Nat. Rev. Dis. Prim.* 2020, 6: 78.
49. Barnett S, Reilly S, Carr L, Ojo I, Beales PL, Charman T. Behavioural phenotype of Bardet-Biedl syndrome. *J Med Genet*. 2002, 39(12):e76.
50. Barreiro-Capurro A, Andres-Lencina JJ, Podlipnik S, et al. Differences in cutaneous melanoma survival between the 7th and 8th edition of the American Joint Committee on Cancer (AJCC). A multicentric population-based study. *Eur. J. Cancer* 2021, 145: 29–37.
51. Bartels CF, Bükülmez H, Padayatti P, et al. Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. *Am. J. Hum. Genet.* 2004, 75: 27–34.
52. Bastian BC, LeBoit PE, Hamm H, et al. Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. *Cancer Res.* 1998, 58: 2170–2175.
53. Battaglia A, Chen Z, Brothman A, Morelli S, Palumbos J, Carey J, Hudgins L, Disteche C. Karyotype/phenotype correlations in duplication 4q: Evidence for a critical region within 4q27-28 for preaxial defects. *Am. J. Med. Genet. Part A* 2005, 134A: 334–337.
54. Beadling C, Jacobson-Dunlop E, Hodi FS, et al. KIT Gene Mutations and Copy Number in Melanoma Subtypes. *Clin. Cancer Res.* 2008, 14: 6821–6828.
55. Beales PL and Forsythe E. Ciliopathies A reference for clinicians, in Bardet–Biedl syndrome. 2013, Oxford University Press.
56. Beales PL, Elcioglu N, Woolf A, Parker D, Flinter F. New criteria for improved diagnosis of Bardet-Biedl syndrome: Results of a population survey. *J. Med. Genet.* 1999, 36, 437–446.
57. Beales PL, et al. New criteria for improved diagnosis of Bardet- Biedl syndrome: results of a population survey. *J Med Genet* 1999, 36(6): 437-46.
58. Belengeanu V, Rozsnyai K, Gug C, Bănăţeanu M, Farcaş S, Belengeanu A. Ambras syndrome: Report on two affected siblings with no prior family history. *Clin. Dysmorphol.* 2004, 13: 265–267.
59. Belengeanu V, Stoicanescu D, Stoian M, Andreescu N, Budisan C. Ichthyosis congenita, harlequin fetus type: A case report. *Adv. Med. Sci.* 2009, 54: 113–115.
60. Bell RJ, Rube HT, Kreig A, et al. The Transcription Factor GABP Selectively Binds and Activates the Mutant TERT Promoter in Cancer. *Science* 2015, 348: 1036–1039.
61. Bellucco FT, Fock RA, de Oliveira-Júnior HR, Perez AB, Melaragno MI. Complex Small Supernumerary Marker Chromosome Leading to Partial 4q/21q Duplications: Clinical Implication and Review of the Literature. *Cytogenet Genome Res* 2018, 156: 173–178.

62. Belousova EA, Filipenko ML, Kushlinskii NE. Circular RNA: New regulatory molecules. *Bull. Exp. Biol. Med.* 2018, 164: 1–13.
63. Benabbad I, Rosilio M, Child CJ, et al. Safety outcomes and near-sdult height gain of growth hormone-treated children with SHOX deficiency: data from an observational study and a clinical trial. *Horm Res. Paediatr.* 2017, 87: 42–50.
64. Benn P, Cuckle H, Pergament E. Non-invasive prenatal testing for aneuploidy: Current status and future prospects. *Ultrasound Obstet. Gynecol.* 2013, 42: 15–33.
65. Bennett DC. How to Make a Melanoma: What Do We Know of the Primary Clonal Events? *Pigment. Cell Melanoma Res.* 2008, 21: 27–38.
66. Benzinou M, Walley A, Lobbens S, et al. Bardet-Biedl syndrome gene variants are associated with both childhood and adult common obesity in French Caucasians. *Diabetes*. 2006, 55(10):2876–2882.
67. Berezovsky A, Rocha DM, Sacai PY, et al. Visual acuity and retinal function in patients with Bardet-Biedl syndrome. Clinics. 2012, 67(2):145–149.
68. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell* 2007, 128: 669–681.
69. Bertelsen B, Melchior L, Jensen LR, Groth C, Glenthøj B, Rizzo R, Debes NM, Skov L, Brøndum-Nielsen K, Paschou P, et al. Intragenic deletions affecting two alternative transcripts of the IMMP2L gene in patients with Tourette syndrome. *Eur. J. Hum. Genet.* 2014, 22: 1283–1289.
70. Bettinelli A, Borsa N, Bellantuono R, Syrèn ML, Calabrese R, Edefonti A, et al. Patients with biallelic mutations in the chloride channel gene CLCNKB: long-term management and outcome. *Am J Kidney Dis.* 2007, 49: 91–8.
71. Bhatia P, Friedlander P, Zakaria EA, Kandil E. Impact of BRAF Mutation Status in the Prognosis of Cutaneous Melanoma: An Area of Ongoing Research. *Ann. Transl. Med.* 2015, 3: 24.
72. Bianchi DW, Chiu RWK. Sequencing of Circulating Cell-free DNA during Pregnancy. *N. Engl. J. Med.* 2018, 379: 464–473.
73. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing. *Obstet. Gynecol.* 2012, 119: 890–901.
74. Bianchi DW, Simpson JL, Jackson LG, et al. Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY I data. National Institute of Child Health and Development Fetal Cell Isolation Study. *Prenat Diagn*. 2002, 22(7): 609-615.
75. Biederman B, Bowen P. Partial trisomy 4q due to familial 2/4 translocation. *Qual. Life Res.* 1976, 33: 147–153.
76. Billingsley G, Bin J, Fieggen K, Duncan JL, Gerth-Kahlert C, Ogata K, Wodak SI, Traboulsi EA, Fishman G, Paterson A, et al. Mutations in chaperonin-like BBS genes are a major contributor to disease development in a multiethnic Bardet-Biedl syndrome patient population. *J. Med. Genet.* 2010, 47, 453–463.
77. Binder G, Ranke MB, Martin DD. Auxology is a valuable instrument for the clinical diagnosis of SHOX haploinsufficiency in school-age children with unexplained short stature. *J. Clin. Endocrinol. Metab* 2003, 88: 4891–4896.
78. Binder G, Rappold GA. SHOX Deficiency Disorders. In *GeneReviews®;* Adam, M.P., Ardinger, H.H., Pagon, R.A., Wallace, S.E., Eds.; GeneReviews®: Seattle, WA, USA, 2005.
79. Binder G. Short stature due to SHOX deficiency: genotype, phenotype, and therapy. *Horm Res. Paediatr.* 2011, 75: 81–89.
80. Birkenhäger R, Otto E, Schürmann MJ, Vollmer M, Ruf EM, Maier-Lutz I, et al. Mutation of BSND causes Bartter syndrome with sensorineural deafness and kidney failure. *Nat Genet.* 2001, 29: 310–4.
81. Blanchard A, Bockenhauer D, Bolignano D, Calo LA, Cosyns E, Devuyst O, et al. Gitelman syndrome: consensus and guidance from a kidney disease: improving global outcomes (KDIGO) controversies conference. *Kidney Int.* 2017, 91: 24-33.
82. Blethen SL, van Wyk JJ, Lorentz WB, Jennette JC. Reversal of Bartter's syndrome by renal transplantation in a child with focal, segmental glomerular sclerosis. *Am J Med Sci.* 1985, 289: 31–6.
83. Blum WF, Ross JL, Zimmermann AG, et al. Jr. GH treatment to final height produces similar height gains in patients with SHOX deficiency and Turner syndrome: results of a multicenter trial.

J. Clin. Endocrinol. Metab. 2013, 98: 1383–1392.
84. Bockenhauer D, van'T Hoff W, Dattani M, Lehnhardt A, Subtirelu M, Hildebrandt F, et al. Secondary nephrogenic diabetes insipidus as a complication of inherited renal diseases. *Nephron Physiol.* 2010, 116: 23–9.
85. Boeckel JN, Jaé N, Heumüller AW, Chen W, Boon RA, Stellos K, Zeiher AM, John D, Uchida S, Dimmeler S. Identification and characterization of hypoxia-regulated endothelial circular RNA. *Circ. Res.* 2015, 117: 884–890.
86. Bögershausen N, Wollnik B. Mutational Landscapes and phenotypic spectrum of SWI/SNF-related intellectual disability disorders. Front Mol Neurosci. 2018; 11:252.
87. Bolling MC, Jongbloed JD, Boven LG, Diercks GF, Smith FJ, McLean WHI, Jonkman MF. Plectin Mutations Underlie Epidermolysis Bullosa Simplex in 8% of Patients. *J. Investig. Dermatol.* 2014, 134: 273–276.
88. Bolognia JL, Schaffer JV, Cerroni L. *Dermatology*, 4th ed.; Elsevier Saunders: Philadelphia, PA, USA, 2017.
89. Bölükbaşi EY, Mumtaz S, Afzal M, Woehlbier U, Malik S, Tolun A. Homozygous mutation in CEP19, a gene mutated in morbid obesity, in Bardet-Biedl syndrome with predominant postaxial polydactyly. *J. Med. Genet.* 2018, 55, 189–197.
90. Bonfante A, Stella M, Rossi G. Partial trisomy 4q: Two cases resulting from a familial translocation t(4;18)(q27;p11). *Qual. Life Res.* 1979, 52: 85–90.
91. Bonnet C, Zix C, Grégoire M-J, Brochet K, Duc M, Rousselet F, Philippe C, Jonveaux P. Characterization of mosaic supernumerary ring chromosomes by array-CGH: Segmental aneusomy for proximal 4q in a child with tall stature and obesity. *Am. J. Med. Genet. Part A* 2006, 140: 233–237.
92. Botton T, Yeh I, Nelson T, et al. Recurrent BRAF Kinase Fusions in Melanocytic Tumors Offer an Opportunity for Targeted Therapy. *Pigment. Cell Melanoma Res.* 2013, 26: 845–851
93. Boukouris S, Mathivanan S. Exosomes in bodily fluids are a highly stable resource of disease biomarkers. *Proteom. Clin. Appl.* 2015, 9: 358–367.
94. Boycott K M, Hartley T, Biesecker L G et al. A Diagnosis for All Rare Genetic Diseases: The Horizon and the Next Frontiers. *Cell* 2019; 177(1): 32-37.
95. Branfield Day L, Quammie C, Heon E, et al. Liver anomalies as a phenotype parameter of Bardet–Biedl syndrome. *Clin Genet*. 2016, 89 (4):507–509.
96. Braun JJ, Noblet V, Kremer S, et al. Value of MRI olfactory bulb evaluation in the assessment of olfactory dysfunction in Bardet–Biedl syndrome. *Clin Genet*. 2016, 90(1):79–83.
97. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 2018, 68: 394–424.
98. Brochard K, Boyer O, Blanchard A, Loirat C, Niaudet P, Macher MA, et al. Phenotype–genotype correlation in antenatal and neonatal variants of Bartter syndrome. *Nephrol Dial Transplant.* 2009, 24: 1455–64.
99. Brosens LA, van Hattem WA, Jansen M, de Leng WW, Giardiello FM, Offerhaus GJA. Gastrointestinal polyposis syndromes. *Curr. Mol. Med.* 2007, 7: 29–46.
100. Buchanan A, Sachs A, Toler T, Tsipis J. NIPT: Current utilization and implications for the future of prenatal genetic counseling. *Prenat. Diagn.* 2014, 34: 850–857.
101. Buday L, Downward J. Many faces of Ras activation. BiochimBiophysActa 2008;1786:178–187.
102. Bülow S. Incidence of associated diseases in familial polyposis coli. In *Seminars in Surgical Oncology* 1987; John Wiley & Sons, Inc.: New York, NY, USA; Volume 3, pp. 84–87.
103. Büscher AK, Cetiner M, Büscher R, et al. Obesity in patients with Bardet-Biedl syndrome: influence of appetite-regulating hormones. *Pediatr Nephrol*. 2012, 27(11):2065–2071.
104. Buyukcelik M, Keskin M, Kilic BD, Kor Y, Balat A. Bartter syndrome and growth hormone deficiency: three cases. *Pediatr Nephrol.* 2012, 27: 2145–8.
105. Caba L, Florea L, Gug C, et al. Circular RNA—Is the Circle Perfect? *Biomolecules* 2021, 11: 1755.
106. Califf RM. Biomarker definitions and their applications. *Exp. Biol. Med.* 2018, 243: 213–221.
107. Cankaya AB, Erdem MA, Isler SC, Cifter M, Olgac V, Kasapoglu C, Oral CK. Oral and maxillofacial considerations in Gardner's syndrome. *Int. J. Med. Sci.* 2012, 9: 137.

108. Cannon-Albright L, Goldgar D, Meyer L, et al. Assignment of a Locus for Familial Melanoma, MLM, to Chromosome 9p13-P22. *Science* 1992, 258: 1148–1152.
109. Cappuccio G, Pinelli M, Torella A, Alagia M, Auricchio R, Staiano A, Nigro V, Brunetti-Pierri N. Expanding the phenotype of *DST*-related disorder: A case report suggesting a genotype/phenotype correlation. *Am. J. Med. Genet. Part A* 2017, 173: 2743–2746.
110. Caputo M, Pigni S, Agosti E, et al. Regulation of GH and GH signaling by nutrients. Cells. 2021; 10(6):1376
111. Cardenas-Rodriguez M, Badano JL. Ciliary biology: Understanding the cellular and genetic basis of human ciliopathies. *Am. J. Med. Genet. Part C Semin. Med. Genet.* 2009, 151C, 263–280.
112. Carey JC, Cassidy SB, Battaglia A, Viskochil D, Eds. *Cassidy and Allanson's Management of Genetic Syndromes*. John Wiley & Sons, 2020.
113.Carota I, Theilig F, Oppermann M, Kongsuphol P, Rosenauer A, Schreiber R, et al. Localization and functional characterization of the human NKCC2 isoforms. *Acta Physiol (Oxf).* 2010, 199: 327–38.
114. Carvajal RD, Antonescu CR, Wolchok JD, et al. KIT as a Therapeutic Target in Metastatic Melanoma. *JAMA* 2011, 305: 2327–2334.
115.Castrop H, Schießl IM. Physiology and pathophysiology of the renal Na-K- 2Cl cotransporter (NKCC2). *Am J Physiol Renal Physiol.* 2014, 307: F991.
116. Caunt CJ, Sale MJ, Smith PD, Cook SJ. MEK1 And MEK2 Inhibitors and Cancer Therapy: The Long and Winding Road. *Nat. Rev. Cancer* 2015, 15: 577–592.
117. Celle L, Lee L, Rintoul N, Savani RC, Long W, Mennuti MT, Krantz ID. Duplication of chromosome region 4q28.3-qter in monozygotic twins with discordant phenotypes. *Am. J. Med. Genet.* 2000, 94: 125–140.
118. Cen J, Liang Y, Huang Y, Pan Y, Shu G, Zheng Z, Liao X, Zhou M, Chen D, Fang Y, et al. Circular RNA circSDHC serves as a sponge for miR-127-3p to promote the proliferation and metastasis of renal cell carcinoma via the CDKN3/E2F1 axis. *Mol. Cancer* 2021, 20: 1–14.
119. Cernakova I, Kvasnicova M, Lovasova Z, Badova N, Drabek J, Bouchalova K, Trojanec R, Hajduch M. A duplication dup(4)(q28q35.2) de novo in a newborn. *Biomed. Pap.* 2006, 150: 113–116.
120. Cervenka J, Djavadi GR, Gorlin RJ. Partial trisomy 4q syndrome: Case report and review. *Hum. Genet.* 1976, 34: 1–7.
121. Cetta F, Dhamo A. Inherited multitumoral syndromes including colorectal carcinoma. *Surg. Oncol.* 2007, 16: 17–23.
122. Cetta F, Montalto G, Gori M, Curia MC, Cama A, Olschwang S. Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: Results from a European cooperative study. *J. Clin. Endocrinol. Metab.* 2000, 85: 286–292.
123. Chamcheu JC, Siddiqui IA, Syed DN, Adhami VM, Liovic M, Mukhtar H. Keratin gene mutations in disorders of human skin and its appendages. *Arch. Biochem. Biophys.* 2011, 508: 123–137.
124. Chang HH, Hemberg M, Barahona M, et al. Transcriptome wide noise controls lineage choice in mammalian progenitor cells. *Nature* 2008, 453; 544–547.
125. Chavanas S, Gache Y, Vailly J, Kanitakis J, Pulkkinen L, Uitto J, Ortonne JP, Meneguzzi G. Splicing modulation of integrin beta4 pre-mRNA carrying a branch point mutation underlies epidermolysis bullosa with pyloric atresia undergoing spontaneous amelioration with ageing. *Hum. Mol. Genet.* 1999, 8: 2097–2105.
126. Chen A, Zhong L, Ju K, Lu T, Lv J, Cao H. Plasmatic circRNA predicting the occurrence of human glioblastoma. *Cancer Manag. Res.* 2020, 12: 2917.
127. Chen B, Huang S. Circular RNA: An emerging non-coding RNA as a regulator and biomarker in cancer. *Cancer Lett.* 2018, 418: 41–50.
128. Chen C-P, Lin M-H, Chern S-R, Chen Y-T, Wu P-S, Kuo Y-L, Lee M-S, Wang W. Directly transmitted 4.5-Mb triplication of 4q12-q13.1: Prenatal diagnosis and molecular cytogenetic characterization. *Taiwan. J. Obstet. Gynecol.* 2014, 53: 123–125.
129. Chen C-P, Su Y-N, Chen Y-T, Chen W-L, Hsu LJ, Wang W. Prenatal diagnosis of directly transmitted benign 4q12-q13.1 quadruplication associated with tandem segmental amplifications of the LPHN3 gene. *Taiwan. J. Obstet. Gynecol.* 2011, 50: 401–404.

130. Chen EZ, Chiu RWK, Sun H, Akolekar R, Chan KCA, Leung TY, Jiang P, Zheng YWL, Lun FMF, Chan LYS et al. Noninvasive Prenatal Diagnosis of Fetal Trisomy 18 and Trisomy 13 by Maternal Plasma DNA Sequencing. *PLoS ONE* 2011, 6: e21791.
131. Chen L-L. The biogenesis and emerging roles of circular RNAs. *Nat. Rev. Mol. Cell Biol.* 2016, 17: 205–211.
132. Chen S, Li T, Zhao Q, Xiao B, Guo J. Using circular RNA hsa_circ_0000190 as a new biomarker in the diagnosis of gastric cancer. *Clin. Chim. Acta* 2017, 466: 167–171.
133. Chen XT, Li ZW, Zhao X; Li ML; Hou PF, Chu SF, Zheng JN, Bai J. Role of Circular RNA in Kidney-Related Diseases. *Front. Pharmacol.* 2021, 12: 286.
134. Cheng D, Wang J, Dong Z, Li X. Cancer-related circular RNA: Diverse biological functions. *Cancer Cell Int.* 2021, 21: 1–16.
135. Cheng F, Zheng B, Si S, Wang J, Zhao G, Yao Z, Niu Z, He W. The Roles of CircRNAs in Bladder Cancer: Biomarkers, Tumorigenesis Drivers, and Therapeutic Targets. *Front. Cell Dev. Biol.* 2021, 9: 666863.
136. Cheng Y, Zhang G, Li G. Targeting MAPK pathway in melanoma therapy. *Cancer Metastasis Rev.* 2013, 32: 567–584.
137. Chiaverini C, Charlesworth AV, Youssef M, Cuny J-F, Rabia SH, Lacour J-P, Meneguzzi G. Inversa Dystrophic Epidermolysis Bullosa Is Caused by Missense Mutations at Specific Positions of the Collagenic Domain of Collagen Type VII. *J. Investig. Dermatol.* 2010, 130: 2508–2511.
138. Chiba K, Lorbeer FK, Shain AH, et al. Mutations in the Promoter of the Telomerase Gene TERT Contribute to Tumorigenesis by a Two-Step Mechanism. *Science* 2017, 357: 1416–1420.
139. Child CJ, Rappold GA, Blum WF. Short stature homeobox-containing (SHOX) gene deficiency: genetics and growth response to growth hormone treatment in comparison with Turner syndrome. In *Handbook of Growth and Growth Monitoring in Health and Disease*, 1st ed.; Preedy, V.R., Eds.; Springer: New York, NY, USA, 2012; Volume 1, pp. 2299–2318.
140. Chioma L, Cappa M. Hypogonadism in male infants and adolescents: new androgen formulations. *Horm Res Paediatr*. 2021, 1–9.
141. Chiu CG, Nakamura Y, Chong KK, et al. Genome-wide characterization of circulating tumor cells identifies novel prognostic genomic alterations in systemic melanoma metastasis. *Clin. Chem.* 2014, 60: 873–885.
142. Cho J-W, Ryu H-W, Kim S-A, Nakano H, Lee K-S. Weber-Cockayne Type Epidermolysis Bullosa Simplex Resulting from a Novel Mutation (c. 608T>C) in the Keratin 5 Gene. *Ann. Dermatol.* 2014, 26: 739–742.
143. Choolani M, Mahyuddin AP, Hahn S. The promise of fetal cells in maternal blood. *Best Pract Res Clin Obstet Gynaecol*. 2012, 26(5): 655-667.
144. Christ-Crain M, Winzeler B, Refardt J. Diagnosis and management of diabetes insipidus for the internist: an update. *J Intern Med*. 2021, 290 (1):73–87.
145. Christensen RD, Henry E, Andres RL, Bennett ST. Reference ranges for blood concentrations of nucleated red blood cells in neonates. *Neonatology*. 2011, 99(4): 289-294.
146. Christensen ST, Morthorst S, Mogensen JB, Pedersen LB. Primary cilia and coordination of receptor tyrosine kinase (RTK) and transforming growth factor β (TGF-β) signaling. *Cold Spring Harb. Perspect. Biol.* 2017, 9, a028167.
147. Christian PH. Cone rod dystrophies. *Orphanet J Rare Dis*. 2007, 2(1):7.
148. Christiano AM, Anton-Lamprecht I, Amano S, Ebschner U, Burgeson RE, Uitto J. Compound heterozygosity for COL7A1 mutations in twins with dystrophic epidermolysis bullosa: A recessive paternal deletion/insertion mutation and a dominant negative maternal glycine substitution result in a severe phenotype. *Am. J. Hum. Genet.* 1996, 58: 682–693.
149. Christiano AM, Fine J-D, Uitto J. Genetic Basis of Dominantly Inherited Transient Bullous Dermolysis of the Newborn: A Splice Site Mutation in the Type VII Collagen Gene. *J. Investig. Dermatol.* 1997, 109: 811–814.
150. Christiano AM, Lee JY-Y, Chen WJ, Laforgia S, Uitto J. Pretibial epidermolysis bullosa: Genetic linkage to COL7A1 and identification of a glycine-to-cysteine substitution in the triple-helical domain of type VII collagen. *Hum. Mol. Genet.* 1995, 4: 1579–1583.
151. Chung HJ, Uitto J. Epidermolysis Bullosa with Pyloric Atresia. *Dermatol. Clin.* 2010, 28: 43–54.

152. Cianfarani S. Safety of pediatric rhGH therapy: an overview and the need for long-term surveillance. *Front. Endocrinol.* 2021,12
153. circAtlas2.0. Available online: http://circatlas.biols.ac.cn/ (accessed on 25 October 2021).
154. Cirenajwis H, Lauss M, Ekedahl H, et al. NF1-Mutated Melanoma Tumors Harbor Distinct Clinical and Biological Characteristics. *Mol. Oncol.* 2017, 11: 438–451.
155. Clark SK, Neale KF, Landgrebe JC, Phillips RKS. Desmoid tumours complicating familial adenomatous polyposis. *Br. J. Surg.* 1999, 86: 1185–1189.
156. Coffin GS, Siris E. Mental retardation with absent fifth fingernail and terminal phalanx. Am J Dis Child. 1970; 119:433–9.
157. Colla A. Duplicación parcial del cromosoma 4 asociada con coloboma ocular bilateral. *Arch. Argent. Pediatr.* 2012, 110: 59–62.
158. Colombo EA, Spaccini L, Volpi L, Negri G, Cittaro D, Lazarevic D, Zirpoli S, Farolfi A, Gervasini C, Cubellis MV, et al. Viable phenotype of ILNEB syndrome without nephrotic impairment in siblings heterozygous for unreported integrin alpha3 mutations. *Orphanet J. Rare Dis.* 2016, 11: 136.
159. Condrat I, He Y, Cosgarea R, Has C. Junctional Epidermolysis Bullosa: Allelic Heterogeneity and Mutation Stratification for Precision Medicine. *Front. Med.* 2019, 5: 363.
160. Contro E, Bernabini D, Farina A. Cell-Free Fetal DNA for the Prediction of Pre-Eclampsia at the First and Second Trimesters: A Systematic Review and Meta-Analysis. *Mol. Diagn. Ther.* 2017, 21: 125–135.
161. Cosgarea I, Ugurel S, Sucker A, et al. Targeted Next Generation Sequencing of Mucosal Melanomas Identifies Frequent NF1 and RAS Mutations. *Oncotarget* 2017, 8: 40683–40692.
162. Coulombe PA, Kerns ML, Fuchs E. Epidermolysis bullosa simplex: A paradigm for disorders of tissue fragility. *J. Clin. Investig.*2009, 119: 1784–1793.
163. Courtens W, Wuyts W, Rooms L, Pera SB, Wauters J. A subterminal deletion of the long arm of chromosome 10: A clinical report and review. *Am. J. Med. Genet. Part A* 2006, 140A: 402–409.
164.Covic M, Covic A, Ardeleanu Ş. Anatomy and physiology of the kidneys. In: Covic A editor. *Nephrology. Theoretical and Practical Principles*. (Iasi: Demiurg Press) 2011. p. 21–35.
165. Covic M, Ştefănescu D, Sandovici I, Gorduza EV. *Genetică Medicală*, 3rd ed.; Polirom Iaşi: Iasi, Romania, 2017.
166. Crew VK, Burton N, Kagan A, Green CA, Levene C, Flinter F, Brady RL, Daniels G, Anstee DJ. CD151, the first member of the tetraspanin (TM4) superfamily detected on erythrocytes, is essential for the correct assembly of human basement membranes in kidney and skin. *Blood* 2004, 104: 2217–2223.
167. Cserhalmi-Friedman PB, Anyane-Yeboa K, Christiano AM. Paternal germline mosaicism in Herlitz junctional epidermolysis bullosa. *Exp. Dermatol.* 2002, 11: 468–470.
168. Cui Y-X, Wang Y-H, Hao L-J, Hou L, Li W, Huang Y-F. Partial trisomy 4q: A case report. *Chin. Med. J.* 2006, 119: 1136–1139.
169. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic Activation of KIT in Distinct Subtypes of Melanoma. *J. Clin. Oncol.* 2006, 24: 4340–4346.
170. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct Sets of Genetic Alterations in Melanoma. *N. Engl. J. Med.* 2005, 353: 2135–2147.
171.da Silva Cunha T, Heilberg IP. Bartter syndrome: causes, diagnosis, and treatment. *Int J Nephrol Renovasc Dis.* 2018, 11: 291.
172. Dang N, Murrell DF. Mutation analysis and characterization of COL7A1 mutations in dystrophic epidermolysis bullosa. *Exp. Dermatol.* 2008, 17: 553–688.
173. Dasgupta B, Yi Y, Chen DY, et al. Proteomic Analysis Reveals Hyperactivation of The Mammalian Target of Rapamycin Pathway in Neurofibromatosis 1-Associated Human and Mouse Brain Tumors. *Cancer Res.* 2005, 65: 2755–2760.
174. Daskalakis M, Till H, Kiess W, et al. Roux-en-Y gastric bypass in an adolescent patient with Bardet-Biedl syndrome, a monogenic obesity disorder. *Obes Surg*. 2010, 20(1):121–125.
175. Dauber A, Rosenfeld RG, Hirschhorn JN. Genetic evaluation of short stature. *J. Clin. Endocrinol. Metab*. 2014, 99: 3080–3092.
176. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF Gene in Human Cancer. *Nature* 2002, 417: 949–954.

177. Davis E, Zhang Q, Liu Q, Diplas B, Davey LM, Hartley J, Stoetzel C, Szymanska K, Ramaswami G, Logan CV, et al. TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. *Nat. Genet.* 2011, 43, 189–196.
178. De Arcangelis A, Hamade H, Alpy F, Normand S, Bruyère E, Lefebvre O, Méchine-Neuville A, Siebert S, Pfister V, Lepage P, et al. Hemidesmosome integrity protects the colon against colitis and colorectal cancer. *Gut* 2017, 66: 1748–1760.
179.de Los Heros P, Pacheco-Alvarez D, Gamba G. Role of WNK kinases in the modulation of cell volume. *Curr Top Membr.* 2018, 81: 207–35.
180. Debta P, Debta FM, Bussari S, Acharya SS, Jeergal VA. Cancellous osteoma of maxilla: A rare case report. *J. Int. Soc. Prev. Community Dent.* 2016, 6: 261.
181.Delpire E, Gagnon KB. SPAK and OSR1: STE20 kinases involved in the regulation of ion homoeostasis and volume control in mammalian cells. *Biochem J*. 2008, 409: 321–31.
182. Denniston AK, Beales PL, Tomlins PJ, et al. Evaluation of visual function and needs in adult patients with Bardet–Biedl syndrome. *Retina*. 2014, 34(11):2282–2289.
183. Desai A, Jha O, Iyer V, et al. Reversible hypogonadism in Bardet-Biedl syndrome. *Fertil Steril.* 2009, 92(1):391.e13–5.
184. Deveault C, Billingsley G, Duncan JL, et al. BBS genotype-phenotype assessment of a multiethnic patient cohort calls for a revision of the disease definition. *Hum Mutat.* 2011, 32(6):610–619.
185. Dey M, Sharma S, Aggarwal S. Prenatal screening methods for aneuploidies. *N. Am. J. Med. Sci.* 2013, 5: 182–190.
186. Diallo LH, Tatin F, David F, Godet AC, Zamora A, Prats AC, Garmy-Susini B, Lacazette E. How are circRNAs translated by non-canonical initiation mechanisms? *Biochimie* 2019, 164: 45–52.
187.Dimke H, Schnermann J. Axial and cellular heterogeneity in electrolyte transport pathways along the thick ascending limb. *Acta Physiol (Oxf).* 2018, 223: e13057.
188. Dinarvand P, Davaro EP, Doan JV, Ising ME, Evans NR, Phillips NJ, Lai J, Guzman M.A. Familial adenomatous polyposis syndrome: An update and review of extraintestinal manifestations. *Arch. Pathol. Lab. Med.* 2019, 143: 1382–1398.
189. Ding L, Getz G, Wheeler DA, et al. Somatic Mutations Affect Key Pathways in Lung Adenocarcinoma. *Nature* 2008, 455: 1069–1075.
190. Diociaiuti A, Pisaneschi E, Rossi S, Condorelli A, Carnevale C, Zambruno G, El Hachem M. Autosomal recessive epidermolysis bullosa simplex due to EXPH5 mutation: Neonatal diagnosis of the first Italian case and literature review. *J. Eur. Acad. Dermatol. Venereol.* 2020, 34: e694–e697.
191. Drera B, Castiglia D, Zoppi N, Gardella R, Tadini G, Floriddia G, De Luca N, Pedicelli C, Barlati S, Zambruno G, et al. Dystrophic epidermolysis bullosa pruriginosa in Italy: Clinical and molecular characterization. *Clin. Genet.* 2006, 70: 339–347.
192. Du M, Han L, Shen P, et al. Long Noncoding RNA LINC02249 Is a Prognostic Biomarker and Correlates with Immunosuppressive Microenvironment in Skin Cutaneous Melanoma. *J. Oncol.* 2022, 2022: 2054901.
193. Du WW, Weining Y, Elizabeth L, Zhenguo Y, Preet D, Burton BY. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res.* 2016, 44: 2846–2858.
194. Dumoulin SC, Perret BP, Bennet AP, et al. Opposite effects of thyroid hormones on binding proteins for steroid hormones (sex hormone-binding globulin and corticosteroid-binding globulin) in humans. *Eur J Endocrinol*. 1995,132(5):594–598. Erratum in: *Eur J Endocrinol* 1995 Sep;133(3):381.
195. Dutrillaux B, Laurent C, Forabosco A, Noel B, Suerinc E, Biemont MC, Cotton JB. La trisomie 4q partielle. Apropos de trois observations. *Ann. Genet.* 1975, 18: 21–27.
196. Easley CJ, Karlinsey JM, Bienvenue JM, et al. A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability. *Proc Natl Acad Sci USA*. 2006, 103(51): 19272-19277.
197. Ebbesen KK, Hansen TB, Kjems J. Insights into circular RNA biology. *RNA Biol.* 2017, 14: 1035–1045.
198.Eder M, Darmann E, Haller MC, Bojic M, Peck-Radosavljevic M, Huditz R, et al. Markers of potassium homeostasis in salt losing tubulopathies— associations with hyperaldosteronism and hypomagnesemia. *BMC Nephrol.*2020, 21 :256.
199. Eggermont AM, Spatz A, Robert C. Cutaneous Melanoma. *Lancet* 2014, 383: 816–827.

200. Eggermont AMM, Blank CU, Mandala M, et al. Longer Follow-Up Confirms Recurrence-Free Survival Benefit of Adjuvant Pembrolizumab in High-Risk Stage III Melanoma: Updated Results From the EORTC 1325-MG/KEYNOTE-054 Trial. *J. Clin. Oncol.* 2020, 38: 3925–3936.
201. El-Ruby M, Hemly NA, Zaki MS. Maternal balanced translocation (4;21) leading to an offspring with partial duplica-tion of 4q and 21q without phenotypic manifestations of Down syndrome. *Genet. Couns.* 2007, 18: 217–226.
202. Enuka Y, Lauriola M, Feldman ME, Sas-Chen A, Ulitsky I, Yarden Y. Circular RNAs are long-lived and display only minimal early alterations in response to a growth factor. *Nucleic Acids Res.* 2016, 44: 1370–1383.
203. Erdogan N, Demir U, Songu M, Ozenler NK, Uluç E, Dirim B. A prospective study of paranasal sinus osteomas in 1889 cases: Changing patterns of localization. *Laryngoscope* 2009, 119: 2355–2359.
204. Eroglu Z, Zaretsky JM, Hu-Lieskovan S, et al. High Response Rate to PD-1 Blockade in Desmoplastic Melanomas. *Nature* 2018, 553: 347–350.
205. Ezquieta B, Cueva E, Oliver A, Gracia R. SHOX intragenic microsatellite analysis in patients with short stature. *J. Pediatr Endocrinol Metab* 2002, 15: 139–148.
206. Faienza MF, Chiarito M, Brunetti G, D'Amato G. Growth plate gene involment and isolated short stature. *Endocrine* 2021, 71: 28–34.
207. Fan L, Cao Q, Liu J, Zhang J, Li B. Circular RNA profiling and its potential for esophageal squamous cell cancer diagnosis and prognosis. *Mol. Cancer* 2019, 18: 16.
208. Fanale D, Taverna S, Russo A, Bazan V. Circular RNA in Exosomes. *Adv. Exp. Med. Biol.* 2018, 1087: 109–117.
209. Farag TI, Teebi AS. High incidence of Bardet Biedl syndrome among the Bedouin. *Clin. Genet.* 2008, 36, 463–464.
210. Fassihi H, Diba VC, Wessagowit V, Dopping-Hepenstal PJC, Jones CA, Burrows NP, McGrath JA. Transient bullous dermolysis of the newborn in three generations. *Br. J. Dermatol.* 2005, 153: 1058–1063.
211. Fassihi H, Wessagowit V, Ashton GHS, Moss C, Ward R, Denyer J, Mellerio JE, McGrath JA. Complete paternal uniparental isodisomy of chromosome 1 resulting in Herlitz junctional epidermolysis bullosa. *Clin. Exp. Dermatol.* 2005, 30: 71–74.
212. Fath MK, Azargoonjahromi A, Soofi A, et al. Current understanding of epigenetics role in melanoma treatment and resistance. *Cancer Cell Int.* 2022, 22: 313.
213. Feng C, He Z, Cai B, et al. Non-invasive prenatal diagnosis of chromosomal aneuploidies and microdeletion syndrome using fetal nucleated red blood cells isolated by nanostructure microchips. *Theranostics*. 2018, 8(5): 1301-1311.
214. Fernandez A, Prieto B, Escudero A, Ladenson JH, Alvarez FV. A monoclonal antibody with potential for aiding non-invasive prenatal diagnosis: utility in screening of pregnant women at risk of pre-eclampsia. *J Histochem Cytochem*. 2005, 53(3): 345-350.
215. Fernández-Medarde A, Santos E. Ras in cancer and developmental diseases. *Genes Cancer* 2011, 2: 344–358.
216. Ferrier A, Sato T, De Repentigny Y, Gibeault S, Bhanot K, O'Meara RW, Lynch-Godrei A, Kornfeld SF, Young KG, Kothary R. Transgenic expression of neuronal dystonin isoform 2 partially rescues the disease phenotype of the dystonia musculorum mouse model of hereditary sensory autonomic neuropathy VI. *Hum. Mol. Genet.* 2014, 23: 2694–2710.
217. Ficari F, Cama A, Valanzano R, Curia MC, Palmirotta R, Aceto G, Esposito DL, Crognale S, Lombardi A, Messerini L, et al. APC gene mutations and colorectal adenomatosis in familial adenomatous polyposis. *Br. J. Cancer* 2000, 82: 348–353.
218. Fine J-D, Eady RA, Bauer EA, Bauer JW, Bruckner-Tuderman L, Heagerty A, Hintner H, Hovnanian A, Jonkman MF, Leigh I, et al. The classification of inherited epidermolysis bullosa (EB): Report of the Third International Consensus Meeting on Diagnosis and Classification of EB. *J. Am. Acad. Dermatol.* 2008, 58: 931–950.
219. Fine J-D, Horiguchi Y, Stein DH, Esterly NB, Leigh IM. Intraepidermal type VII collagen: Evidence for abnormal intracytoplasmic processing of a major basement membrane protein in rare patients with dominant and possibly localized recessive forms of dystrophic epidermolysis bullosa. *J. Am. Acad. Dermatol.* 1990, 22: 188–195.

220. Fine J-D, Johnson L, Weiner M, Stein A, Cash S, DeLeoz J, Devries D, Suchindran C. Pseudosyndactyly and musculoskeletal contractures in inherited epidermolysis bullosa: Experience of the national epidermolysis bullosa registry, 1986–2002. *J. Hand Surg.* 2005, 30: 14–22.
221. Fine J-D, Johnson LB, Weiner M, Li K-P, Suchindran C. Epidermolysis bullosa and the risk of life-threatening cancers: The National EB Registry experience, 1986–2006. *J. Am. Acad. Dermatol.* 2009, 60: 203–211.
222. Fine J-D. Epidemiology of Inherited Epidermolysis Bullosa Based on Incidence and Prevalence Estimates from the National Epidermolysis Bullosa Registry. *JAMA Dermatol.* 2016, 152: 1231–1238.
223. Fine J-D. Inherited epidermolysis bullosa. *Orphanet J. Rare Dis.* 2010, 5: 12.
224. Fleck BJ, Pandya A, Vanner L, Kerkering K, Bodurtha J. Coffin-Siris syndrome: Review and presentation of new cases from a questionnaire study. Am J Med Genet. 2001; 99(1): 1-7
225. Florea L, Caba L, Gorduza EV. Bardet–Biedl syndrome—Multiple kaleidoscope images: insight into mechanisms of genotype–phenotype correlations. *Genes*. 2021,12(9):1353.
226. Focșa IO, Budișteanu M and Bălgrădean M. Clinical and genetic heterogeneity of primary ciliopathies (Review). *Int J Mol Med* 2021, 48(3): 176.
227.Focșa IO, et al. A case of Bardet-Biedl syndrome caused by a recurrent variant in BBS12: A case report. *Biomed Rep* 2021, 15(6):103.
228. Forsyth RL, Gunay-Aygun M. Bardet-Biedl Syndrome Overview. In: Adam MP, Ardinger HH, Pagon RA, et al. editors. *GeneReviews® [Internet]*. Seattle: University of Washington, Seattle; 2003. Available from https://www.ncbi.nlm.nih.gov/books/NBK1363/. Accessed May 21, 2022.
229.Forsythe E et al. Managing Bardet-Biedl Syndrome-Now and in the Future. *Front Pediatr* 2018, 6: 23.
230. Forsythe E, Beales PL. Bardet-Biedl syndrome. *Eur J Hum Genet*. 2013, 21(1):8–13.
231. Forsythe E, Kenny J, Bacchelli C, Beales PL. Managing Bardet–Biedl syndrome—now and in the future. *Front Pediatr*. 2018, 6:23.
232. Forsythe E, Sparks K, Best S, et al. Risk factors for severe renal disease in Bardet-Biedl syndrome. *J Am Soc Nephrol*. 2017, 28(3):963–970.
233. Forsythe E, Sparks K, Hoskins BE, et al. Genetic predictors of cardiovascular morbidity in Bardet-Biedl syndrome. *Clin Genet*. 2015, 87 (4):343–349.
234. Francke, U. Quinacrine mustard fluorescence of human chromosomes: Characterization of unusual translocations. *Am. J. Hum. Genet.* 1972, 24: 189–213.
235. Frey L, Klümper N, Schmidt D, Kristiansen G, Toma M, Ritter M, Alajati A, Ellinger J. CircEHD2, CircNETO2 and CircEGLN3 as Diagnostic and Prognostic Biomarkers for Patients with Renal Cell Carcinoma. *Cancers* 2021, 13: 2177.
236. Friedl W, Caspari R, Sengteller M, Uhlhaas S, Lamberti C, Jungck M, Kadmon M, Wolf M, Fahnenstich J, Gebert J, et al. Can APC mutation analysis contribute to therapeutic decisions in familial adenomatous polyposis? Experience from 680 FAP families. *Gut* 2001, 48: 515–521.
237. Fryns JP, Berghe HVD. Partial duplication of the long arm of chromosome. *Ann. Génét.* 1980, 23: 52–53.
238. Fu L, Jiang Z, Li T, Hu Y, Guo J. Circular RNA s in hepatocellular carcinoma: Functions and implications. *Cancer Med.* 2018, 7: 3101–3109.
239. Fu S, Wu H, Zhang H, et al. DNA methylation/hydroxymethylation in melanoma. *Oncotarget* 2017, 8: 78163.
240. Fujita KI, Yamazaki T, Harada K, Seno S, Matsuda H, Masuda S. URH49 exports mRNA by remodeling complex formation and mediating the NXF1-dependent pathway. *Biochim. Biophys. Acta Gene Regul. Mech.* 2020, 1863: 194480.
241. Fukami M, Seki A, Ogata T. SHOX haploinsufficiency as a cause of syndromic and nonsyndromic short stature. *Mol. Syndromol.* 2016, 7: 3–11.
242.Gallagher H, Soar J, Tomson C. New guideline for perioperative management of people with inherited salt-wasting alkaloses. *Br J Anaesth*. 2016, 116: 746–9.
243. Gallagher SJ, Tiffen JC, Hersey P. Histone modifications, modifiers and readers in melanoma resistance to targeted and immune therapy. *Cancer* 2015, 7: 1959–1982.
244.Gamba G. The thiazide-sensitive Na-Cl cotransporter: molecular biology, functional properties,

and regulation by WNKs. *Am J Physiol Renal Physiol.* 2009, 297: F838–48.
245. Ganani D, Malovitski K, Sarig O, Gat A, Sprecher E, Samuelov L. Epidermolysis bullosa simplex due to bi-allelic DST mutations: Case series and review of the literature. *Pediatr. Dermatol.* 2021, 38: 436–441.
246. Gandini S, Zanna I, De Angelis SP, et al. Circulating Tumour DNA and Melanoma Survival: A Systematic Literature Review and Meta-Analysis. *Crit. Rev. Oncol. Hematol.* 2021, 157: 103187.
247. Gardella R, Castiglia D, Posteraro P, Bernardini S, Zoppi N, Paradisi M, Tadini G, Barlati S, McGrath J, Zambruno G, et al. Genotype–Phenotype Correlation in Italian Patients with Dystrophic Epidermolysis Bullosa. *J. Investig. Dermatol.* 2002, 119: 1456–1462.
248.Garnier A, Dreux S, Vargas-Poussou R, Oury JF, Benachi A, Deschênes G, et al. Bartter syndrome prenatal diagnosis based on amniotic fluid biochemical analysis. *Pediatr Res.* 2010, 67: 300–3.
249. Garvey WT, Mechanick JI, Brett EM, et al. Reviewers of the AACE/ACE Obesity Clinical Practice Guidelines. American association of clinical endocrinologists and American college of endocrinology comprehensive clinical practice guidelines for medical care of patients with obesity. *Endocr Pract*. 2016, 22 Suppl 3:1–203.
250. Gast A, Scherer D, Chen B, et al. Somatic Alterations in the Melanoma Genome: A High-Resolution Array-Based Comparative Genomic Hybridization Study. *Genes Chromosom. Cancer* 2010, 49: 733–745.
251. Gawron K, Łazarz-Bartyzel K, Potempa J, Chomyszyn-Gajewska M. Gingival fibromatosis: clinical, molecular and therapeutic issues. Orphanet journal of rare diseases 2016: 11(1), 9.
252. Gedde-Dahl T Jr, Dupuy BM, Jonassen R, Winberg J-O, Anton-Lamprecht I, Olaisen B, Olalsen B. Junctional epidermoly- sis bullosa inversa (locus EBR2A) assigned to 1q31 by linkage and association to LAMC1. *Hum. Mol. Genet.* 1994, 3:1387–1391.
253. Gerson KD, Truong S, Haviland MJ, O'Brien BM, Hacker MR, Spiel MH. Low fetal fraction of cell-free DNA predicts placental dysfunction and hypertensive disease in pregnancy. *Pregnancy Hypertens.* 2019, 16: 148–153.
254. Geuens T, Bouhy D, Timmerman V. The hnRNP family: Insights into their role in health and disease. *Hum. Genet.* 2016, 135: 851–867.
255. Ghafouri-Fard S, Khoshbakht T, Hussen BM, et al. Circ_CDR1as: A circular RNA with roles in the carcinogenesis. *Pathol. Res. Pract.* 2022, 236: 153968.
256. Giagulli VA, Kaufman JM, Vermeulen A. Pathogenesis of the decreased androgen levels in obese men. *J Clin Endocrinol Metab*. 1994, 79 (4):997–1000.
257. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for an- euploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2017, 50(3): 302-314.
258.Gil-Peña H, Mejia N, Alvarez-Garcia O, Loredo V, Santos F. Longitudinal growth in chronic hypokalemic disorders. *Pediatr Nephrol.* 2010, 25: 733–7.
259. Giunta EF, Arrichiello G, Curvietto M, et al. Epigenetic Regulation in Melanoma: Facts and Hopes. *Cells* 2021, 10: 2048.
260. Giunta EF, De Falco V, Napolitano S, et al. Optimal Treatment Strategy for Metastatic Melanoma Patients Harboring BRAF-V600 Mutations. *Ther. Adv. Med. Oncol.* 2020, 12: 1758835920925219.
261. Goldenberg P. An Update on Common Chromosome Microdeletion and Microduplication Syndromes. *Pediatr. Ann.* 2018, 47: e198–e203.
262. Goodman BK, Capone GT, Hennessey J, Thomas GH. Familial tandem duplication of band q31.1 to q32.3 on chromo-some 4 with mild phenotypic effect. *Am. J. Med. Genet.* 1997, 73: 119–124.
263. Gorduza EV, Socolov DG, Socolov RV. Prenatal Biochemical and Ultrasound Markers in Chromosomal Anomalies. In *Congenital Anomalies-From the Embryo to the Neonate*, 1st ed.; IntechOpen: Zagreb, Croatia, 2018; Volume 1, pp. 365–396.
264. Gostyńska KB, Nijenhuis M, Lemmink H, Pas H, Pasmooij AM, Lang KK, Castañón MJ, Wiche G, Jonkman MF. Mutation in exon 1a of PLEC, leading to disruption of plectin isoform 1a, causes autosomal-recessive skin-only epidermolysis bullosa simplex. *Hum. Mol. Genet.* 2015, 24: 3155–3162.

265. Gourdol O, David L, Colon S, et al. Renal involvement in the Laurence-Moon-Bardet-Biedl syndrome. Apropos of 3 cases. *Pediatrie*. 1984, 39 (3):175–181.
266. Goydos JS, Shoen SL. Acral Lentiginous Melanoma. *Cancer Treat. Res.* 2016, 167: 321–329.
267. Grechukhina O, Gressel GM, Munday W, et al. Endometrial carcinoma in a 26-year-old patient with Bardet-Biedl syndrome. Case Rep Obstet Gynecol. 2018, 2018:1952351.
268. Green JS, Parfrey PS, Harnett JD, et al. The cardinal manifestations of Bardet-Biedl syndrome, a form of Laurence-Moon-Biedl syndrome. *N Engl J Med*. 1989, 321(15):1002–1009.
269. Groen EJ, Roos A, Muntinghe FL, Enting RH, de Vries J, Kleibeuker JH, Witjes MJ, Links TP, van Beek AP. Extra-intestinal manifestations of familial adenomatous polyposis. *Ann. Surg. Oncol.* 2008, 15: 2439–2450.
270. Grønbaek K, Hother C, Jones PA. Epigenetic changes in cancer. *APMIS* 2007, 115: 1039–1059.
271. Gu L-H, Kim S-C, Ichiki Y, Park J, Nagai M, Kitajima Y. A usual frameshift and delayed termination codon mutation in keratin 5 causes a novel type of epidermolysis bullosa simplex with migratory circinate erythema. *J. Investig. Dermatol.* 2003, 121: 482–485.
272. Guarnerio J, Bezzi M, Jeong JC, Pafenholz SV, Berry K, Naldini MM, Lo-Coco F, Tay Y, Beck AH, Pandolfi PP. Onco- genic role of fusion-circRNAs derived from cancer-associated chromosomal translocations. *Cell* 2016, 165: 289–302.
273. Guerrero-Aspizua S, Conti CJ, Escamez MJ, Castiglia D, Zambruno G, Youssefian L, Vahidnezhad H, Requena L, Itin P, Tadini G, et al. Assessment of the risk and characterization of non-melanoma skin cancer in Kindler syndrome: Study of a series of 91 patients. *Orphanet J. Rare Dis.* 2019, 14: 183.
274. Gug C, Burada F, Ioana M, Riza AL, Moldovan M, Mozos I, Raţiu A, Martiniuc V, Gorduza EV. Polyploidy in First and Second Trimester Pregnancies in Romania—A Retrospective Study. *Clin Lab.* 2020, 66: 517–527.
275. Gug C, Caba L, Mozos I, Stoian D, Atasie D, Gug M, Gorduza EV. Rare splicing mutation in COL1A1 gene identified by whole exomes sequencing in a patient with osteogenesis imperfecta type I followed by prenatal diagnosis: A case report and review of the literature. *Gene* 2020, 741: 144565.
276. Gug C, Chiriac VD, Catu D, Budau G, Costa SD, Navolan DB. Prenatal Cytogenetic Diagnosis in Timisoara: 1 Year Experience. *Timis. Med. J.* 2003, 53: 113–117.
277. Gug C, Gorduza EV, Lăcătuşu A, Vaida MA, Bîrsăşteanu F, Puiu M, Stoicănescu D. CHARGE syndrome associated with de novo (I1460Rfs*15) frameshift mutation of CHD7 gene in a patient with arteria lusoria and horseshoe kidney. *Exp. Ther. Med.* 2020, 20; 479–485.
278. Gug C, Hutanu D, Vaida M, Doroş G, Popa C, Stroescu R, Furău G, Furău C, Grigorita L, Mozos I. De novo unbalanced translocation t(15;22)(q26.2;q12) with velo-cardio-facial syndrome: A case report and review of the literature. *Exp. Ther. Med.* 2018, 16: 3589–3595.
279. Gug C, Mihaescu A, Mozos I. Two mutations in the thiazide-sensitive NaCl co-transporter gene in a Romanian Gitelman syndrome patient: Case report. *Ther. Clin. Risk Manag.* 2018, 14: 149–155.
280. Gug C, Ratiu A, Navolan D, Drăgan I, Groza I-M, Păpurică M, Vaida M-A, Mozoş I, Jurcă MC. Incidence and Spectrum of Chromosome Abnormalities in Miscarriage Samples: A Retrospective Study of 330 Cases. *Cytogenet. Genome Res.* 2019, 158: 171–183.
281. Gug C, Stoicanescu D, Mozos I, Nussbaum L, Cevei M, Stambouli D, Pavel AG, Doros G. De novo 8p21.3→p23.3 Duplication With t(4;8)(q35;p21.3) Translocation Associated With Mental Retardation, Autism Spectrum Disorder, and Congenital Heart Defects: Case Report With Literature Review. *Front. Pediatr.* 2020, 8: 375.
282. Gundewar S, Kothari DS, Mokal NJ, Ghalme A. Osteomas of the craniofacial region: A case series and review of literature. *Indian J. Plast. Surg.* 2013: 46, 479.
283. Guo DF, Cui H, Zhang Q, et al. The BBSome controls energy homeostasis by mediating the transport of the leptin receptor to the plasma membrane. *PLoS Genet*. 2016, 12(2):e1005890.
284. Guo D-F, Rahmouni K. Molecular basis of the obesity associated with Bardet–Biedl syndrome. *Trends Endocrinol. Metab.* 2011, 22, 286–293.
285. Guo F, Yang J, Huang Y, Qi Y, Hou Y, Peng H, Wang D, Wang Y, Luo X, Li Y, et al. Association between fetal fraction at the second trimester and subsequent spontaneous preterm birth. *Prenat. Diagn.* 2019, 39: 1191–1197.

286. Guo J, Si L, Kong Y, et al. Phase II, Open-Label, Single-Arm Trial of Imatinib Mesylate in Patients with Metastatic Melanoma Harboring C-Kit Mutation or Amplification. *J. Clin. Oncol.* 2011, 29: 2904–2909.
287. Guo L, Degenstein L, Dowling J, Yu Q-C, Wollmann R, Perman B, Fuchs E. Gene targeting of BPAG1: Abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. *Cell* 1995, 81: 233–243.
288. Guo S, Hu C, Zhai X, Sun D. Circular RNA 0006602 in plasma exosomes: A new potential diagnostic biomarker for hepatocellular carcinoma. *Am. J. Transl. Res.* 2021, 13: 6001.
289. Guo S, Li B, Chen Y, Zou D, Yang S, Zhang Y, Wu N, Sheng L, Huang H, Ouyang G, et al. Hsa_circ_0012152 and Hsa_circ_0001857 accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. *Front. Oncol.* 2020, 10: 1655.
290. Guo W, Wang H, Li C. Signal pathways of melanoma and targeted therapy. *Signal Transduct. Target. Ther.* 2021, 6: 424.
291. Guo Y, Huang Q, Zheng J, Hsueh CY, Yuan X, Heng Y, Zhou L. Diagnostic role of dysregulated circular RNA hsa_circ_0036722 in laryngeal squamous cell carcinoma. *OncoTargets Ther.* 2020, 13: 5709.
292. Guran T, Ekinci G, Atay Z, et al. Radiologic and hormonal evaluation of pituitary abnormalities in patients with Bardet-Biedl syndrome. *Clin Dysmorphol*. 2011, 20(1):26–31.
293. Guria A, Sharma P, Natesan S, Pandi G. Circular RNAs—The road less traveled. *Front. Mol. Biosci.* 2020, 6: 146.
294. Gürsoy S, Hazan F, Aykut A, et al. Detection of SHOX gene variations in patients with skeletal abnormalities with or without short stature. *J. Clin. Res. Pediatr. Endocrinol.* 2020, 12: 358–365.
295. Halal F, Vekemans M, Chitayat D. Interstitial tandem direct duplication of the long arm of chromosome 4 (q23–q27) and possible assignment of the structural gene encoding human aspartylglucosaminidase to this segment. *Am. J. Med. Genet.* 1991, 39: 418–421.
296. Half E, Bercovich D, Rozen P. Familial adenomatous polyposis. *Orphanet J. Rare Dis.* 2009, 4: 1–23.
297. Hall A, Marshall CJ, Spurr NK, Weiss RA. Identification of Transforming Gene in Two Human Sarcoma Cell Lines as A New Member of The Ras Gene Family Located on Chromosome 1. *Nature* 1983, 303: 396–400.
298. Hamada H, Arinami T, Kubo T, Hamaguchi H, Iwasaki H. Fetal nucleated cells in maternal peripheral blood: frequency and relationship to gestational age. *Hum Genet*. 1993, 91(5): 427-432.
299. Hamlington B, Ivey LE, Brenna E, et al. Characterization of courtesy stigma perceived by parents of overweight children with Bardet-Biedl syndrome. *PLoS One*. 2015,10(10):e0140705.
300. Hammami-Hauasli N, Raghunath M, Bruckner-Tuderman L, Küster W. Transient Bullous Dermolysis of the Newborn Associated with Compound Heterozygosity for Recessive and Dominant COL7A1 Mutations. *J. Investig. Dermatol.* 1998, 111: 1214–1219.
301. Hammersen J, Has C, Naumann-Bartsch N, Stachel D, Kiritsi D, Söder S, Tardieu M, Metzler M, Bruckner-Tuderman L, Schneider H. Genotype, Clinical Course, and Therapeutic Decision Making in 76 Infants with Severe Generalized Junctional Epidermolysis Bullosa. *J. Investig. Dermatol.* 2016, 136: 2150–2157.
302. Han YM, Kang GM, Byun K, Ko HW, Kim J, Shin M-S, Kim H-K, Gil SY, Yu JH, Lee B, et al. Leptin-promoted cilia assembly is critical for normal energy balance. *J. Clin. Investig.* 2014, 124, 2193–2197.
303. Hanniford D, Ulloa-Morales A, Karz A, et al. Epigenetic silencing of CDR1as drives IGF2BP3-mediated melanoma invasion and metastasis. *Cancer Cell* 2020, 37: 55–70.
304. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013, 495: 384–388.
305. Harel A, Bergman R, Indelman M, Sprecher E. Epidermolysis Bullosa Simplex with Mottled Pigmentation Resulting from a Recurrent Mutation in KRT14. *J. Investig. Dermatol.* 2006, 126: 1654–1657.
306. Harnett JD, Green JS, Cramer BC, et al. spectrum of renal disease in Laurence-Moon-Biedl syndrome. *N Engl J Med*. 1988, 319(10):615–618.
307.Hart TC, Pallos D, Bowden DW, Bolyard J, Pettenati MJ, Cortelli JR. Genetic linkage of

hereditary gingival fibromatosis to chromosome 2p21. Am J Hum Genet. 1998; 62:876–883.
308. Hart TC, Zhang Y, Gorry MC et al. A mutation in the SOS1 gene causes hereditary gingival fibroma tosis type 1. Am J Hum Genet. 2002; 70:943–954.
309. Hartwig TS, Ambye L, Werge L, Weiergang MP, Nørgaard P, Sørensen S, Jørgensen FS. Non-Invasive Prenatal Testing (NIPT) in pregnancies with trisomy 21, 18 and 13 performed in a public setting—Factors of importance for correct interpretation of results. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2018, 226: 35–39.
310. Has C, Bauer JW, Bodemer C, Bolling MC, Bruckner-Tuderman L, Diem A, Fine J-D, Heagerty A, Hovnanian A, Marinkovich MP, et al. Consensus reclassification of inherited epidermolysis bullosa and other disorders with skin fragility. *Br. J. Dermatol.* 2020, 183: 614–627.
311. Has C, Castiglia D, Del Rio M, Diez MG, Piccinni E, Kiritsi D, Kohlhase J, Itin P, Martin L, Fischer J, et al. Kindler syndrome: Extension of FERMT1 mutational spectrum and natural history. *Hum. Mutat.* 2011, 32: 1204–1212.
312. Has C, Chang Y-R, Volz A, Hoeping D, Kohlhase J, Bruckner-Tuderman L. Novel Keratin 14 Mutations in Patients with Severe Recessive Epidermolysis Bullosa Simplex. *J. Investig. Dermatol.* 2006, 126: 1912–1914.
313. Has C, Fischer J. Inherited epidermolysis bullosa: New diagnostics and new clinical phenotypes. *Exp. Dermatol.* 2019, 28; 1146–1152.
314. Has C, Nyström A, Saeidian AH, Bruckner-Tuderman L, Uitto J. Epidermolysis bullosa: Molecular pathology of connective tissue components in the cutaneous basement membrane zone. *Matrix Biol.* 2018, 71–72: 313–329.
315. Has C, South A, Uitto J. Molecular Therapeutics in Development for Epidermolysis Bullosa: Update 2020. *Mol. Diagn. Ther.* 2020, 24: 299–309.
316. Has C, Spartà G, Kiritsi D, Weibel L, Moeller A, Vega-Warner V, Waters A, He Y, Anikster Y, Esser P, et al. Integrin α3 Mutations with Kidney, Lung, and Skin Disease. *N. Engl. J. Med.* 2012, 366: 1508–1514.
317. Has C. The "Kelch" Surprise: KLHL24, a New Player in the Pathogenesis of Skin Fragility. *J. Investig. Dermatol.* 2017, 137: 1211–1212.
318. Hatta N, Takata M, Shimizu H. Spontaneous disappearance of intraepidermal type VII collagen in a patient with dystrophic epidermolysis bullosa. *Br. J. Dermatol.* 1995, 133: 619–624.
319. Hatzimichael E, Lagos K, Sim VR, et al. Epigenetics in diagnosis, prognostic assessment and treatment of cancer: An update. *Exp. Clin. Sci. J.* 2014, 13: 954–976.
320. Hawkes CP, Grimberg A, Dzata VE, De Leon DD. Adding glucagon stimulated GH testing to the diagnostic fast increases the detection of GH sufficient children. Horm Res Paediatr. 2016; 85(4):265-272.
321. Haws RM, Gordon G, Han JC, et al. The efficacy and safety of setmelanotide in individuals with Bardet-Biedl syndrome or Alström syndrome: phase 3 trial design. *Contemp Clin Trials Commun*. 2021, 22:100780.
322. Haws RM, Joshi A, Shah SA, Alkandari O, Turman MA. Renal transplantation in Bardet–Biedl Syndrome. *Pediatr. Nephrol.* 2016, 31, 2153–2161.
323. Haws RM, McIntee TJ, Green CB. Cutaneous findings in Bardet-Biedl syndrome. *Int J Dermatol*. 2019, 58(10):1160–1164.
324. Hayward NK, Wilmott JS, Waddell N et al. Whole-Genome Landscapes of Major Melanoma Subtypes. *Nature* 2017, 545: 175–180.
325. He AT, Liu J, Li F, Yang BB. Targeting circular RNAs as a therapeutic approach: Current strategies and challenges. *Signal Transduct. Target. Ther.* 2021, 6: 185.
326. He L, Hannon GJ. MicroRNAs: Small RNAs with a big role in gene regulation. *Nat. Rev. Genet.* 2004, 5: 522–531.
327. He Y, Maier K, Leppert J, Hausser I, Schwieger-Briel A, Weibel L, Theiler M, Kiritsi D, Busch H, Boerries M, et al. Monoallelic Mutations in the Translation Initiation Codon of KLHL24 Cause Skin Fragility. *Am. J. Hum. Genet.* 2016, 99: 1395–1404.
328. He Z, Guo F, Feng C, et al. Fetal nucleated red blood cell analysis for non-invasive prenatal diagnostics using a nanostructure microchip. *J Mater Chem B.* 2017, 5(2): 226-235.
329. Health Quality Ontario. Noninvasive Prenatal Testing for Trisomies 21, 18, and 13, Sex Chromosome Aneuploidies, and Microdeletions: A Health Technology Assessment. *Ont. Health*

Technol. Assess. Ser. 2019, 19: 1–166.
330. Hedberg-Oldfors C, Abramsson A, Osborn DPS, Danielsson O, Fazlinezhad A, Nilipour Y, Hübbert L, Nennesmo I, Visuttijai K, Bharj J, et al. Cardiomyopathy with lethal arrhythmias associated with inactivation of KLHL24. *Hum. Mol. Genet.* 2019, 28: 1919–1929.
331.Hennings JC, Andrini O, Picard N, Paulais M, Huebner AK, Cayuqueo IKL, et al. The ClC-K2 chloride channel is critical for salt handling in the distal nephron. *J Am Soc Nephrol.* 2017, 28: 209–17.
332. Hestand MS, Bessem M, Van Rijn P, De Menezes RX, Sie D, Bakker I, Boon EMJ, Sistermans EA, Weiss MM. Fetal fraction evaluation in non-invasive prenatal screening (NIPS). *Eur. J. Hum. Genet.* 2018, 27: 198–202.
333.HGNC, HUGO Gene Nomenclature Committee. *The Resource for Approved Human Gene Nomenclature*. (2022). Available online at: http://www. genenames.org/ (accessed March 28, 2022).
334. Hisado-Oliva A, Ruzafa-Martin A, Sentchordi L, et al. Mutations in C-natriuretic peptide (NPPC): A novel cause of autosomal dominant short stature. *Genet. Med.* 2018, 20: 91–97.
335. Hjortshøj TD, Grønskov K, Brondum-Nielsen K, Rosenberg T. A novel founder BBS1 mutation explains a unique high prevalence of Bardet-Biedl syndrome in the Faroe Islands. *Br. J. Ophthalmol.* 2008, 93, 409–413.
336. Hjortshøj TD, Grønskov K, Philp AR, Nishimura DY, Riise R, Sheffield V, Rosenberg T, Brøndum-Nielsen K. Bardet-Biedl syndrome in Denmark-report of 13 novel sequence variations in six genes. *Hum. Mutat.* 2010, 31, 429–436.
337. Hodi FS, Friedlander P, Corless CL, et al. Major Response to Imatinib Mesylate in KIT-Mutated Melanoma. *J. Clin. Oncol.* 2008, 26: 2046–2051.
338. Horbinski C, Carter EM, Heard PL, Sathanoori M, Hu J, Vockley J, Gunn S, Hale DE, Surti U, Cody JD. Molecular and clinical characterization of a recurrent cryptic unbalanced t(4q;18q) resulting in an 18q deletion and 4q duplication. *Am. J. Med. Genet. Part A* 2008, 146A: 2898–2904.
339. Hou Y, Yang J, Qi Y, Guo F, Peng H, Wang D, Wang Y, Luo X, Li Y, Yin A. Factors affecting cell-free DNA fetal fraction: Statistical analysis of 13,661 maternal plasmas for non-invasive prenatal screening. *Hum. Genom.* 2019, 13: 1–8.
340. Hovnanian A, Rochat A, Bodemer C, Petit E, Rivers CA, Prost C, Fraitag S, Christiano AM, Uitto J, Lathrop M, et al. Characterization of 18 New Mutations in COL7A1 in Recessive Dystrophic Epidermolysis Bullosa Provides Evidence for Distinct Molecular Mechanisms Underlying Defective Anchoring Fibril Formation. *Am. J. Hum. Genet.* 1997, 61: 599–610.
341. Hoyer J, Ekici AB, Endele S, et al. Haploinsufficiency of ARID1B, a member of the SWI/SNF-a chromatin-remodeling complex, is a frequent cause of intellectual disability. Am J Hum Genet. 2012; 90(3):565-572.
342. Hsu CJ, Meers O, Buschbeck M, Heidel FH. The role of MacroH2A histone variants in cancer. *Cancer* 2021, 13: 3003.
343. Hu H, Wang L, Wu J, Zhou P, Fu J, Sun J, Cai W, Liu H, Yang Y. Noninvasive prenatal testing for chromosome aneuploidies and subchromosomal microdeletions/microduplications in a cohort of 8141 single pregnancies. *Hum. Genom.* 2019, 13: 14.
344. Hu Y, Zhu Y, Zhang W, Lang J, Ning L. Utility of plasma circBNC2 as a diagnostic biomarker in epithelial ovarian cancer. *OncoTargets Ther.* 2019, 12: 9715.
345. Hua R, Barrett AN, Tan TZ, et al. Detection of aneuploidy from single fetal nucleated red blood cells using whole genome sequencing. *Prenat Diagn*. 2015, 35(7): 637-644.
346. Huang CE, Ma GC, Jou HJ, et al. Noninvasive prenatal diagnosis of fetal aneuploidy by circulating fetal nucleated red blood cells and extravillous trophoblasts using silicon-based nanostructured mi- crofluidics. *Mol Cytogenet*. 2017, 10: 44.
347. Huang FW, Hodis E, Xu MJ, et al. Highly Recurrent TERT Promoter Mutations in Human Melanoma. *Science* 2013, 339: 957–959.
348. Huang R, Barber TA, Schmidt MA, et al. A microfluidics approach for the isolation of nucleated red blood cells (NRBCs) from the peripheral blood of pregnant women. *Prenat Diagn*. 2008, 28(10): 892-899.
349. Huang X, Zhang W, Shao Z. Prognostic and diagnostic significance of circRNAs expression in lung cancer. *J. Cell. Physiol.* 2019, 234: 18459–18465.
350. Huang Z, Fong CY, Gauthaman K, Sukumar P, Choolani M, Bongso A. Novel approaches to

manipulating foetal cells in the maternal circulation for non-invasive prenatal diagnosis of the unborn child. *J Cell Biochem*. 2011, 112(6): 1475-1485.
351. Huber V, Vallacchi V, Fleming V, et al. Tumor- Derived MicroRNAs Induce Myeloid Suppressor Cells and Predict Immunotherapy Resistance in Melanoma. *J. Clin. Investig.* 2018, 128: 5505–5516.
352. Hudecova I, Sahota D, Heung MMS, Jin Y, Lee WS, Leung TY, Lo YMD, Chiu RW. Maternal Plasma Fetal DNA Fractions in Pregnancies with Low and High Risks for Fetal Chromosomal Aneuploidies. *PLoS ONE* 2014, 9: e88484.
353. Hut PH, Vlies PV, Verlind E, Buys CH, Scheffer H, Jonkman MF, Shimizu H. Exempting Homologous Pseudogene Sequences from Polymerase Chain Reaction Amplification Allows Genomic Keratin 14 Hotspot Mutation Analysis. *J. Investig. Dermatol.* 2000, 114: 616–619.
354. Ida M, Nakamura T, Utsunomiya J. Osteomatous changes and tooth abnormalities found in the jaws of patients with adenomatosis coli. *Oral Surg. Oral Med. Oral Pathol.* 1981, 52: 2–11.
355. Imhoff O, Marion V, Stoetzel C, et al. Bardet-Biedl syndrome: a study of the renal and cardiovascular phenotypes in a French cohort. *Clin J Am Soc Nephrol*. 2011, 6(1):22–29.
356. International Diabetes federation: the IDF consensus worldwide definition on the metabolic syndrome. Available from:https://idf.org/our-activities
357. Irvine A, Rugg E, Lane E, Hoare S, Peret C, Hughes A, Heagerty A. Molecular confirmation of the unique phenotype of epidermolysis bullosa simplex with mottled pigmentation. *Br. J. Dermatol.* 2001, 144: 40–45.
358. Irving M, Hanson H, Turnpenny P, Brewer C, Ogilvie CM, Davies A, Berg J. Deletion of the distal long arm of chromosome 10; is there a characteristic phenotype? A report of 15 de novo and familial cases. *Am. J. Med. Genet.* 2003, 123A: 153–163.
359. Issa M, Potter AM, Blank CE. Multiple congenital defects associated with trisomy for long arm of No. *J. Med. Genet.* 1976, 13: 326–329.
360. Ivanov A, Memczak S, Wyler E, Torti F, Porath HT, Orejuela MR, Piechotta M, Levanon EY, Landthaler M, Dieterich C, et al. Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals. *Cell Rep.* 2015, 10: 170–177.
361. James AWM, Le Chang BS, Shrestha S, et al. Cytogenetics of melanoma: A review. *J. Assoc. Genet. Technol.* 2014, 40: 209–218.
362. Jang SI, Lee EJ, Hart PS, Ramaswami M, Pallos D, Hart TC. Germ line gain of function with SOS1 mutation in hereditary gingival fibromatosis. J BiolChem 2007; 282:20245–20255.
363. Jansen MW, Brandenburg H, Wildschut HI, et al. The effect of chorionic villus sampling on the number of fetal cells isolated from maternal blood and on maternal serum alpha-fetoprotein levels. *Prenat Diagn*. 1997, 17(10): 953-959.
364. Järvinen HJ, Peltomäki P. The complex genotype–phenotype relationship in familial adenomatous polyposis. *Eur. J. Gastroenterol. Hepatol.* 2004: 16, 5–8.
365. Jasperson KW, Patel SG, Ahnen DJ. APC-Associated Polyposis Conditions. In *GeneReviews® [Internet]*; Adam MP, Ardinger HH, Pagon R, Wallace SE, Eds.; 18 December 1998 [Updated 2 February 2017]; University of Washington: Seattle, WA, USA, 1993–2021. Available online: https://www.ncbi.nlm.nih.gov/books/NBK1345/ (accessed on 6 June 2021).
366.Jeck N, Reinalter SC, Henne T, Marg W, Mallmann R, Pasel K, et al. Hypokalemic salt-losing tubulopathy with chronic renal failure and sensorineural deafness. *Pediatrics.* 2001, 108: E5.
367. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 2013, 19: 141–157.
368. Jee YH, Andrade AC, Baron J, Nilsson O. Genetics of Short Stature. *Endocrinol. Metab. Clin. N. Am.* 2017, 46: 259–281.
369. Jenkins EC, Curcuru-Giordano FM, Krishna SG, Cantarella J. De novo occurrence of 46,XX,t(4;13) (q31;q14) in a mentally retarded girl. *Ann. de Génétique* 1975, 18: 117–120.
370.Jentsch TJ, Pusch M. CLC chloride channels and transporters: structure, function, physiology, and disease. *Physiol Rev.* 2018, 98: 1493–590.
371. Jeong S. SR proteins: Binders, regulators, and connectors of RNA. *Mol. Cells* 2017, 40: 1–9.
372. Jeziorowska A, Ciesla W, Houck GE Jr, Yao XL, Harris MS, Truszczak B, Skorski M, Jakubowski L, Jenkins EC, Kaluzewski B. Cytogenetic and molecular identification of a de novo

direct duplication of the long arm of chromosome 4(q21.3→q31.3). *Am. J. Med. Genet.* 1993, 46: 83–87.
373. Ji Y, Eichler EE, Schwartz S, Nicholls RD. Structure of Chromosomal Duplicons and their Role in Mediating Human Genomic Disorders. *Genome Res.* 2000, 10: 597–610.
374. Jia Y, Liu M, Wang S. CircRNA hsa_circRNA_0001776 inhibits proliferation and promotes apoptosis in endometrial cancer via downregulating LRIG2 by sponging miR-182. *Cancer Cell Int.* 2020, 20: 1–13.
375. Jiang F, Ren J, Chen F, Zhou Y, Xie J, Dan S, Su Y, Xie J, Yin B, Su W, et al. Noninvasive Fetal Trisomy (NIFTY) test: An advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies. *BMC Med Genom.* 2012, 5: 57.
376. Jiang X, Zhu Y, Sun H, Gu F. A Novel Mutation p.L461P in KRT5 Causing Localized Epidermolysis Bullosa Simplex. *Ann. Dermatol.* 2021, 33: 11–17.
377. Jiang XM, Li ZL, Li JL, Xu Y, Leng KM, Cui YF, Sun DJ. A novel prognostic biomarker for cholangiocarcinoma: circRNA Cdr1as. *Eur. Rev. Med. Pharmacol. Sci.* 2018, 22: 365–371.
378. Jin H, Nachury M. The BBSome. *Curr. Biol.* 2009, 19, R472–R473.
379. Jobard F, Bouadjar B, Caux F, Hadj-Rabia S, Has C, Matsuda F, Weissenbach J, Lathrop M, Prud'Homme J-F, Fischer J. Identification of mutations in a new gene encoding a FERM family protein with a pleckstrin homology domain in Kindler syndrome. *Hum. Mol. Genet.* 2003, 12: 925–935.
380. Jonklaas J, Bianco AC, Bauer AJ, et al. Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association task force on thyroid hormone replacement. *Thyroid.* 2014, 24(12):1670–1751.
381. Jonklaas J. Sex and age differences in levothyroxine dosage requirement. *Endocr Pract.* 2010, 16(1):71–79.
382. Jonkman MF, Heeres K, Pas HH, van Luyn MJ, Elema JD, Corden LD, Smith FJ, McLean WI, Ramaekers FC, Burton M, et al. Effects of Keratin 14 Ablation on the Clinical and Cellular Phenotype in a Kindred with Recessive Epidermolysis Bullosa Simplex. *J. Investig. Dermatol.* 1996, 107: 764–769.
383. Jonkman MF, Pasmooij AM. Revertant Mosaicism—Patchwork in the Skin. *N. Engl. J. Med.* 2009, 360: 1680–1682.
384. Journe F, Boufker HI, Van Kempen L, et al. TYRP1 mRNA Expression in Melanoma Metastases Correlates with Clinical Outcome. *Br. J. Cancer* 2011, 105: 1726–1732.
385. Kadotani T, Watanabe Y, Kiyuna T, Takeuchi S. A case of partial trisomy 4q resulting from familial (4;9)(q23;p24) translocation. *Proc. Jpn. Acad. Ser. B* 1981, 57: 374–377.
386.Kahle KT, Rinehart J, Giebisch G, Gamba G, Hebert SC, Lifton RP. A novel protein kinase signaling pathway essential for blood pressure regulation in humans. *Trends Endocrinol Metab.* 2008, 19: 91–5.
387. Kamb A, Shattuck-Eidens D, Eeles R, et al. Analysis of the P16 Gene (CDKN2) as a Candidate for the Chromosome 9p Melanoma Susceptibility Locus. *Nat. Genet.* 1994, 8: 23–26.
388. Kanemaru H, Fukushima S, Yamashita J, et al. The circulating microRNA-221 level in patients with malignant melanoma as a new tumor marker. *J. Dermatol. Sci.* 2011, 61: 187–193.
389. Kant SG, Cervenkova I, Balek L, et al. A novel variant of FGFR3 causes proportionate short stature. Eur. J. Endocrinol. 2015, 172: 763–770.
390. Kapoor A, Goldberg MS, Cumberland LK, et al. The histone variant MACROH2A suppresses melanoma progression through regulation of CDK8. *Nature* 2010, 468: 1105–1109.
391. Karimian E, Chagin AS, Sävendahl L. Genetic regulation of the growth plate. *Front. Endocrinol.* 2012, 2: 1–10.
392.Kataoka M, Kido J, Shinohara Y, Nagata T. Druginduced gingival overgrowtha review. Biol Pharm Bull. 2005;28:1817–1821.
393. Kather J, Salgado M A C, Salgado U F L, Cortelli J R, Pallos D. Clinical and histomorphometric characteristics of three different families with hereditary gingival fibromatosis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2008; 105(3): 348352.
394. Katsanis SH, Katsanis N. Molecular genetic testing and the future of clinical genomics. Nature Reviews Genetics 2013; 14(6): 415.
395. Kaur P, Chaudhry C, Neelam H, Panigrahi I. Bardet-Biedl syndrome presenting with laryngeal

web and bifid epiglottis. *BMJ Case Rep*. 2021, 14(1): e236325.
396. Kehrer-Sawatzki H, Daumiller E, Müller-Navia J, Kendziorra H, Rossier E, Du Bois G, Barbi G. Interstitial deletion del(10)(q25.2q25.3∼26.11)—Case report and review of the literature. *Prenat. Diagn.* 2005, 25: 954–959.
397. Keith BP, Robertson DL, Hentge KE. Locus heterogeneity disease genes encode proteins with high interconnectivity in the human protein interaction network. *Front. Genet.* 2014, 5, 434.
398. Kelly-Mancuso G, Kopelan B, Azizkhan RG, Lucky AW. Junctional epidermolysis bullosa incidence and survival: 5-year experience of the Dystrophic Epidermolysis Bullosa Research Association of America (DebRA) nurse educator, 2007 to 2011. *Pediatr. Dermatol.* 2014, 31: 159–162.
399. Kerr EN, Bhan A, Heon E. Exploration of the cognitive, adaptive and behavioral functioning of patients affected with Bardet–Biedl syndrome. *Clin Genet*. 2016, 89(4):426–433.
400. Khan SA, Muhammad N, Khan MA, Kamal A, Rehman ZU, Khan S. Genetics of human Bardet–Biedl syndrome, an updates. *Clin Genet.* 2016, 90 (1):3–15.
401.Kim JY, Kim GA, Song JH, Lee SW, Han JY, Lee JS, et al. A case of living-related kidney transplantation in Bartter's syndrome. *Yonsei Med J*. 2000, 41: 662–5.
402. Kinyó Á, Kovács AL, Degrell P, Kálmán E, Nagy N, Kárpáti S, Gyulai R, Saeidian AH, Youssefian L, Vahidnezhad H, et al. Homozygous ITGA3 Missense Mutation in Adults in a Family with Syndromic Epidermolysis Bullosa (ILNEB) without Pulmonary Involvement. *J. Investig. Dermatol.* 2021, 141: 2752–2756.
403. Kiritsi D, Has C, Bruckner-Tuderman L. Laminin 332 in junctional epidermolysis bullosa. *Cell Adhes. Migr.* 2013, 7: 135–141.
404. Kiritsi D, Kern JS, Schumann H, Kohlhase J, Has C, Bruckner-Tuderman L. Molecular mechanisms of phenotypic variability in junctional epidermolysis bullosa. *J. Med. Genet.* 2011, 48: 450–457.
405. Kiritsi D, Tsakiris L, Schauer F. Plectin in Skin Fragility Disorders. *Cells* 2021, 10: 2738.
406.Kitanaka S, Sato U, Maruyama K, Igarashi T. A compound heterozygous mutation in the BSND gene detected in Bartter syndrome type IV. *Pediatr Nephrol.* 2006, 21:190–3.
407. Kleinfinger P, Lohmann L, Luscan A, Trost D, Bidat L, Debarge V, Castaigne V, Senat M-V, Brechard M.-P, Guilbaud L, et al. Strategy for Use of Genome-Wide Non-Invasive Prenatal Testing for Rare Autosomal Aneuploidies and Unbalanced Structural Chromosomal Anomalies. *J. Clin. Med.* 2020, 9: 2466.
408. Klink BU, Gatsogiannis C, Hofnagel O, Wittinghofer A, Raunser S. Author response: Structure of the human BBSome core complex. *eLife*, 2020, 9, e53910.
409. Koelper NC, Sammel MD, Dugoff L, Bender WR. Association of Fetal Fraction of Cell-Free DNA and Hypertensive Disorders of Pregnancy. *Am. J. Perinatol.* 2018, 36: 311–316.
410. Koemeter-Cox AI, Sherwood TW, Green JA, et al. Primary cilia enhance kisspeptin receptor signaling on gonadotropin-releasing hormone neurons. *Proc Natl Acad Sci U S A*. 2014, 111(28):10335–10340.
411. Koh J, Enders GH, David Dynlacht B, Harlow ED. Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition. *Nature* 1995, 375: 506–510.
412. Kolvraa S, Christensen B, Lykke-Hansen L, Philip J. The fetal erythroblast is not the optimal target for non-invasive prenatal diagnosis: preliminary results. *J Histochem Cytochem*. 2005, 53(3): 331-336.
413.Kömhoff M, Reinalter SC, Gröne HJ, Seyberth HW. Induction of microsomal prostaglandin E2 synthase in the macula densa in children with hypokalemic salt-losing tubulopathies. *Pediatr Res.* 2004, 55: 261.
414. Kondo T, Sekizawa A, Saito H, Jimbo M, Sugito Y, Okai T. Fate of fetal nucleated erythrocytes circulating in maternal blood: apoptosis is induced by maternal oxygen concentration. *Clin Chem*. 2002, 48(9): 1618-1620.
415.Konrad M, Nijenhuis T, Ariceta G, Bertholet-Thomas A, Calo LA, Capasso G, et al. Diagnosis and management of Bartter Syndrome: executive summary of the consensus and recommendations from the European rare kidney disease reference network working group for tubular disorders. *Kidney Int.* 2021, 99: 324–35.
416.Konrad M, Vollmer M, Lemmink HH, Van Den Heuvel LP, Jeck N, Vargas- Poussou R, et al.

Mutations in the chloride channel gene CLCNKB as a cause of classic Bartter syndrome. *J Am Soc Nephrol.* 2000, 11: 1449–59.
417. Konstantinov NK, Ulff-Møller CJ, Dimitrov S. Histone variants and melanoma: Facts and hypotheses. *Pigment. Cell Melanoma Res.* 2016, 29: 426–433.
418. Koscinski I, Mark M, Messaddeq N, et al. Reproduction function in male patients with Bardet Biedl syndrome. *J Clin Endocrinol Metab*. 2020;105 (12):e4417–29. Erratum in: *J Clin Endocrinol Metab*. 2021;106(4):e1936.
419. Koskenvuo L, Ristimäki A, Lepistö A. Comparison of sporadic and FAP-associated desmoid-type fibromatoses. *J. Surg. Oncol.* 2017, 116: 716–721.
420. Koss-Harnes D, Høyheim B, Anton-Lamprecht I, Gjesti A, Jørgensen RS, Jahnsen FL, Olaisen B, Wiche G, Gedde-Dahl T. A Site-Specific Plectin Mutation Causes Dominant Epidermolysis Bullosa Simplex Ogna: Two Identical De Novo Mutations. *J. Investig. Dermatol.* 2002, 118: 87–93.
421. Koumbaris G, Achilleos A, Nicolaou M, Loizides C, Tsangaras K, Kypri E, Mina P, Sismani C, Velissariou V, Christopoulou G, et al. Targeted capture enrichment followed by NGS: Development and validation of a single comprehensive NIPT for chromosomal aneuploidies, microdeletion syndromes and monogenic diseases. *Mol. Cytogenet.* 2019, 12: 48.
422.Kousi M, Söylemez O, Ozanturk A, et al. Evidence for secondary-variant genetic burden and non-random distribution across biological modules in a recessive ciliopathy. *Nat Genet* 2020, 52(11): 1145-1150.
423. Kowalczyk M, Srebniak M, Tomaszewska A. Chromosome abnormalities without phenotypic consequences. *J. Appl. Genet.*2007, 48: 157–166.
424. Kowalewski C, Bremer J, Gostynski A, Wertheim-Tysarowska K, Wozniak K, Bal J, Jonkman M, Pasmooij A. Amelioration of junctional epidermolysis bullosa due to exon skipping. *Br. J. Dermatol.* 2016, 174: 1375–1379.
425. Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. *Endocr Rev*. 2010, 31(5):702–755.
426. Krauthammer M, Kong Y, Bacchiocchi A, et al. Exome Sequencing Identifies Recurrent Mutations in *NF1* And Rasopathy Genes in Sun-Exposed Melanomas. *Nat. Genet.* 2015, 47: 996–1002.
427. Kronenberg HM. Developmental regulation of the growth plate. *Nature* 2003, 423: 332–336.
428. Kumagai Y, Umegaki-Arao N, Sasaki T, Nakamura Y, Takahashi H, Ashida A, Tsunemi Y, Kawashima M, Shimizu A, Ishiko A, et al. Distinct phenotype of epidermolysis bullosa simplex with infantile migratory circinate erythema due to frameshift mutations in the V2 domain of KRT5. *J. Eur. Acad. Dermatol. Venereol.* 2017, 31: e241–e243.
429. Kwon KH, Jeon YJ, Hwang HS, et al. A high yield of fetal nucleated red blood cells isolated using optimal osmolality and a double-density gradient system. *Prenat Diagn*. 2007, 27(13): 1245-1250.
430. Lafranchi S. Thyroiditis and acquired hypothyroidism. *Pediatr Ann*. 1992, 21(1):29,32–9.
431.Laghmani K, Beck BB, Yang SS, Seaayfan E, Wenzel A, Reusch B, et al. Polyhydramnios, transient antenatal Bartter's syndrome, and MAGED2 mutations. *N Engl J Med.* 2016, 374: 1853–63.
432. Lahtz C, Stranzenbach R, Fiedler E, et al. Methylation of PTEN as a Prognostic Factor in Malignant Melanoma of the Skin. *J. Investig. Dermatol.* 2010, 130: 620–622.
433. Lai-Cheong JE, Tanaka A, Hawche G, Emanuel P, Maari C, Taskesen M, Akdeniz S, Liu L, McGrath, JA. Kindler syndrome: A focal adhesion genodermatosis. *Br. J. Dermatol.* 2009, 160: 233–242.
434. LaLonde E, Rentas S, Lin F, Dulik MC, Skraban CM, Spinner NB. Genomic Diagnosis for Pediatric Disorders: Revolution and Evolution. *Front. Pediatr.* 2020, 8: 373.
435. Landi MT, Bishop DT, MacGregor S, et al. Genome-wide association meta-analyses combining multiple risk phenotypes provide insights into the genetic architecture of cutaneous melanoma susceptibility. *Nat. Genet.* 2020, 52; 494–504.
436. Lane EB, Rugg EL, Navsaria H, Leigh IM, Heagerty AHM, Ishida-Yamamoto A, Eady RAJ. A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. *Nature* 1992, 356: 244–246.

437. Larjava H, Plow EF, Wu C. Kindlins: Essential regulators of integrin signalling and cell–matrix adhesion. *EMBO Rep.* 2008, 9:1203–1208.
438. Laurent GS, Wahlestedt C, Kapranov P. The Landscape of long noncoding RNA classification. *Trends Genet.* 2015, 31: 239–251.
439. Lazăr AD, Dinescu S, Costache M. The non-coding landscape of cutaneous malignant melanoma: A possible route to efficient targeted therapy. *Cancers* 2020, 12: 3378.
440. Le TN, Nestler JE, Strauss JF 3rd, et al. Sex hormone-binding globulin and type 2 diabetes mellitus. *Trends Endocrinol Metab*. 2012, 23(1):32–40.
441.Lee SE, Han KH, Jung YH, Lee HK, Kang HG, Moon KC, et al. Renal transplantation in a patient with Bartter syndrome and glomerulosclerosis. *Korean J Pediatr.* 2011, 54: 36–9.
442. Lee J, Chung YD. Ciliary subcompartments: How are they established and what are their functions? *BMB Rep.* 2015, 48, 380–387.
443. Lee SE, Choi JY, Kim S-E, Kim S-C. A novel deletion mutation in the 2B domain of KRT5 in epidermolysis bullosa simplex with childhood-onset migratory circinate erythema. *Eur. J. Dermatol.* 2018, 28: 123–125.
444.Legrand A, Treard C, Roncelin I, Dreux S, Bertholet-Thomas A, Broux F, et al. Prevalence of novel MAGED2 mutations in antenatal Bartter syndrome. *Clin J Am Soc Nephrol.* 2018, 13: 242–50.
445. Leitch CC, Zaghloul NA, Davis E, Stoetzel C, Diaz-Font A, Rix S, Al-Fadhel M, Lewis RA, Eyaid W, Banin E, et al. Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. *Nat. Genet.* 2008, 40, 443–448.
446. Leonardi GC, Candido S, Falzone L, et al. Cutaneous Melanoma and the Immunotherapy Revolution (Review). *Int. J. Oncol.* 2020, 57: 609–618.
447. Leroith D, Farkash Y, Bar-Ziev J, et al. Hypothalamic-pituitary function in the Bardet-Biedl syndrome. *Isr J Med Sci*. 1980, 16(7):514–518.
448. Lestringant G, Allegra M, Gagnoux-Palacios L, Gache Y, Roques S, Ortonne J-P, Meneguzzi G. Rapid Decay of α6 Integrin Caused by a Mis-Sense Mutation in the Propeller Domain Results in Severe Junctional Epidermolysis Bullosa with Pyloric Atresia. *J. Investig. Dermatol.* 2003, 121: 1336–1343.
449. Leucci E. Melanoma addiction to the long non-coding RNA SAMMSON. *Nature* 2016, 531, 518–522.
450. Lewandowski RC, Kukolich MK, Sears JW, Mankinen CB. Partial deletion 10q. *Qual. Life Res.* 1978, 42: 339–343.
451. Li A, Wang WC, McAlister V, Zhou Q, Zheng X. Circular RNA in colorectal cancer. *J. Cell. Mol. Med.* 2021, 25: 3667–3679.
452. Li S, Sun X, Miao S, Lu T, Wang Y, Liu J, Jiao W. hsa_circ_0000729, a potential prognostic biomarker in lung adenocarcinoma. *Thorac. Cancer* 2018, 9: 924–930.
453. Li T, Shao Y, Fu L, Xie YI, Zhu L, Sun W, Yu R, Xiao B, Guo J. Plasma circular RNA profiling of patients with gastric cancer and their droplet digital RT-PCR detection. *J. Mol. Med.* 2018, 96: 85–96.
454. Li W, Zhong C, Jiao J, Li P, Cui B, Ji C, Ma D. Characterization of hsa_circ_0004277 as a new biomarker for acute myeloid leukemia via circular RNA profile and bioinformatics analysis. *Int. J. Mol. Sci.* 2017, 18: 597.
455. Li X, Ma F, Wu L, Zhang X, Tian J, Li J, Cao J, Ma Y, Zhang L, Wang L. Identification of Hsa_circ_0104824 as a potential biomarkers for breast cancer. *Technol. Cancer Res. Treat.* 2020, 19: 1533033820960745.
456. Li Z, Cheng Y, Wu F, Wu L, Cao H, Wang Q, Tang W. The emerging landscape of circular RNAs in immunity: Breakthroughs and challenges. *Biomark. Res.* 2020, 8: 25.
457. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, et al. Exon-intron circular RNAs regulate transcription in the nucleus. *Nat. Struct. Mol. Biol.* 2015, 22: 256–264.
458. Li, Z, Zhou YE, Yang G, He S, Qiu X, Zhang L, Deng Q, Zheng F. Using circular RNA SMARCA5 as a potential novel biomarker for hepatocellular carcinoma. *Clin. Chim. Acta* 2019, 492: 37–44.
459. Lim TH, Tan A, Goh VH. Enrichment of fetal trophoblasts and nucleated erythrocytes from maternal blood by an immunomagnetic colloid system. *Hum Genet*. 1999, 104(5): 399-404.
460. Lin J, Cai D, Li W, Yu T, Mao H, Jiang S, Xiao B. Plasma circular RNA panel acts as a

novel diagnostic biomarker for colorectal cancer. *Clin. Biochem.* 2019, 74: 60–68.
461. Lin S, Rawlins L, Turner C, Doyle E, Sleep T. Novel ocular findings with 5p deletion and partial trisomy of distal 4q. *Can. J. Ophthalmol.* 2017, 53: e89–e90.
462. Lin S, Zhou Y, Fang Q, Wu J, Zhang Z, Ji Y, Luo Y. Chromosome 10q26 deletion syndrome: Two new cases and a review of the literature. *Mol. Med. Rep.* 2016, 14: 5134–5140.
463. Lindstrand A, Frangakis S, Carvalho CM, Richardson EB, McFadden KA, Willer JR, Pehlivan D, Liu P, Pediaditakis IL, Sabo A, et al. Copy-Number variation contributes to the mutational load of Bardet-Biedl syndrome. *Am. J. Hum. Genet.* 2016, 99, 318–336.
464. Liou JD, Pao CC, Hor JJ, Kao SM. Fetal cells in the maternal circulation during first trimester in pregnancies. *Hum Genet*. 1993, 92(3): 309-311.
465. Liu H, Gao Y, Hu Z, Lin L, Yin X, Wang J, Chen D, Chen F, Jiang H, Ren J, et al. Performance Evaluation of NIPT in Detection of Chromosomal Copy Number Variants Using Low-Coverage Whole-Genome Sequencing of Plasma DNA. *PLoS ONE* 2016, 11: e0159233.
466. Liu J, Liu T, Wang X, He A. Circles reshaping the RNA world: From waste to treasure. *Mol. Cancer* 2017, 16: 58.
467. Liu X, Wang X, Li J, Hu S, Deng Y, Yin H, Bao X, Zhang QC, Wang G, Wang B, et al. Identification of mecciRNAs and their roles in the mitochondrial entry of proteins. *Sci. China Life Sci.* 2020, 63: 1429–1449.
468. Liu X, Yang Y, Shan G. Identification and detection of mecciRNAs. *Methods* 2021, 196: 147-152.
469. Liu XX, Yang YE, Liu X, Zhang MY, Li R, Yin YH, Qu YQ. A two-circular RNA signature as a noninvasive diagnostic biomarker for lung adenocarcinoma. *J. Transl. Med.* 2019, 17: 1–13.
470. Liu Y, Yue Z, Wang H, Li M, Wu X, Lin H, Han W, Lan S, Sun L. A novel ITGA3 homozygous splice mutation in an ILNEB syndrome child with slow progression. *Clin. Chim. Acta* 2021, 523: 430–436.
471. Lo YMD, Chan KCA, Sun H, Chen EZ, Jiang P, Lun FMF, Zheng YW, Leung TY, Lau TK, Cantor CR, et al. Maternal Plasma DNA Sequencing Reveals the Genome-Wide Genetic and Mutational Profile of the Fetus. *Sci. Transl. Med.* 2010, 2: 61ra91.
472. Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997, 350: 485–487.
473. Long GV, Menzies AM, Nagrial AM, et al. Prognostic and Clinicopathologic Assimilations of Oncogenes BRAF in Metastatic Melanoma. *J. Clin. Oncol.* 2011, 29: 1239–1246.
474. Lopez AJ, Wood MA. Role of nucleosome remodeling in neurodevelopmental and intellectual disability disorders. Front Behav Neurosci. 2015;9: 100
475. Lorentzen E, Kikkawa M. Decision letter: Structure and activation mechanism of the BBSome membrane protein trafficking complex. *eLife*, 2019, 9, e53322.
476. Lu AT, Xue L, Salfati EL, et al. GWAS of epigenetic aging rates in blood reveals a critical role for TERT. *Nat. Commun.* 2018, 9: 387.
477. Lu J, Li Y. Circ_0079593 facilitates proliferation, metastasis, glucose metabolism and inhibits apoptosis in melanoma by regulating the miR-516b/GRM3 axis. *Mol. Cell. Biochem.* 2020, 475: 227–237.
478. Lü L, Sun J, Shi P, Kong W, Xu K, He B, Zhang S, Wang J. Identification of circular RNAs as a promising new class of diagnostic biomarkers for human breast cancer. *Oncotarget* 2017, 8: 44096.
479. Lu X, Wang C, Sun Y, Tang J, Tong K, Zhu J. Noninvasive prenatal testing for assessing foetal sex chromosome aneu-ploidy: A retrospective study of 45,773 cases. *Mol. Cytogenet.* 2021, 14: 1.
480. Lukas J, Bartkova J, Rohde M, et al. Cyclin D1 is dispensable for G1 control in retinoblastoma gene-deficient cells independently of cdk4 activity. *Mol. Cell. Biol.* 1995, 15: 2600–2611.
481. Lundin C, Zech L, Sjörs K, Wadelius C, Annerén G. Trisomy 4q syndrome: Presentation of a new case and review of the literature. *Ann. Génét.* 2002, 45: 53–57.
482. M'Hamdi O, Redin C, Stoetzel C, Ouertani I, Chaabouni M, Maazoul F, M'Rad R, Mandel JL, Dollfus H, Muller J. Clinical and genetic characterization of Bardet-Biedl syndrome in Tunisia: Defining a strategy for molecular diagnosis. *Clin. Genet.* 2013, 85, 172–177.
483. Ma GC, Lin WH, Huang CE, et al. A silicon-based coral-like nanostructured microfluidics to isolate rare cells in human circulation: validation by SK-BR-3 cancer cell line and its utility in

circulating fetal nucleated red blood cells. *Micromachines*. 2019, 10(2): 132.
484. Ma J, Du WW, Zeng K, Wu N, Fang L, Lyu J, Yee AJ, Yang BB. An antisense circular RNA circSCRIB enhances cancer progression by suppressing parental gene splicing and translation. *Mol. Ther.* 2021, 29: 2754–2768.
485. Ma M. Cilia and polycystic kidney disease. *Semin. Cell Dev. Biol.* 2021, *110*, 139–148.
486. Machol K, Rousseau J, Ehresmann S, et al. Expanding the spectrum of BAF-related disorders: de novo variants in SMARCC2 cause a syndrome with intellectual disability and developmental delay. Am J Hum Genet. 2019; 104(1):164–78.
487. Machuca STV. Duplicación parcial 4q en un neonato originado por reordenamiento der (20), t(4;20) (q21;q13.1)mat. *Rev. Peru. Investig. Matern. Perinat.* 2016, 5: 75–81.
488. Maertens O, Johnson B, Hollstein P, et al. Elucidating Distinct Roles for *NF1* In Melanomagenesis. *Cancer Discov.* 2013, 3: 338–349.
489.Mallmann MR, et al. Prenatal Diagnosis of Hydro(metro)colpos: A Series of 20 Cases. *Fetal Diagnosis and Therapy* 2019, 45(1): 62-68.
490. Maltby EL, Barnes IC, Bennett CP. Duplication involving band 4q32 with minimal clinical effect. *Am. J. Med. Genet.* 1999, 83: 431.
491. Mann GJ, Pupo GM, Campain AE, et al. BRAF Mutation, NRAS Mutation, and the Absence of an Immune-Related Expressed Gene Profile Predict Poor Outcome in Patients with Stage III Melanoma. *J. Investig. Dermatol.* 2013, 133: 509–517.
492. Mannavola F, D'oronzo S, Cives M, et al. Extracellular vesicles and epigenetic modifications are hallmarks of melanoma progression. *Int. J. Mol. Sci.* 2019, 21: 52.
493. Mao R, Krautscheid P, Graham RP, Ganguly A, Shankar S, Ferber M, Hegde M. Genetic testing for inherited colorectal cancer and polyposis, 2021 revision: A technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* 2021, 1–11.
494. Marchini A, Ogata T, Rappold GA. A Track Record on SHOX: From Basic Research to Complex Models and Therapy. *Endocr Rev*. 2016, 37: 417–448.
495. Mariath LM, Santin JT, Schuler-Faccini L, Kiszewski AE. Inherited epidermolysis bullosa: Update on the clinical and genetic aspects. *An. Bras. Dermatol.* 2020, 95: 551–569.
496. Mariman ECM, Vink RG, Roumans NJT, Bouwman FG, Stumpel CTRM, Aller EEJG, van Baak M, Wang P. The cilium: A cellular antenna with an influence on obesity risk. *Br. J. Nutr.* 2016, 116, 576–592.
497. Marion V, Schlicht D, Mockel A, et al. Bardet-Biedl syndrome highlights the major role of the primary cilium in efficient water reabsorption. *Kidney Int*. 2011, 79(9):1013–1025.
498. Marion V, Stutzmann F, Gerard M, et al. Exome sequencing identifies ´ mutations in LZTFL1, a BBSome and smoothened trafficking regulator, in a family with Bardet–Biedl syndrome with situs inversus and insertional polydactyly. *J Med Genet*. 2012, 49(5):317–321.
499. Marstrand-Joergensen MR, Jensen RB, Aksglaede L, et al. Prevalence of SHOX haploinsufficiency among short statured children. *Pediatr. Res.* 2017, 81: 335–334.
500. Martin CL, Waggoner DJ, Wong A, Uhrig SA; Roseberry J, Hedrick JF, Pack S, Russell KL, Zackai EH, Dobyns W, et al. "Molecular rulers" for calibrating phenotypic effects of telomere imbalance. *J. Med. Genet.* 2002, 39: 734–740.
501. Martin GA, Viskochil D, Bollag G, et al. The GAP-Related Domain of the Neurofibromatosis Type 1 Gene Product Interacts with ras p21. *Cell* 1990, 63: 843–849.
502. Martins L, Seoane-Collazo P, Contreras C, et al. A functional link between AMPK and orexin mediates the effect of BMP8B on energy balance. *Cell Rep*. 2016,16(8):2231–2242.
503. Mary L, Chennen K, Stoetzel C, et al. Bardet-Biedl syndrome: antenatal presentation of forty-five fetuses with biallelic pathogenic variants in known Bardet-Biedl syndrome genes. *Clin Genet*. 2019, 95(3):384–397.
504. Mason K, Page L, Balikcioglu PG. Screening for hormonal, monogenic, and syndromic disorders in obese infants and children. *Pediatr Ann*. 2014, 43(9):e218–e224.
505. Matoso E, Melo JB, Ferreira SI, Jardim A, Castelo TM, Weise A, Carreira IM. Insertional translocation leading to a 4q13 duplication including theEPHA5gene in two siblings with attention-deficit hyperactivity disorder. *Am. J. Med. Genet. Part A* 2013, 161: 1923–1928.
506. Matsumura T, Goto K, Yamanaka G, Lee JH, Zhang C, Hayashi YK, Arahata K. Chromosome 4q;10q translocations; Comparison with different ethnic populations and FSHD patients. *BMC*

Neurol. 2002, 2: 7.
507. Mattei M-G, Mattei J-F, Bernard R, Giraud F. Partial trisomy 4 resulting from a complex maternal rearrangement of chromosomes 2, 4, and 18 with interstitial translocation. *Qual. Life Res.* 1979, 51: 55–61.
508. McCague EA, Lamichhane R, Holt N, Schrier Vergano SA. Growth charts for individuals with Coffin-Siris syndrome. Am J Med Genet Part A. 2020; 182:2253–62.
509. McGrath J, Schofield O, Eady R. Epidermolysis bullosa pruriginosa: Dystrophic epidermolysis bullosa with distinctive clinicopathological features. *Br. J. Dermatol.* 1994, 130: 617–625.
510. McGrath JA, Ashton GH, Mellerio JE, McMillan JR, Eady RA, Salas-Alanis JC, Swensson O. Moderation of Phenotypic Severity in Dystrophic and Junctional Forms of Epidermolysis Bullosa Through In-Frame Skipping of Exons Containing Non- Sense or Frameshift Mutations. *J. Investig. Dermatol.* 1999, 113: 314–321.
511. McGrath JA, Stone KL, Begum R, et al. Germline Mutation in EXPH5 Implicates the Rab27B Effector Protein Slac2-b in Inherited Skin Fragility. *Am. J. Hum. Genet.* 2012, 91; 1115–1121.
512. McGrath JA. Recently Identified Forms of Epidermolysis Bullosa. *Ann. Dermatol.* 2015, 27: 658–666.
513. McKanna T, Ryan A, Krinshpun S, et al. Fetal fraction-based risk algorithm for non-invasive prenatal testing: Screening for trisomies 13 and 18 and triploidy in women with low cell-free fetal DNA. *Ultrasound Obstet. Gynecol.* 2019, 53: 73–79.
514. McLean WI, Irvine AD, Hamill KJ, Whittock NV, Coleman-Campbell CM, Mellerio JE, Ashton GS, Dopping-Hepenstal PJ, Eady RA, Jamil T, et al. An unusual N-terminal deletion of the laminin 3a isoform leads to the chronic granulation tissue disorder laryngo-onycho-cutaneous syndrome. *Hum. Mol. Genet.* 2003, 12: 2395–2409.
515. Mehta L, Duckett DP, Youn I.D. Behaviour disorder in monosomy 10qter. *J. Med. Genet.* 1987, 24: 185–186.
516. Mehta SL, Dempsey RJ, Vemuganti R. Role of circular RNAs in brain development and CNS diseases. *Prog. Neurobiol.* 2020, 186: 101746.
517. Mellerio JE, Ashton GH, Mohammedi R, Eady RA, McGrath JA, Lyon CC, Kirby B, Harman KE, Salas-Alanis JC, Atherton DJ, et al. Allelic Heterogeneity of Dominant and Recessive COL7A1 Mutations Underlying Epidermolysis Bullosa Pruriginosa. *J. Investig. Dermatol.* 1999, 112: 984–987.
518. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013, 495: 333–338.
519. Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, Wu M. CircRNA: Functions and properties of a novel potential biomarker for cancer. *Mol. Cancer* 2017, 16: 94.
520. Meng X, Li X, Zhang P, Wang J, Zhou Y, Chen M. Circular RNA: An emerging key player in RNA world. *Brief. Bioinform.* 2017, 18: 547–557.
521. Menzies SW, Moloney FJ, Byth K et al. Dermoscopic Evaluation of Nodular Melanoma. *JAMA Dermatol.* 2013, 149: 699–709.
522. Meves A, Stremmel C, Gottschalk K, Fässler R. The Kindlin protein family: New members to the club of focal adhesion proteins. *Trends Cell Biol.* 2009, 19: 504–513.
523. Meyer JR, Krentz AD, Berg RL, et al. Kidney failure in Bardet–Biedl syndrome. *Clin Genet.* 2022, 101(4):429–441.
524. Meyer-Mueller C, Osborn MJ, Tolar J, Boull C, Ebens CL. Revertant Mosaicism in Epidermolysis Bullosa. *Biomedicines* 2022, 10: 114.
525. Michielin O, Atkins MB, Koon HB, et al. Evolving Impact of Long-Term Survival Results on Metastatic Melanoma Treatment. *J. Immunother. Cancer* 2020, 8: e000948.
526. Mieusset R, Fouda PJ, Vaysse P, et al. Increase in testicular temperature in case of cryptorchidism in boys. *Fertil Steril*. 1993, 59(6):1319–1321.
527. Mikelsaar RV, Lurie IW, EIlus T. "Pure" partial trisomy 4q25-qter owing to a de novo 4;22 translocation. *J. Med. Genet.* 1996, 33: 344–345.
528. Milazzo G, Mercatelli D, Di Muzio G, et al. Histone deacetylases (HDACs): Evolution, specificity, role in transcriptional complexes, and pharmacological actionability. *Genes* 2020, 11: 556.

529. Miller ND, Nance MA, Wohler ES, Hoover-Fong JE, Lisi E, Thomas GH, Pevsner J. Molecular (SNP) analyses of overlapping hemizygous deletions of 10q25.3 to 10qter in four patients: Evidence for HMX2 and HMX3 as candidate genes in hearing and vestibular function. *Am. J. Med. Genet. Part A* 2009, 149A: 669–680.
530. Minear MA, Lewis C, Pradhan S, Chandrasekharan S. Global perspectives on clinical adoption of NIPT. *Prenat. Diagn.* 2015, 35: 959–967.
531. Mitchison HM, Valente EM. Motile and non-motile cilia in human pathology: From function to phenotypes. *J. Pathol.* 2017, 241, 294–309.
532. Miyake N, Tsurusaki Y, Matsumoto N. Numerous BAF complex genes are mutated in Coffin-Siris syndrome. Am J Med Genet C Semin Med Genet. 2014; 166C:257–61.
533. Mockel A, Perdomo Y, Stutzmann F, et al. Retinal dystrophy in Bardet–Biedl syndrome and related syndromic ciliopathies. *Prog Retin Eye Res*. 2011, 30(4):258–274.
534. Mohamed AM, El-Bassyouni, H.T.; El-Gerzawy, A.M.; Hammad, S.A.; Helmy, N.A.; Kamel, A.K.; Ismail SI, Issa MY, Eid O, Zaki MS. Cytogenomic characterization of 1q43q44 deletion associated with 4q32.1q35.2 duplication and phenotype correlation. *Mol. Cytogenet.* 2018, 11: 57.
535. Mohr AM, Mott JL. Overview of microRNA biology. *Semin. Liver Dis.* 2015, 35: 3–11.
536. Monteleon CL, Lee IY, Ridky TW. Exophilin-5 Supports Lysosome-Mediated Trafficking Required for Epidermal Differentia- tion. *J. Investig. Dermatol.* 2019, 139: 2219–2222.e6.
537. Moog U, de Die-Smulders CE, Scheffer H, van der Vlies P, Henquet CJ, Jonkman MF. Epidermolysis bullosa sim-plex with mottled pigmentation: Clinical aspects and confirmation of the P24L mutation in the KRT5 gene in further pa-tients. *Am. J. Med. Genet.* 1999, 86: 376–379.
538. Moon KR, Choi YD, Kim JM, et al. Genetic Alterations in Primary Acral Melanoma and Acral Melanocytic Nevus in Korea: Common Mutated Genes Show Distinct Cytomorphological Features. *J. Investig. Dermatol.* 2018, 138: 933–945.
539. Morin PJ, Weeraratna AT. The APC tumor suppressor pathway. In *Tumor Suppressor Genes*; Humana Press: Totowa, NJ, USA, 2003; pp. 21–40.
540. Mork ME, You YN, Ying J, Bannon SA, Lynch PM, Rodriguez-Bigas MA, Vilar E. High prevalence of hereditary cancer syndromes in adolescents and young adults with colorectal cancer. *J. Clin. Oncol.* 2015, 33: 3544.
541. Mozaffarian G, Nakhjavani MK, Farrahi A. The Laurence-Moon-Bardet-Biedl syndrome: unresponsiveness to the action of testosterone, a possible mechanism. *Fertil Steril*. 1979, 31(4):417–422.
542. Mozos I, Gug C, Mozos C, Stoian D, Pricop M, Jianu, D. Associations between Intrinsic Heart Rate, P Wave and QT Interval Durations and Pulse Wave Analysis in Patients with Hypertension and High Normal Blood Pressure. *Int. J. Environ. Res. Public Health* 2020, 17: 4350.
543. Mozos I, Jianu D, Gug C, Stoian D. Links between High-Sensitivity C-Reactive Protein and Pulse Wave Analysis in Middle- Aged Patients with Hypertension and High Normal Blood Pressure. *Dis. Markers* 2019, 2019: 2568069.
544. Mujahid S, Huda MS, Beales P, et al. Adjustable gastric banding and sleeve gastrectomy in Bardet-Biedl syndrome. *Obes Surg*. 2014, 24 (10):1746–1748.
545. Mujahid S, Hunt KF, Cheah YS, et al. The endocrine and metabolic characteristics of a large Bardet-Biedl syndrome clinic population. *J Clin Endocrinol Metab*. 2018, 103(5):1834–1841.
546. Müller FB, Küster W, Wodecki K, Almeida H, Bruckner-Tuderman L, Krieg T, Korge BP, Arin MJ. Novel and recurrent mutations in keratin *KRT5* and *KRT14* genes in epidermolysis bullosa simplex: Implications for disease phenotype and keratin filament assembly. *Hum. Mutat.* 2006, 27: 719–720.
547. Muller J, Stoetzel C, Vincent MC, Leitch CC, Laurier V, Danse JM, Hellé S, Marion V, Bennouna-Greene V, Vicaire S, et al. Identification of 28 novel mutations in the Bardet–Biedl syndrome genes: The burden of private mutations in an extensively heterogeneous disease. *Qual. Life Res.* 2010, 127, 583–593.
548. Muraki K, Katano R, Hiraki Y, Ueda K, Fujita H. A case of an interstitial tandem direct duplication of long arm of chromosome 4: 46, XY, dup (4) (q25q31.3) de novo. *Hiroshima J. Med. Sci.* 1997, 46: 105–108.
549. Murata T, Masunaga T, Shimizu H, Takizawa Y, Ishiko A, Hatta N, Nishikawa T. Glycine substitution mutations by different amino acids in the same codon of COL7A1 lead to

heterogeneous clinical phenotypes of dominant dystrophic epidermolysis bullosa. *Arch. Dermatol. Res.* 2000, 292: 477–481.
550. Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peränen J, Merdes A, Slusarski D, Scheller RH, Bazan JF, Sheffield V, et al. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* 2007, 129, 1201–1213.
551. Nager AR, Goldstein JS, Herranz-Pérez V, Portran D, Ye F, Garcia-Verdugo JM, Nachury MV. An actin network dispatches ciliary gpcrs into extracellular vesicles to modulate signaling. *Cell* 2017, 168, 252–263.
552. Nagy N, Tanaka A, Techanukul T, McGrath JA. Common IL-31 Gene Haplotype Associated with Non-atopic Eczema is Not Implicated in Epidermolysis Bullosa Pruriginosa. *Acta Dermatol. Venereol.* 2010, 90: 631–632.
553. Nakamura H, Sawamura D, Goto M, Sato-Matsumura KC, LaDuca J, Lee JY-Y, Masunaga T, Shimizu H. The G2028R glycine substitution mutation in COL7A1 leads to marked inter-familiar clinical heterogeneity in dominant dystrophic epidermolysis bullosa. *J. Dermatol. Sci.* 2004, 34: 195–200.
554. Narrandes S, Xu W. Gene expression detection assay for cancer clinical use. *J. Cancer* 2018, 9: 2249.
555. National Center for Biotechnology Information. ClinVar; [VCV000858116.2]. Available online: https://www.ncbi.nlm.nih.gov/ clinvar/variation/VCV000858116.2 (accessed on 8 August 2021).
556. Natsug K. Plectin-related skin diseases. *J. Dermatol. Sci.* 2015, 77: 139–145.
557. Natsuga K, Nishie W, Shinkuma S, Arita K, Nakamura H, Ohyama M, Osaka H, Kambara T, Hirako Y, Shimizu H. Plectin deficiency leads to both muscular dystrophy and pyloric atresia in epidermolysis bullosa simplex. *Hum. Mutat.* 2010, 31: E1687–E1698.
558. Navarro EG, Romero MM, Expósito IL, Velasco CM, Llamas JG, Ramón FH, Jimenez RD. De novo interstitial tandem dupli-cation of chromosome 4(q21–q28). *Am. J. Med. Genet.* 1996, 62: 297–299.
559. Navarro P, Oldfield A, Legoupi J, Festuccia N, Dubois A, Attia M, Schoorlemmer J, Rougeulle C, Chambers I, Avner P. Molecular coupling of Tsix regulation and pluripotency. *Nat. Cell Biol.* 2010, 468: 457–460.
560. Naylor RW, Watson E, Williamson S, Preston R, Davenport JB, Thornton N, Lowe M, Williams M, Lennon R. Basement membrane defects in CD151-associated glomerular disease. *Pediatr. Nephrol.* 2022, 1–11.
561. Neagu M, Constantin C, Cretoiu SM, Zurac S. miRNAs in the Diagnosis and Prognosis of Skin Cancer. *Front. Cell Dev. Biol.* 2020, 8: 71.
562. Nemescu D, Constantinescu D, Martiniuc V, Onofriescu M, Carasevici E. Detection of nucleated red blood cells in maternal circulation by magnetic sorting and in situ hybridization. *Gineco Ro*. 2011, 7(1): 15-18.
563. Nemescu D, Martiniuc V, Gorduza V, Onofriescu M. Fetal aneuploidy diagnosis through rapid fluorescence in situ hybridization (FISH) on uncultured amniocytes. *Rev Romana Med Lab*. 2011,19(2): 161-167.
564. Nicolaides K. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat. Diagn.* 2011, *31*: 7–15.
565. Niederlova V, Modrak M, Tsyklauri O, et al. Meta-analysis of genotype-phenotype associations in Bardet-Biedl syndrome uncovers differences among causative genes. *Hum. Mutat*. 2019, 40(11):2068–2087.
566. Nieuwenhuis MH, Vasen HFA. Correlations between mutation site in APC and phenotype of familial adenomatous polyposis (FAP): A review of the literature. *Crit. Rev. Oncol. Hematol.* 2007, 61: 153–161.
567. Nikolaev SI, Rimoldi D, Iseli C, et al. Exome Sequencing Identifies Recurrent Somatic MAP2K1 and MAP2K2 Mutations in Melanoma. *Nat. Genet.* 2011, 44: 133–139.
568. Nikolaou V, Stratigos A. Emerging Trends in the Epidemiology of Melanoma. *Br. J. Dermatol.* 2014, 170: 11–19.
569. Nilsson O. Aggrecanopathies highlight the need for genetic evaluation of ISS children. *Eur. J. Endocrinol.* 2020, 183: C9–C10.

570. Nusliha A, Dalpatadu U, Amarasinghe B, Chandrasinghe PC, Deen KI. Congenital hypertrophy of retinal pigment epithelium (CHRPE) in patients with familial adenomatous polyposis (FAP); a polyposis registry experience. *BMC Res. Notes* 2014, 7: 1–4.
571. Nyström A, Thriene K, Mittapalli VR, Kern JS, Kiritsi D, Dengjel J, Bruckner-Tuderman L. Losartan ameliorates dystrophic epidermolysis bullosa and uncovers new disease mechanisms. *EMBO Mol. Med.* 2015, 7: 1211–1228.
572. O'Dea D, Parfrey PS, Harnett JD, Hefferton D, Cramer BC, Green J. The importance of renal impairment in the natural history of Bardet-Biedl syndrome. *Am J Kidney Dis*. 1996, 27(6):776–783.
573. Ogata T. SHOX haploinsufficiency and its modifying factors. *Pediatr. Endocrinol. Metab.* 2002, 5: 1289–1294.
574. Oku T, Takayama T, Sato Y, Sato Y, Takada K, Hayashi T, Takahashi M, Kuroda M, Kato J, Niitsu Y. A case of Gardner syndrome with a mutation at codon 1556 of APC: A suggested case of genotype–phenotype correlation in dental abnormality. *Eur. J. Gastroenterol. Hepatol.* 2004, 16: 101–105.
575. Oliveira CS, Alves C. The role of the SHOX gene in the pathophysiology of Turner syndrome. *Endocrinol. Nutr.* 2011, 58: 433– 442.
576. Olson AJ, Krentz AD, Finta KM, Okorie UC, Haws RM. Thoraco-abdominal abnormalities in Bardet-Biedl syndrome: situs inversus and heterotaxy. *J Pediatr*. 2019, 204:31–37.
577. *Online Mendelian Inheritance in Man, OMIM*®; McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University Baltimore: Baltimore, MD, USA; Available online: https://omim.org/ (accessed on 14 October 2022).
578.Oppermann M, Mizel D, Huang G, Li C, Deng C, Theilig F, et al. Macula densa control of renin secretion and preglomerular resistance in mice with selective deletion of the B isoform of the Na. K, 2Cl co-transporter. *J Am Soc Nephrol.* 2006, 17: 2143-52.
579.Oppermann M, Mizel D, Kim SM, Chen L, Faulhaber-Walter R, Huang Y, et al. Renal function in mice with targeted disruption of the A isoform of the Na- K-2Cl co-transporter. *J Am Soc Nephrol.* 2007. 18: 440–8.
580. Orabona GDA, Salzano G, Iaconetta G, Piombino P, Ponzo L, Santella A, Astarita F, Solari D, Salzano FA, Califano L. Facial osteomas: Fourteen cases and a review of literature. *Eur. Rev. Med. Pharmacol. Sci.* 2015, 19: 1796–1802.
581. Orouji E, Utikal J. Tackling malignant melanoma epigenetically: Histone lysine methylation. *Clin. Epigenet.* 2018, 10: 145.
582.Orphanet Report Series. *«Prevalence of Rare Diseases: Bibliographic Data», Orphanet Report Series, Rare Diseases Collection, Janvier 2022*. (2022). Available online athttps://www.orpha.net/orphacom/cahiers/docs/GB/
Prevalence_of_rare_diseases_by_alphabetical_list.pdf (accessed March 15, 2022).
583. Otsuka T, Fujinaka H, Imamura M, Tanaka Y, Hayakawa H, Tomizawa S. Duplication of chromosome 4q: Renal pathology of two siblings. *Am. J. Med. Genet. Part A* 2005, 134A: 330–333.
584. Pacho F, Zambruno G, Calabresi V, Kiritsi D, Schneider H. Efficiency of translation termination in humans is highly dependent upon nucleotides in the neighbourhood of a (premature) termination codon. *J. Med. Genet.* 2011, 48: 640–644.
585. Pagon RA, Wallace SE, Bean J.H., Mirzaa G, Amemiya A, Eds.; University of Washington: Seattle, WA, USA, 1993; 14 July 2003 (Updated 23 July 2020). Available online: https://www.ncbi.nlm.nih.gov/books/NBK1363/ (accessed on 27 June 2021).
586. Palomaki GE, Kloza EM, Lambert-Messerlian GM, van den Boom D, Ehrich M, Deciu C, Bombard AT, Haddow JE. Circulating cell free DNA testing: Are some test failures informative? *Prenat. Diagn.* 2015, 35: 289–293.
587. Pampena R, Kyrgidis A, Lallas A, et al. A Meta-Analysis of Nevus-Associated Melanoma: Prevalence and Practical Implications. *J. Am. Acad. Dermatol.* 2017, 77: 938–945.
588. Pan X, Zhang C, Li X, Chen S, Huijuan G, Zhang Y, Chen F, Jiang H, Jiang F, Zhang H, et al. Noninvasive fetal sex determination by maternal plasma sequencing and application in X-linked disorder counseling. *J. Matern Fetal Neonatal Med.* 2014, 27: 1829–1833.
589. Panny A, Glurich I, Haws RM, Acharya A. Oral and craniofacial anomalies of Bardet-Biedl syndrome: dental management in the context of a rare disease. *J Dent Res*. 2017, 96(12):1361–1369.

590. Papagelopoulos PJ, Mavrogenis AF, Mitsiokapa EA, Papaparaskeva KT, Galanis EC, Soucacos PN. Current trends in the management of extra-abdominal desmoid tumours. *World J. Surg. Oncol.* 2006, 4: 1–8.
591. Park TJ, Mitchell B, Abitua P, Kintner C, Wallingford JB. Dishevelled controls apical docking and planar polarization of basal bodies in ciliated epithelial cells. *Nat. Genet.* 2008, 40, 871–879.
592. Parsons DW, Jones S, Zhang X, et al. An Integrated Genomic Analysis of Human Glioblastoma Multiforme. *Science* 2008, 321: 1807–1812.
593. Pascanu I, Pop R, Barbu CG, et al. Development of synthetic growth charts for Romanian population. *Acta Endocrinol.* 2016,12:309–318.
594. Pascanu I, Pop R, Barbu CG, et al. Endocrine care development of synthetic growth charts for Romanian population. Acta Endocrinol. 2016; XII:309–18.
595. Patel SG, Ahnen DJ. Colorectal cancer in the young. *Curr. Hepatol. Rep.* 2018, 20: 1–12.
596. Patel V, Raghuveer T, Persons D, Myers S. Trisomy 4q Syndrome: A rare Syndrome Phenotypically Similar to Trisomy. *Internet J. Pediatr. Neonatol.* 2006, 7: 133–172.
597. Payne M, Anderson JA, Cook J. Gardner's syndrome—A case report. *Br. Dent. J.* 2002, 193: 383–384.
598. Pearlman R, Frankel WL, Swanson B, Zhao W, Yilmaz A, Miller K, Bacher J, Bigley C, Nelsen L, Goodfellow PJ, et al. Prevalence and spectrum of germline cancer susceptibility gene mutations among patients with early-onset colorectal cancer. *JAMA Oncol.* 2017, 3: 464–471.
599. Pearson HA. Life-span of the fetal red blood cell. *J Pediatr*. 1967, 70(2): 166-171.
600. Pêgo S P B, de Faria P R, Santos L A N, Coletta R D, de Aquino S N, MartelliJúnior H. Ultrastruc tural evaluation of gingival connective tissue in hereditary gingival fibromatosis. Oral surgery, oral medicine, oral pathology and oral radiology 2016; 122(1): 8188.
601. Peiker G, Boehm W, Carol W. Laurence-Moon-Bardet-Biedl-Syndrom und Endometriumkarzinom [Laurence-Moon-Bardet-Biedl syndrome and endometrial carcinoma]. *Zentralbl Gynakol*. 1978,100(17):1126–1130.
602. Peng W, Chen JQ, Liu C, et al. Loss of PTEN Promotes Resistance to T Cell–Mediated Immunotherapy. *Cancer Discov.* 2016, 6: 202–216.
603. Peoples R, Franke Y, Wang Y-K, Pérez-Jurado L, Paperna T, Cisco M, Francke U. A Physical Map, Including a BAC/PAC Clone Contig, of the Williams-Beuren Syndrome–Deletion Region at 7q11. *Am. J. Hum. Genet.* 2000, 66: 47–68.
604. Persikov AV, Pillitteri RJ, Amin P, Schwarze U, Byers PH, Brodsky B. Stability related bias in residues replacing glycines within the collagen triple helix (Gly-Xaa-Yaa) in inherited connective tissue disorders. *Hum. Mutat.* 2004, 24: 330–337.
605.Peters M, Jeck N, Reinalter S, Leonhardt A, Tönshoff B, ünter Klaus G, et al. Clinical presentation of genetically defined patients with hypokalemic salt-losing tubulopathies. *Am J Med.* 2002, 112: 183–90.
606. Pfendner E, Rouan F, Uitto J. Progress in epidermolysis bullosa: The phenotypic spectrum of plectin mutations. *Exp. Dermatol.* 2005, 14: 241–249.
607. Pfendner EG, Bruckner AL. Epidermolysis Bullosa Simplex. In *GeneReviews®*; Adam MP, Ardinger H, Pagon RA, Wallace SE, Eds.; University of Washington: Seattle, WA, USA, 1998. Available online: https://www.ncbi.nlm.nih.gov/books/ NBK1369/ (accessed on 5 March 2022).
608. Pfendner EG, Lucky AW. Epidermolysis Bullosa with Pyloric Atresia. In *GeneReviews®*; Adam MP, Ardinger HH, Pagon RA, Wallace SE, Eds.; University of Washington: Seattle, WA, USA, 2008. Available online: https://www.ncbi.nlm.nih.gov/ books/NBK1157/ (accessed on 6 March 2022).
609. Pfendner EG, Sadowski SG, Uitto J. Epidermolysis Bullosa Simplex: Recurrent and De Novo Mutations in the KRT5 and KRT14 Genes, Phenotype/Genotype Correlations, and Implications for Genetic Counseling and Prenatal Diagnosis. *J. Investig. Dermatol.* 2005, 125: 239–243.
610. PhenoModifier: A Genetic Modifier Database for Elucidating the Genetic Basis of Human Phenotypic Variation. Available online: https://www.biosino.org/PhenoModifier/index (accessed on 26 July 2021).
611. Pho LN, Champine M, Leachman SA, Kohlmann W. Inherited gene mutations in melanoma. In *Genetics of Melanoma*; Springer: New York, NY, USA, 2016; pp. 117–149.

612. Pichert G, Jacobs C. (Eds.) *Rare Hereditary Cancers: Diagnosis and Management*; Springer International Publishing: Cham, Switzerland, 2016; Volume 205.
613. Pierre S, Coumoul X . Understanding SOS (Son of Sevenless). Biochemical pharmacology 2011; 82(9): 10491056.
614. Pin-Jung C, Pai-Chi T, Zhu Y, et al. Noninvasive prenatal diagnostics: recent developments using circulating fetal nucleated cells. *Curr Obstet Gynecol Rep*. 2019, 8(1): 1-8.
615. Pollock PM, Harper UL, Hansen KS, et al. High Frequency of BRAF Mutations in Nevi. *Nat. Genet.* 2003, 33: 19–20.
616. Pomeroy J, Krentz AD, Richardson JG, Berg RL, VanWormer JJ, Haws RM. Bardet-Biedl syndrome: weight patterns and genetics in a rare obesity syndrome. *Pediatr Obes*. 2021,16(2): e12703.
617. Pope B, Tsumagari K, Battaglia D, Ryba T, Hiratani I, Ehrlich M, Gilbert DM. DNA Replication Timing Is Maintained Genome-Wide in Primary Human Myoblasts Independent of D4Z4 Contraction in FSH Muscular Dystrophy. *PLoS ONE* 2011, 6: e27413.
618. Popescu R, Grămescu M, Caba L, Pânzaru M-C, Butnariu L, Braha E, Popa S, Rusu C, Cardos G, Zeleniuc M, et al. A Case of Inherited t(4;10)(q26;q26.2) Chromosomal Translocation Elucidated by Multiple Chromosomal and Molecular Analyses. Case Report and Review of the Literature. *Genes* 2021, 12: 1957.
619. Potocki L, Chen K-S, Park SS, Osterholm DE, Withers MA, Kimonis V, Summers AM, Meschino WS, Anyane-Yeboa K, Kashork CD, et al. Molecular mechanism for duplication 17p11.2—The homologous recombination reciprocal of the Smith-Magenis microdeletion. *Nat. Genet.* 2000, 24: 84–87.
620. Potrony M, Badenas C, Aguilera P, et al. Update in Genetic Susceptibility in Melanoma. *Ann. Transl. Med.* 2015, 3: 210.
621. Poulin MA, Laframboise R, Blouin MJ. Association of bifid epiglottis and laryngeal web with Bardet-Biedl syndrome: a case report. *Int J Pediatr Otorhinolaryngol*. 2019,122:138–140.
622. Preda C, Ungureanu MC, Leustean L, Cristea C, Vulpoi C. Ethical issues related to the use of human growth hormone in idiopathic short stature. Revista Romana de Bioetica 2013; 11(4): 31-37.
623. Prevalence and Incidence of Rare Diseases: Bibliographic Data. Orphanet Report Series Number 1 January 2020. Available online: https://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf (accessed on 5 November 2020).
624. Prieto B, Candenas M, Ladenson JH, Alvarez FV. Comparison of different CD71 monoclonal antibodies for enrichment of fetal cells from maternal blood. *Clin Chem Lab Med*. 2002, 40(2): 126-131.
625. Prodinger C, Chottianchaiwat S, Mellerio JE, McGrath JA, Ozoemena L, Liu L, Moore W, Laimer M, Petrof G, Martinez AE. The natural history of laryngo-onycho-cutaneous syndrome: A case series of six pediatric patients and literature review. *Pediatr. Dermatol.* 2021, 38: 1094–1101.
626. Prodinger C, Reichelt J, Bauer JW, Laimer M. Epidermolysis bullosa: Advances in research and treatment. *Exp. Dermatol.* 2019, 28: 1176–1189.
627. Proteinatlas the Human Protein Atlas. Available online: https://www.proteinatlas.org (accessed on 19 December 2019).
628. Pucci M, Asiáin PR, Sáez ED, Jantus-Lewintre E, Malarani M, Khan S, Fontana S, Naing A, Passiglia F, Raez LE, et al. Extracellular vesicles as miRNA nano-shuttles: Dual role in tumor progression. *Target. Oncol.* 2018, 13: 175–187.
629. Puricelli E, Bettinelli A, Borsa N, Sironi F, Mattiello C, Tammaro F, et al. Long- term follow-up of patients with Bartter syndrome type I and II. *Nephrol Dial Transplant.* 2010, 25: 2976–81.
630. Putoux A, Attie-Bitach T, Martinovic J, Gubler MC. Phenotypic variability of Bardet-Biedl syndrome: focusing on the kidney. *Pediatr Nephrol*. 2012, 27(1):7–15.
631. Putoux A, Mougou-Zerelli S, Thomas S, et al. BBS10 mutations are common in 'Meckel'-type cystic kidneys. *J Med Genet*. 2010, 47(12):848–852.
632. Qi Y, Yang J, Hou Y, Hu R, Wang D, Peng H, Yin A. Potential influence of parental copy number variations on non-invasive prenatal testing (NIPT): Two case reports. *Mol. Cytogenet.* 2020, 13: 18.
633. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, Sun W, Dou K, Li H. Circular RNA: A new star of noncoding RNAs. *Cancer Lett.* 2015, 365: 141–148.
634. Quintos JB, Guo MH, Dauber A. Idiopathic short stature due to novel heterozygous mutation

of the aggrecan gene. *J. Pediatr Endocrinol. Metab* 2015, 28: 927–932.
635.Ranade VV, Somberg JC. Bioavailability and pharmacokinetics of magnesium after administration of magnesium salts to humans. *Am J Ther.* 2001, 8: 345.
636. Ransohoff KJ, Jaju PD, Tang JY, et al. Familial skin cancer syndromes: Increased melanoma risk. *J. Am. Acad. Dermatol.* 2016, 74: 423–434.
637. Rapaport R, Wit JM, Savage MO. Growth failure: 'idiopathic' only after a detailed diagnostic evaluation. *Endocr. Connect.* 2021, 10: 125–138.
638. Rappold G, Blum WF, Shavrikova EP, et al. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. *J. Med. Genet.* 2007, 44: 306–313.
639. Ravnan JB, Tepperberg JH, Papenhausen P, Lamb AN, Hedrick J, Eash D, Ledbetter DH, Martin CL. Subtelomere FISH analysis of 11 688 cases: An evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. *J. Med. Genet.* 2006, 43: 478–489.
640. Reading JP, Huffman JL, Wu JC, et al. Nucleated erythrocytes in maternal blood: quantity and quality of fetal cells in enriched populations. *Hum Reprod*. 1995, 10(9): 2510-2515.
641.Reinalter SC, Jeck N, Brochhausen C, Watzer B, Nüsing RM, Seyberth HW, et al. Role of cyclooxygenase-2 in hyperprostaglandin E syndrome/antenatal Bartter syndrome. *Kidney Int.* 2002, 62: 253–60.
642. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol*. 2017, 18(9):533–547.
643. Ren S, Xin Z, Xu Y, Xu J, Wang G. Construction and analysis of circular RNA molecular regulatory networks in liver cancer. *Cell Cycle* 2017, 16: 2204–2211.
644. Rial-Pensado E, Freire-Agulleiro O, Ríos M, et al. Obesity induces resistance to central action of BMP8B through a mechanism involving the BBSome. *Mol Metab*. 2022, 59:101465.
645. Riegel M, Baumer A, Jamar M, Delbecque K, Herens C, Verloes A, Schinzel A. Submicroscopic terminal deletions and duplications in retarded patients with unclassified malformation syndromes. *Qual. Life Res.* 2001, 109: 286–294.
646. Riley EP, Infante MA, Warren KR. Fetal alcohol spectrum disorders: an overview. Neuropsychol Rev. 2011; 21(2):73-80.
647. Rimoin DL, Pyeritz RE, Korf B. (Eds.) *Emery and Rimoin's Essential Medical Genetics*; Elsevier: Amsterdam, The Netherlands, 2013.
648. Rinaldi R, De Bernardo C, Assumma M, Grammatico B, Buffone E, Poscente M, Grammatico P. Cytogenetic and molecular characterization of a de novo 4q24qter duplication and correlation to the associated phenotype. *Am. J. Med. Genet.* 2003, 118A: 122–126.
649. Rolnik DL, Costa FDS, Lee TJ, Schmid M, McLennan AC. Association Between Fetal Fraction on Cell-Free DNA Testing and First-Trimester Markers for Pre-eclampsia. *Obstet. Gynecol. Surv.* 2019, 74: 265–266.
650. Romero MC, Mialdea OG, Company AV, Tapia MC, Piñera JG. Duplicación parcial del cromosoma 4q (q31, q35): Síndrome aurículo-acro-renal. In *Anales de Pediatría*; Elsevier: Amsterdam, The Netherlands, 2008; Volume 68, pp. 361–364.
651. Rouabhi M, Guo DF, Morgan DA, et al. BBSome ablation in SF1 neurons causes obesity without comorbidities. *Mol Metab*. 2021, 48:101211.
652. Rousselle P, Beck K. Laminin 332 processing impacts cellular behavior. *Cell Adhes. Migr.* 2013, 7: 122–134.
653. Rugg EL, McLean WH, Lane EB, Pitera R, McMillan JR, Dopping-Hepenstal PJ, Navsaria H, Leigh IM, et al. A functional "knockout" of human keratin 14. *Genes Dev.* 1994, 8: 2563–2573.
654. Ruivo CF, Adem B, Silva M, Melo SA. The biology of cancer exosomes: Insights and new perspectives. *Cancer Res.* 2017, 77: 6480–6488.
655. Russo ML, Blakemore KJ. A historical and practical review of first trimester aneuploidy screening. *Semin. Fetal Neonatal Med.* 2014, 19: 183–187.
656. Sag SO, Gorukmez O, Ture M, Gulten T, Yakut T. De novo partial trisomy distal 4q: A case report. *Genet. Couns.* 2014, 25: 423–428.
657. Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. *Ultrasound Obstet Gynecol*. 2019, 54(4): 442-451.

658. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS ONE* 2012, 7: e30733.
659. Salzman J. Circular RNA expression: Its potential regulation and function. *Trends Genet.* 2016, 32; 309–316.
660. Samura O. Update on noninvasive prenatal testing: A review based on current worldwide research. *J. Obstet. Gynaecol. Res.* 2020, 46: 1246–1254.
661. Sánchez-Jimeno C, Escámez M, Ayuso C, Trujillo-Tiebas M, del Río M. Genetic Diagnosis of Epidermolysis Bullosa: Recommendations from an Expert Spanish Research Group. *Actas Dermo-Sifiliográficas* 2018, 109: 104–122.
662. Sand M, Bechara FG, Sand D, Gambichler T, Hahn SA, Bromba M, Stockfleth E, Hessam S. Circular RNA expression in basal cell carcinoma. *Epigenomics* 2016, 8: 619–632.
663. Sanfilippo JS, Lara-Torre E, Edmonds DK, et al. Clinical Pediatric and Adolescent Gynecology. New York: Informa Healthcare; 2009:450.
664. Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *Proc. Natl. Acad. Sci. USA* 1976, 73: 3852–3856.
665. Sangu N, Okamoto N, Shimojima K, Ondo Y, Nishikawa M, Yamamoto T. A de novo microdeletion in a patient with inner ear abnormalities suggests that the 10q26.13 region contains the responsible gene. *Hum. Genome Var.* 2016, 3: 16008.
666. Sathishkumar D, Orrin E, Terron-Kwiatkowski A, Browne F, Martinez AE, Mellerio JE, Ogboli M, Hoey S, Ozoemena L, Liu L, et al. The p.Glu477Lys Mutation in Keratin 5 Is Strongly Associated with Mortality in Generalized Severe Epidermolysis Bullosa Simplex. *J. Investig. Dermatol.* 2016, 136: 719–721.
667. Savarirayan R, Tofts L, Irving M, et al. Safe and persistent growth-promoting effects of vosoritide in children with achondroplasia: 2-year results from an open-label, phase 3 extension study. *Genet. Med.* 2021, 23: 2443–2447.
668. Sawamura D, Goto M, Sakai K, Nakamura H, McMillan JR, Akiyama M, Shirado O, Oyama N, Satoh M, Kaneko F, et al. Possible Involvement of Exon 31 Alternative Splicing in Phenotype and Severity of Epidermolysis Bullosa Caused by Mutations in PLEC1. *J. Investig. Dermatol.* 2007, 127: 1537–1540.
669. Sawamura D, Nakan H, Matsuzaki Y. Overview of epidermolysis bullosa. *J. Dermatol.* 2010, 37: 214–219.
670. Schaefer E, Lauer J, Durand M, et al. Mesoaxial polydactyly is a major feature in Bardet–Biedl syndrome patients with LZTFL1 (BBS17) mutations. *Clin Genet.* 2014, 85(5):476–481.
671. Schaefer E, Lauer J, Durand M, Pelletier V, Obringer C, Claussmann A, Braun J-J, Redin C, Mathis C, Muller J, et al. Mesoaxial polydactyly is a major feature in Bardet-Biedl syndrome patients withLZTFL1(BBS17) mutations. *Clin. Genet.* 2014, 85, 476–481.
672. Scheidecker S, Hull S, Perdomo Y, et al. Predominantly cone-system dysfunction as rare form of retinal degeneration in patients with molecularly confirmed Bardet-Biedl syndrome. *Am J Ophthalmol*. 2015, 160(2):364–372.
673. Schrier SA, Bodurtha JN, Burton B, et al. The Coffin-Siris syndrome: a proposed diagnostic approach and assessment of 15 overlapping cases. Am J Med Genet A. 2012; 158A:1865–76.
674. Schrott HG, Sakaguchi S, Francke U, Luzzatti L, Fialkow PJ. Translocation, t(4q-;13q+), in three generations resulting in partial trisomy of the long arm of chromosome 4 in the fourth generation. *J. Med. Genet.* 1974, 11: 201–205.
675. Schumann H, Has C, Kohlhase J, Bruckner-Tuderman L. Dystrophic epidermolysis bullosa pruriginosa is not associated with frequentFLGgene mutations. *Br. J. Dermatol.* 2008, 159: 464–469.
676. Schwab G, Kriz K. Two sisters with Laurence Bardet-Biedl-Moon syndrome and concomitant carcinoma of the body of the uterus. *Geburtshilfe Frauenheilkd.* 1980, 40(3):279–281.
677. Schwieger-Briel A, Fuentes I, Castiglia D, Barbato A, Greutmann M, Leppert J, Duchatelet S, Hovnanian A, Burattini S, Yubero MJ, et al. Epidermolysis Bullosa Simplex with KLHL24 Mutations Is Associated with Dilated Cardiomyopathy. *J. Investig. Dermatol.* 2019, 139: 244–249.
678. Scigliano S, Grégoire M, Schmitt M, Jonveaux P, LeHeup B. Terminal deletion of the long arm of chromosome. *Clin. Genet.* 2004, 65: 294–298.
679. Scott F, Bonifacio M, Sandow R, Ellis K, Smet ME, McLennan A. Rare autosomal trisomies:

Important and not so rare. *Prenat Diagn.* 2018, 38: 765–771.
680. Seaver LH, Irons M. ACMG practice guideline: Genetic evaluation of short stature. Genet Med. 2009;11:465.
681. Sekizawa A, Purwosunu Y, Matsuoka R, et al. Recent advances in non-invasive prenatal DNA diagnosis through analysis of maternal blood. *J Obstet Gynaecol Res*. 2007, 33(6): 747-764.
682. Sekizawa A, Samura O, Zhen DK, Falco V, Farina A, Bianchi DW. Apoptosis in fetal nucleated erythrocytes circulating in maternal blood. *Prenat Diagn*. 2000, 20(11): 886-889.
683. Seo S, Baye LM, Schulz NP, Beck JS, Zhang Q, Slusarski DC, Sheffield VC. BBS6, BBS10, and BBS12 form a complex with CCT/TRiC family chaperonins and mediate BBSome assembly. *Proc. Natl. Acad. Sci. USA* 2010, 107, 1488–1493.
684. Seo S, Guo DF, Bugge K, Morgan DA, Rahmouni K, Sheffield VC. Requirement of Bardet-Biedl syndrome proteins for leptin receptor signaling. *Hum Mol Genet*. 2009, 18(7):1323–1331.
685. Seppo A, Frisova V, Ichetovkin I, et al. Detection of circulating fetal cells utilizing automated microscopy: potential for noninvasive prenatal diagnosis of chromosomal aneuploidies. *Prenat Diagn*. 2008, 28(9): 815-821.
686. Septer S, Slowik V, Morgan R, Dai H, Attard T. Thyroid cancer complicating familial adenomatous polyposis: Mutation spectrum of at-risk individuals. *Hered. Cancer Clin. Pract.* 2013, 11: 1–6.
687.Seys E, Andrini O, Keck M, Mansour-Hendili L, Courand PY, Simian C, et al. Clinical and genetic spectrum of Bartter syndrome type 3. *J Am Soc Nephrol*. 2017, 28: 2540–52.
688. Shaffer L, Theisen A. Disorders caused by chromosome abnormalities. *Appl. Clin. Genet.* 2010, 3: 159–174.
689. Shain AH, Bastian BC. From Melanocytes to Melanomas. *Nat. Rev. Cancer* 2016, 16: 345–358.
690. Shain AH, Garrido M, Botton T, et al. Exome Sequencing of Desmoplastic Melanoma Identifies Recurrent NFKBIE Promoter Mutations and Diverse Activating Mutations in The MAPK Pathway. *Nat. Genet.* 2015, 47: 1194–1199.
691. Shain AH, Joseph NM, Yu R, et al. Genomic and Transcriptomic Analysis Reveals Incremental Disruption of Key Signaling Pathways During Melanoma Evolution. *Cancer Cell* 2018, 34: 45–55.
692. Shain AH, Yeh I, Kovalyshyn I, et al. The Genetic Evolution of Melanoma from Precursor Lesions. *N. Engl. J. Med.* 2015, 373: 1926–1936.
693. Shan NL, Kahn A, Pusztai L. Intratumour heterogeneity, from hypothesis to the clinic. *Br. J. Cancer* 2022,1-2.
694. Shang Q, Yang Z, Jia R, Ge S. The novel roles of circRNAs in human cancer. *Mol. Cancer* 2019, 18: 6.
695. Shashi V, Berry MN, Santos C, Pettenati MJ. Partial duplication of 4q12q13 leads to a mild phenotype. *Am. J. Med. Genet.*1999, 86: 51–53.
696. Shaw J, Scotchman E, Chandler N, Chitty L. Non-invasive prenatal testing for aneuploidy, copy number variants and single gene disorders. *Reproduction* 2020, 160: A1–A11.
697. Sheffield VC, Carml R, Kwltek-Black A, Rokhlina T, Nishlmura D, Duyk GM, Elbedour K, Sunden SL, Stone EM. Identification of a Bardet-Biedl syndrome locus on chromosome 3 and evaluation of an efficient approach to homozygosity mapping. *Hum. Mol. Genet.* 1994, 3, 1331–1335.
698. Sheffield VC, Zhang Q, Heon E, Drack AV, Stone EM, Carmi R. The Bardet-Biedl syndrome. *Epstein's Inborn Errors Dev.* 2016, 237–240.
699. Shenoy RD, Shenoy V, Shetty V. Chromosomal Abnormalities in Syndromic Orofacial Clefts: Report of Three Children. *Case Rep. Genet.* 2018, 2018: 1928918.
700. Shetty AK, Shah HJ, Patil MA, Jhota KN. Idiopathic gingival enlargement and its management. J Indian Soc Periodontol 2010; 14:263–265.
701. Shi B-J, Zhu X-J, Liu Y, Hao J, Yan G-F, Wang S-P, Wang X-Y, Diao Q-C. Transient bullous dermolysis of the newborn: A novel de novo mutation in the *COL7A1* gene. *Int. J. Dermatol.* 2015, 54: 438–442.
702. Shi H, Cordin O, Minder CM, Linder P, Xu RM. Crystal structure of the human ATP-dependent splicing and export factor UAP56. *Proc. Natl. Acad. Sci. USA* 2004, 101: 17628–17633.
703. Siañez-González C, Pezoa-Jares R, Salas-Alanis JC. Congenital epidermolysis bullosa: A

review. *Actas Dermo-Sifiliográficas* 2009, 100: 842–856.
704. Sigalotti L, Covre A, Fratta E, et al. Whole Genome Methylation Profiles as Independent Markers of Survival in Stage IIIC Melanoma Patients. *J. Transl. Med.* 2012, 10: 185.
705. Silla IO, Rueda D, Rodríguez Y, García JL, de la Cruz Vigo F, Perea J. Early-onset colorectal cancer: A separate subset of colorectal cancer. *World J. Gastroenterol.* 2014, 20: 17288.
706. Silveira LG, Tusset C, Latronico AC. Impact of mutations in kisspeptin and neurokinin B signaling pathways on human reproduction. *Brain Res*. 2010, 1364:72–80.
707. Sim JCH, White SM, Lockhart PJ. ARID1B-mediated disorders: Mutations and possible mechanisms. Intractable Rare Dis Res. 2015;4:17–23
708. Singla V. The primary cilium as the cell's antenna: Signaling at a sensory organelle. *Science* 2006, 313, 629–633.
709. Sinha A, Tekkis PP, Gibbons DC, Phillips RK, Clark SK. Risk factors predicting desmoid occurrence in patients with familial adenomatous polyposis: A meta-analysis. *Colorectal Dis.* 2011, 13: 1222–1229.
710. Sireteanu A, Popescu R, Braha EE, et al. Detection of chromosomal imbalances using combined MLPA kits in patients with syndromic intellectual disability. *Rev. Romana Med. Lab.* 2014, 22: 157–164.
711. Skrlec I, Wagner J, Puseljić S, Heffer M, Stipoljev F. Partial monosomy 2p and partial trisomy 4q due to paternal trans-location t(2;4)(p25.1;q31.3). *Coll. Antropol.* 2014, 38: 759–762.
712. Smith BB, Barbara DW, Hyder JA, Smith MM. Anesthetic considerations for patients with Bardet–Biedl syndrome: a case series and review of the literature. *Paediatr Anaesth*. 2016, 26(4):429–437.
713. Soura E, Eliades PJ, Shannon K, et al. Hereditary melanoma: Update on syndromes and management: Genetics of familial atypical multiple mole melanoma syndrome. *J. Am. Acad. Dermatol.* 2016, 74: 395–407.
714. Spurna Z, Capkova P, Srovnal J, et al. Clinical impact of variants in non- coding regions of SHOX-Current knowledge. *Gene* 2022, 15: 146238.
715. Starke S, Jost I, Rossbach O, Schneider T, Schreiner S, Hung LH, Bindereif A. Exon circularization requires canonical splice signals. *Cell Rep.* 2015, 10: 103–111.
716. Steinhagen E, Guillem JG, Chang G, Salo-Mullen EE, Shia J, Fish S, Stadler ZK, Markowitz AJ. The prevalence of thyroid cancer and benign thyroid disease in patients with familial adenomatous polyposis may be higher than previously recognized. *Clin. Colorectal Cancer* 2012, 11: 304–308.
717. Stella M, Falzone L, Caponnetto A, Gattuso G, Barbagallo C, Battaglia R, Mirabella F, Broggi G, Altieri R, Certo F, et al. Serum extracellular vesicle-derived circHIPK3 and circSMARCA5 are two novel diagnostic biomarkers for glioblastoma multi- forme. *Pharmaceuticals* 2021, 14: 618.
718. Stene J, Stengel-Rutkowski S. Genetic risks of familial reciprocal and Robertsonian translocation carriers. In *The Cytogenetics of Mammalian Autosomal Rearrangements*; Daniel, A., Ed.; Alan R Liss, Inc.: New York, NY, USA, 1988; pp. 1–54.
719. Stenson PD, Mort M, Ball EV, Evans K, Hayden M, Heywood S, Hussain M, Phillips AD, Cooper DN. The Human Gene Mutation Database: Towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Qual. Life Res.* 2017, 136: 665–677. Available online: http://www.hgmd.cf.ac.uk/ac/all.php (accessed on 26 June 2021).
720. Stephens PJ, Greenman CD, Fu B, et al. Massive Genomic Rearrangement Acquired in a Single Catastrophic Event During Cancer Development. *Cell* 2011, 144: 27–40.
721. Stoffel EM, Koeppe E, Everett J, Ulintz P, Kiel M, Osborne J, Williams L, Hanson K, Gruber SB, Rozek LS. Germline genetic features of young individuals with colorectal cancer. *Gastroenterology* 2018, 154: 897–905.
722. Stone EM, Andorf JL, Whitmore SS, et al. Clinically focused molecular investigation of 1000 consecutive families with inherited retinal disease. *Ophthalmology*. 2017, 124(9):1314–1331.
723. Strong A, Li D, Mentch F, Bedoukian E, Hartung EA, Meyers K, Skraban C, Wen J, Medne L, Glessner J, et al. Ciliopathies: Coloring outside of the lines. *Am. J. Med. Genet. Part A*, 2021, 185, 687–694.
724. Sun S, Karsdal MA. Type VI collagen. In *Biochemistry of Collagens, Laminins and Elastin*; Academic Press: Cambridge, MA, USA, 2016; pp. 49–55.

725. Sun X, Lu J, Ma X. An efficient method for noninvasive prenatal diagnosis of fetal trisomy 13, trisomy 18, and trisomy 21. *PLoS ONE* 2019, 14: e0215368.
726. Surana RB, Conen PE. Partial trisomy 4 resulting from a 4-18 reciprocal translocation. *Ann. Génét.* 1972, 15: 191–194.
727. Suzuki H, Tsukahara T. A view of pre-mRNA splicing from RNase R resistant RNAs. *Int. J. Mol. Sci.* 2014, 15: 9331–9342.
728. Suzumori N, Sekizawa A, Ebara T, Samura O, Sasaki A, Akaishi R, Wada S, Hamanoue H, Hirahara F, Izumi H, et al. Fetal cell-free DNA fraction in maternal plasma for the prediction of hypertensive disorders of pregnancy. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2018, 224; 165–169.
729. Szeverenyi I, Cassidy AJ, Chung CW, Lee BTK, Common JEA, Ogg SC, Chen H, Sim SY, Goh WLP, Ng KW, et al. The Human Intermediate Filament Database: Comprehensive information on a gene family involved in many human diseases. *Hum. Mutat.* 2008, 29: 351–360.
730.Takahashi M, Yanagida N, Okano M, Ishizaki A, Meguro J, Kukita K, et al. A first report: living related kidney transplantation on a patient with Bartter's syndrome. *Transplant Proc.* 1996, 28: 1588.
731. Tanabe S, Akiba T, Katoh M, Satoh T. Terminal deletion of chromosome 10q: Clinical features and literature review. *Pediatr. Int.* 1999, 41: *565*–567.
732. Taneja PA, Prosen TL, De Feo E, Kruglyak KM, Halks-Miller M, Curnow KJ, Bhatt S. Fetal aneuploidy screening with cell-free DNA in late gestation. *J. Matern. Neonatal Med.* 2017, 30: 338–342.
733. Tang K, Zhang H, Li Y, et al. Circular RNA as a potential biomarker for melanoma: A systematic review. *Front. Cell Dev. Biol.* 2021, 9: 638548.
734. Tas F, Erturk K. BRAF V600E Mutation as a Prognostic Factor in Cutaneous Melanoma Patients. *Dermatol. Ther.* 2020, 33: e13270.
735. Taylor KM, Francke U, Brown MG, George DL, Kaufhold M, Opitz JM. Inverted tandem ("mirror") duplications in human chromosomes: Inv dup 8p, 4q, 22q. *Am. J. Med. Genet.* 1977, 1: 3–19.
736. Thapa M, Asamoah A, Gowans GC, Platky KC, Barch MJ, Mouchrani P, Rajakaruna C, Hersh JH. Molecular characterization of distal 4q duplication in two patients using oligonucleotide array-based comparative genomic hybridization (oaCGH) analysis. *Am. J. Med. Genet. Part A* 2014, 164: 1069–1074.
737. Thurik FF, Ruiter ML-D, Javadi A, Kwee A, Woortmeijer H, Page-Christiaens GCML, Franx A, Van Der Schoot CE, Koster MPH. Absolute first trimester cell-free DNA levels and their associations with adverse pregnancy outcomes. *Prenat. Diagn.* 2016, 36: 1104–1111.
738. Tian L, Wu X, Lin Y, Liu Z, Xiong F, Han Z, Zhou Y, Zeng Q, Wang Y, Deng J, et al. Characterization and potential function of a novel pre-implantation embryo-specific RING finger protein: TRIML. *Mol. Reprod. Dev.* 2009, 76: 656–664.
739. Tieder M, Levy M, Gubler MC, Gagnadoux MF, Broyer M. Renal abnormalities in the Bardet-Biedl syndrome. *Int J Pediatr Nephrol*. 1982, 3 (3):199–203.
740. Tiffen JC, Gunatilake D, Gallagher SJ, et al. Targeting activating mutations of EZH2 leads to potent cell growth inhibition in human melanoma by derepression of tumor suppressor genes. *Oncotarget* 2015, 6: 27023.
741. Tiret A, Parc C. Fundus lesions of adenomatous polyposis. *Curr. Opin. Ophthalmol.* 1999, *10*, 168–172.
742. Tonella L, Pala V, Ponti R, et al. Prognostic and predictive biomarkers in stage III melanoma: Current insights and clinical implications. *Int. J. Mol. Sci.* 2021, 22: 4561.
743. Tong L, Hodgkins PR, Denyer J, Brosnahan D, Harper J, Russell-Eggitt I, I Taylor DS, Atherton D. The eye in epidermolysis bullosa. *Br. J. Ophthalmol.* 1999, 83: 323–326.
744. Troeger C, Zhong XY, Burgemeister R, et al. Approximately half of the erythroblasts in maternal blood are of fetal origin. *Mol Hum Reprod*. 1999, 5(12): 1162-1165.
745. Truta B, Allen BA, Conrad PG, Kim YS, Berk T, Gallinger S, Bapat B, Terdiman JP, Sleisenger MH. Genotype and phenotype of patients with both familial adenomatous polyposis and thyroid carcinoma. *Fam. Cancer* 2003, 2: 95–99.
746.Tsang SH, Aycinena AR, and Sharma T. Ciliopathy: bardet- Biedl syndrome. *Adv Exp Med Biol* 2018, 1085: 171-174.

747. Tsyklauri O, Niederlova V, Forsythe E, et al. Bardet-Biedl syndrome ciliopathy is linked to altered hematopoiesis and dysregulated self-tolerance. *EMBO Rep*. 2021, 22(2):e50785.
748. Turcan I, Pasmooij AMG, Van den Akker PC, Lemmink H, Sinke RJ, Jonkman MF. Association of Epidermolysis Bullosa Simplex with Mottled Pigmentation andEXPH5Mutations. *JAMA Dermatol.* 2016, 152: 1137–1141.
749. Turczynski S, Titeux M, Pironon N, Cohn H, Murrell D, Hovnanian A. Marked intrafamilial phenotypic heterogeneity in dystrophic epidermolysis bullosa caused by inheritance of a mild dominant glycine substitution and a novel deep intronic recessive *COL7A1* mutation. *Br. J. Dermatol.* 2016, 174: 1122–1125.
750.Ueki R, Okutani R, Fukushima A, Tsuji T, Ohata H, Tashiro C. Perioperative endocrinological findings in a patient with Bartter's syndrome and living-related renal transplantation. *J Anesth.* 2000, 14: 105–8.
751. Uenoyama Y, Pheng V, Tsukamura H, et al. The roles of kisspeptin revisited: inside and outside the hypothalamus. *J Reprod Dev*. 2016, 62 (6):537–545.
752. Uitto J, Richard G, McGrath J. Diseases of epidermal keratins and their linker proteins. *Exp. Cell Res.* 2007, 313: 1995–2009.
753. UniProt: Universal Protein Knowledgebase. Available from: https://www.uniprot.org/. Accessed May 25, 2022.
754. Uong A, Zon LI. Melanocytes in Development and Cancer. *J. Cell Physiol.* 2010, *222*: 38–41.
755. Vahidnezhad H, Youssefian L, Saeidian AH, Mahmoudi H, Touati A, Abiri M, Kajbafzadeh A-M, Aristodemou S, Liu L, McGrath J, et al. Recessive mutation in tetraspanin CD151 causes Kindler syndrome-like epidermolysis bullosa with multi-systemic manifestations including nephropathy. *Matrix Biol.* 2018, 66: 22–33.
756. Vahidnezhad H, Youssefian L, Saeidian AH, Mozafari N, Barzegar M, Sotoudeh S, Daneshpazhooh M, Isaian A, Zeinali S, Uitto J. KRT5 and KRT14 Mutations in Epidermolysis Bullosa Simplex with Phenotypic Heterogeneity, and Evidence of Semidominant Inheritance in a Multiplex Family. *J. Investig. Dermatol.* 2016, 136: 1897–1901.
757. Vahidnezhad H, Youssefian L, Saeidian AH, Uitto J. Phenotypic Spectrum of Epidermolysis Bullosa: The Paradigm of Syndromic versus Non-Syndromic Skin Fragility Disorders. *J. Investig. Dermatol.* 2019, 139: 522–527.
758. Vaida MA, Streian CG, Gug C, Damen NS, Jianu AM, Grigorita A, Grigorita L. Morphological Study of Fossa Ovalis in Formalin-Fixed Human Hearts and Its Clinical Importance. *Medicina* 2021, 57*:* 1254.
759. Van Allen EM, Wagle N, Sucker, A.; Treacy, D.J.; Johannessen, C.M.; Goetz, E.M.; Place, C.S.; Taylor-Weiner A, Whittaker S, Kryukov GV, et al. The Genetic Landscape of Clinical Resistance to RAF Inhibition in Metastatic Melanoma. *Cancer Discov.* 2014, 4: 94–109.
760. van der Meij KR, Sistermans EA, Macville MV, Stevens SJ, Bax CJ, Bekker MN, Bilardo CM, Boon EM, Boter M, Diderich KE, et al. TRIDENT-2: National Implementation of Genome-wide Non-invasive Prenatal Testing as a First-Tier Screening Test in the Netherlands. *Am. J. Hum. Genet.* 2019, 105: 1091–1101.
761. van der Sluijs PJ, Jansen S, Vergano SA, et al. The ARID1B spectrum in 143 patients: from nonsyndromic intellectual disability to Coffin–Siris syndrome. Genet Med. 2019; 21(6):1295-1307.
762. van der Steen M, Pfundt R, Maas SJWH, et al. ACAN gene mutations in short children born SGA and response to growth hormone treatment. *J. Clin. Endocrinol. Metab.* 2017, 102: 1458–1467.
763. Vannelli S, Baffico M, Buganza R, et al. SHOX deficiency in children with growth impairment: evaluation of known and new auxological and radiological indicators. *Ital. J. Pediatr.* 2020, 46: 1–6.
764. Vardabasso C, Gaspar-Maia A, Hasson D, et al. Histone variant H2A.Z.2 mediates proliferation and drug sensitivity ofmalignant melanoma. *Mol. Cell* 2015, 59: 75–88.
765. Vardabasso C, Hasson D, Ratnakumar K, et al. Histone variants: Emerging players in cancer biology. *Cell. Mol. Life Sci.* 2014, 71: 379–404.
766.Vargas-Poussou R. Pathophysiological aspects of the thick ascending limb and novel genetic defects: helix syndrome and transient antenatal Bartter syndrome. *Pediatr Nephrol.* 2021, 37: 239–52.

767. Varki R, Sadowski S, Pfendner E, Uitto J. Epidermolysis bullosa. I. Molecular genetics of the junctional and hemidesmosomal variants. *J. Med. Genet.* 2006, 43: 641–652.
768. Varki R, Sadowski S, Uitto J, Pfendner E. Epidermolysis bullosa. II. Type VII collagen mutations and phenotype-genotype correlations in the dystrophic subtypes. *J. Med. Genet.* 2007, 44: 181–192.
769. Vasko A, Drivas TG, Schrier Vergano SA. Genotype-phenotype correlations in 208 individuals with coffin-siris syndrome. Genes (Basel). 2021;12(6): 937
770. Vasko A, Schrier Vergano SA. Language impairments in individuals with Coffin-Siris syndrome. Front Neurosci. 2022; 15: 802583.
771. Vasques GA, Andrade NLM, Jorge AAL. Genetic causes of isolated short stature. *Arch. Endocrinol. Metab* 2019, 63: 70–78.
772. Vasques GA, Funari MFA, Ferreira FM, et al. IHH gene mutations causing short stature with nonspecific skeletal abnormalities and response to growth hormone therapy. *J. Clin. Endocrinol Metab* 2018, 103: 604–614.
773. Verfaillie A, Imrichova H, Atak ZK, et al. Decoding the regulatory landscape of melanomareveals TEADS as regulators of the invasive cell state. *Nat. Commun.* 2015, 6: 6683.
774. Vergano SA, Sluijs PJ van der, Santen G. ARID1B-Related Disorder. 2019. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle: University of Washington, Seattle: 1993-2022.
775. Vergano SS, Deardorff MA. Clinical features, diagnostic criteria, and management of Coffin-Siris syndrome. Am J Med Genet Part C Semin Med Genet. 2014; 166:252–6.
776. Vermeer MC, Bolling MC, Bliley JM, Gomez KFA, Pavez-Giani MG, Kramer D, Romero-Herrera PH, Westenbrink BD, Diercks GF, van den Berg MP, et al. Gain-of-function mutation in ubiquitin ligase KLHL24 causes desmin degradation and dilatation in hiPSC-derived engineered heart tissues. *J. Clin. Investig.* 2021, 131: e140615.
777. Vidal AF. Read-through circular RNAs reveal the plasticity of RNA processing mechanisms in human cells. *RNA Biol.* 2020, 17: 1823–1826.
778. Viquez OM, Yazlovitskaya EM, Tu T, Mernaugh G, Secades P, McKee KK, Georges-Labouesse E, De Arcangelis A, Quaranta V, Yurchenco P, et al. Integrin alpha6 maintains the structural integrity of the kidney collecting system. *Matrix Biol.* 2016, 57–58: 244–257.
779. Vogel W, Siebers JW, Gunkel J, Haas B, Knörr-Gärtner H, Niethammer DG, Noel B. Uneinheitlicher Phänotyp bei Partialtrisomie 4q. *Qual. Life Res.* 1975, 28: 103–112.
780. Vossaert L, Wang Q, Salman R, et al. Reliable detection of subchromosomal deletions and duplications using cell-based noninvasive prenatal testing. *Prenat Diagn*. 2018, 38(13): 1069-1078.
781. Wagstaff W, Mwamba RN, Grullon K et al. Melanoma: Molecular Genetics, Metastasis, Targeted Therapies, Immunotherapies, and Therapeutic Resistance. *Genes Dis.* 2022, 9: 1608–1623.
782. Wallace IR, McKinley MC, Bell PM, et al. Sex hormone binding globulin and insulin resistance. *Clin Endocrinol*. 2013, 78(3):321–329. doi:10.1111/cen.12086
783. Wallace MR, Marchuk DA, Andersen LB, et al. Type 1 Neurofibromatosis Gene: Identification of a Large Transcript Disrupted in Three NF1 Patients. *Science* 1990, 249: 181–186.
784.Walsh PR, Tse Y, Ashton E, Iancu D, Jenkins L, Bienias M, et al. Clinical and diagnostic features of Bartter and Gitelman syndromes. *Clin Kidney J.* 2018, 11: 302–9.
785. Wang D, Liang S, Zhang X, Dey SK, Li Y, Xu C, Yu Y, Li M, Zhao G, Zhang Z. Targeted next-generation sequencing approach for molecular genetic diagnosis of hereditary colorectal cancer: Identification of a novel single nucleotide germline insertion in adenomatous polyposis coli gene causes familial adenomatous polyposis. *Mol. Genet. Genom. Med.* 2019, 7: e00505.
786. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. *Nature* 2008, 456: 470–476.
787. Wang M, Yang Y, Xu J, Bai W, Ren X, Wu H. CircRNAs as biomarkers of cancer: A meta-analysis. *BMC Cancer* 2018, 18: 303.
788. Wang M, Zhang L, Ren W, Li S, Zhi K, Zheng J, Gao L. Diagnostic Value of CircRNAs as Potential Biomarkers in Oral Squamous Cell Carcinoma: A Meta-Analysis. *Front. Oncol.* 2021, 11: 693284.
789. Wang W, Zuidema A, Te Molder L, Nahidiazar L, Hoekman L, Schmidt T, Coppola S,

Sonnenberg A. Hemidesmosomes modulate force generation via focal adhesions. *J. Cell Biol.* 2020, 219: e201904137.
790. Wang Y, Li S, Wang W, Dong Y, Zhang M, Wang X, Yin C. Cell-free DNA screening for sex chromosome aneuploidies by non-invasive prenatal testing in maternal plasma. *Mol. Cytogenet.* 2020, 13: 1–8.
791. Wang, Y.; Wang, H.; Zheng, R.; Wu, P.; Sun, Z.; Chen, J.; Zhang, L.; Zhang, C.; Qian, H.; Jiang, J.; et al. Circular RNA ITCH suppresses metastasis of gastric cancer via regulating miR-199a-5p/Klotho axis. *Cell Cycle* 2021, 20: 522–536.
792. Wapner R, Babiarz JE, Levy B, Stosic M, Zimmermann B, Sigurjonsson S, Wayham N, Ryan A, Banjevic M, Lacroute P, et al. Expanding the scope of noninvasive prenatal testing: Detection of fetal microdeletion syndromes. *Am. J. Obstet. Gynecol.* 2015, 212: 332.e1–332.e9.
793. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N. Engl. J Med.* 2012, 367: 2175–2184.
794. Watanabe G, Kiyoi Y, Takeyama I, Kawana S, Yamamoto M. Inherited chromosomal translocation in two families (t(4q-;13q+) and t(5q-;13q+)). *Tohoku J. Exp. Med.* 1977, 121: 179–184.
795. Waters AM, Beales PL. Ciliopathies: An expanding disease spectrum. *Pediatr. Nephrol.* 2011, 26, 1039–1056.
796. Watson CN, Belli A, Di Pietro V. Small non-coding RNAs: New class of biomarkers and potential therapeutic targets in neurodegenerative disease. *Front. Genet.* 2019, 10: 364.
797. Wax JR, Cartin A, Chard R, Lucas FL, Pinette MG. Noninvasive prenatal testing: Impact on genetic counseling, invasive prenatal diagnosis, and trisomy 21 detection. *J. Clin. Ultrasound* 2015, 43: 1–6.
798. Weckselblatt B, Rudd MK. Human Structural Variation: Mechanisms of Chromosome Rearrangements. *Trends Genet.* 2015, 31: 587–599.
799. Wei X, Ao Z, Cheng L, et al. Highly sensitive and rapid isolation of fetal nucleated red blood cells with microbead-based selective sedimentation for non-invasive prenatal diagnostics. *Nanotechnology*. 2018, 29(43): 434001.
800.eihbrecht K, et al. Keeping an Eye on Bardet-Biedl Syndrome: A Comprehensive Review of the Role of Bardet-Biedl Syndrome Genes in the Eye. *Med Res Arch* 2017, 5(9).
801. Weihbrecht K, Goar WA, Pak T, et al. Keeping an eye on Bardet-Biedl syndrome: a comprehensive review of the role of Bardet-Biedl syndrome genes in the eye. *Med Res Arch.* 2017, 5:9, 1–21.
802. Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell* 1995, 81: 323–330.
803. Weinel S, Lucky AW, Uitto J, Pfendner EG, Choo D. Dystrophic Epidermolysis Bullosa with One Dominant and One Recessive Mutation of the COL7A1 Gene in a Child with Deafness. *Pediatr. Dermatol.* 2008, 25: 210–214.
804. Wheway G, Mitchison HM. Genomics England research consortium opportunities and challenges for molecular understanding of ciliopathies–The 100,000 genomes project. *Front. Genet.* 2019, 10, 127.
805. Wheway G, Nazlamova L, Hancock JT. Signaling through the primary cilium. *Front Cell Dev Biol*. 2018, 6:8.
806. Whittaker SR, Theurillat JP, Van Allen E et al. A Genome-Scale RNA Interference Screen Implicates NF1 Loss in Resistance to RAF Inhibition. *Cancer Discov.* 2013, 3: 350–362.
807. Whittle AJ, Carobbio S, Martins L, et al. BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. *Cell*. 2012,149(4):871–885.
808. Wiens CJ, Tong Y, Esmail MA, Oh E, Gerdes J, Wang J, Tempel W, Rattner JB, Katsanis N, Park H-W, et al. Bardet-Biedl syndrome-associated small GTPase ARL6 (BBS3) functions at or near the ciliary gate and modulates Wnt signaling. *J. Biol. Chem.* 2010, 285, 16218–16230.
809. Wiesner T, He J, Yelensky R, et al. Kinase Fusions Are Frequent in Spitz Tumors and Spitzoid Melanomas. *Nat. Commun.* 2014, 5: 3116.
810. Wiesner T, Kiuru M, Scott SN, et al. NF1 Mutations Are Common in Desmoplastic Melanoma. *Am. J. Surg. Pathol.* 2015, 39: 1357–1362.
811. Wiik MU, Talseth-Palmer BA. Familial Adenomatous Polyposis. In *Handbook of Tumor Syndromes*; Liu D, Ed.; CRC Press— Taylor & Francis Group: Boca Raton, FL, USA, 2020.

812. Wijn MA, Keller JJ, Giardiello FM, Brand HS. Oral and maxillofacial manifestations of familial adenomatous polyposis. *Oral Dis.* 2007, 13: 360–365.
813. Wilting RH, Dannenberg JH. Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. *Drug Resist. Update* 2012, 15: 21–38.
814. Wilusz JE. A 360 view of circular RNAs: From biogenesis to functions. *Wiley Interdiscip. Rev. RNA* 2018, 9: e1478.
815. Wilusz JE. Controlling translation via modulation of tRNA levels. *Wiley Interdiscip. Rev. RNA* 2015, 6: 453–470.
816. World Health Organisation. WHO Child growth standards: Height-for-age girls 2 to 5 years (percentiles).[https://cdn.who.int/media/docs/default-source/child-growth/child-growth-standards/indicators/length-height-for-age/hfa-girls-2-5-percentiles.pdf?sfvrsn=adc3b954_7]
817. World Health Organization. Congenital Anomalies. Available online: https://www.who.int/health-topics/congenital-anomalies# tab=tab (accessed on 12 February 2021).
818. Wright CF, Fitzgerald TW, Jones WD, et al. Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. Lancet (London, England). 2015; 385:1305–14.
819. Wright JT. Oral Manifestations in the Epidermolysis Bullosa Spectrum. *Dermatol. Clin.* 2010, 28: 159–164.
820. Wu J, Guo X, Wen Y, Huang S, Yuan X, Tang L, Sun H. N6-methyladenosine modification opens a new chapter in circular RNA biology. *Front. Cell Dev. Biol.* 2021, 9: 2031.
821. Xian J, Su W, Liu L, Rao B, Lin M, Feng Y, Qiu F, Chen J, Zhou Q, Zhao Z, et al. Identification of Three Circular RNA Cargoes in Serum Exosomes as Diagnostic Biomarkers of Non–Small-Cell Lung Cancer in the Chinese Population. *J. Mol. Diagn.* 2020, 22: 1096–1108.
822. Xiao S, Bu L, Zhu L et al. A new locus for hereditary gingival fibromatosis (GINGF2) maps to 5q13 q22. Genomics 2001; 74:180–185.
823. Xu K, Qiu Z, Xu L, Qiu X, Hong L, Wang J. Increased levels of circulating circular RNA (hsa_circ_0013587) may serve as a novel biomarker for pancreatic cancer. *Biomark. Med.* 2021, 15: 977–985.
824. Yalcin S, Philip PA. (Eds.) *Textbook of Gastrointestinal Oncology*; Springer Nature: Cham, Switzerland, 2019.
825. Yamada T, Sekizawa A, Fujii Y, Hirose T, Samura O, Suzumori N, Miura K, Sawai H, Hirahara F, Murotsuki J. Maternal age-specific risk for trisomy 21 based on the clinical performance of NIPT and empirically derived NIPT age-specific positive and negative predictive values in Japan. *J. Hum. Genet.* 2018, 63: 1035–1040.
826. Yan Y, Wu Q, Zhang L, Wang X, Dan S, Deng D, Sun L, Yao L, Ma Y, Wang L. Detection of submicroscopic chromosomal aberrations by array-based comparative genomic hybridization in fetuses with congenital heart disease. *Ultrasound Obstet. Gynecol.* 2013, 43: 404–412.
827. Yang F, Liu DY, Guo JT, Ge N, Zhu P, Liu X, Wang S, Wang GX, Sun SY. Circular RNA circ-LDLRAD3 as a biomarker in diagnosis of pancreatic cancer. *World J. Gastroenterol.* 2017, 23: 8345.
828. Yang R, Duan Y, Kong Q, Li W, Xu J, Xia X, Sang H. What do we learn from dystrophic epidermolysis bullosa, nails only? Idiopathic nail dystrophy may harbor a COL7A1 mutation as the underlying cause. *J. Dermatol.* 2020, 47: 782–786.
829. Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, Jin Y, Yang Y, Chen LL, Wang Y, et al. Extensive translation of circular RNAs driven by N 6-methyladenosine. *Cell Res.* 2017, 27: 626–641.
830. Yao H, Jiang F, Hu H, Gao Y, Zhu Z, Zhang H, Wang Y, Guo Y, Liu L, Yuan Y, et al. Detection of fetal sex chromosome aneuploidy by massively parallel sequencing of maternal plasma DNA: Initial experience in a Chinese hospital. *Ul-trasound Obstet. Gynecol.* 2014, 44: 17–24.
831. Yatsenko S, Kruer M, Bader P, Corzo D, Schuette J, Keegan C, Nowakowska B, Peacock S, Cai W, Peiffer D, et al. Identification of critical regions for clinical features of distal 10q deletion syndrome. *Clin. Genet.* 2009, 76: 54–62.
832. Yau M, Rapaport R. Growth hormone stimulation testing: to test or not to test? That is one of the questions. Front Endocrinol (Lausanne). 2022; 0:1099.
833. Yeh I, Botton T, Talevich E, et al. Activating MET Kinase Rearrangements in Melanoma and Spitz Tumours. *Nat. Commun.* 2015, 6: 7174.
834. Yenamandra V, Akker PVD, Lemmink H, Jan S, Diercks G, Vermeer M, Berg MVD, van der

Meer P, Pasmooij A, Sinke R, et al. Cardiomyopathy in patients with epidermolysis bullosa simplex with mutations in KLHL24. *Br. J. Dermatol.* 2018, 179: 1181–1183.
835. Yin WB, Yan MG, Fang X, Guo JJ, Xiong W, Zhang RP. Circulating circular RNA hsa_circ_0001785 acts as a diagnostic biomarker for breast cancer detection. *Clin. Chim. Acta* 2018, 487: 363–368.
836. Yobb TM, Somerville MJ, Willatt L, Firth HV, Harrison K, MacKenzie J, Gallo N, Morrow BE, Shaffer LG, Babcock M, et al. Microduplication and Triplication of 22q11.2: A Highly Variable Syndrome. *Am. J. Hum. Genet.* 2005, 76: 865–876.
837. Yoo CB, Jones PA. Epigenetic therapy of cancer: Past, present and future. *Nat. Rev. Drug Discov.* 2006, 5: 37–50.
838. You X, Vlatkovic I, Babic A, Will T, Epstein I, Tushev G, Akbalik G, Wang M, Glock C, Quedenau C et al. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat. Neurosci.* 2015, 18: 603–610.
839. Young J, Xu C, Papadakis GE, et al. Clinical management of congenital hypogonadotropic hypogonadism. *Endocr Rev.* 2019, 40(2):669–710.
840. Yu D, Zhang K, Han M, Wei P, Ying C, Yunfeng W, Hongyan J, Ling D, Qiying Z, Xiaojie S, et al. Noninvasive prenatal testing for fetal subchromosomal copy number variations and chromosomal aneuploidy by low-pass whole-genome sequencing. *Mol. Genet. Genom. Med.* 2019, 7: e674.
841. Yu SCY, Chan KCA, Zheng YWL, Jiang P, Liao GJW, Sun H, Akolekar R, Leung TY, Go ATJI, van Vugt JMG, et al. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. *Proc. Natl. Acad. Sci. USA* 2014, 111: 8583–8588.
842. Yu W, Lv Y, Yin S, Liu H, Li X, Liang B, Kong L, Liu C. Screening of fetal chromosomal aneuploidy diseases using noninvasive prenatal testing in twin pregnancies. *Expert Rev. Mol. Diagn.* 2019, 19: 189–196.
843. Yu X, Zheng H, Tse G, et al. Long non-coding RNAs in melanoma. *Cell Prolif.* 2018, 51: e12457.
844. Yu Y, Yao RE, Wang L, et al. De novo mutations in ARID1B associated with both syndromic and non-syndromic short stature. BMC Genomics. 2015; 16:1–10.
845. Yuen WY, Jonkman MF. Risk of squamous cell carcinoma in junctional epidermolysis bullosa, non-Herlitz type: Report of 7 cases and a review of the literature. *J. Am. Acad. Dermatol.* 2011, 65: 780–789.
846. Yun M, Wu J, Workman JL, Li B. Readers of histone modifications. *Cell Res.* 2011, 21: 564–578.
847. Zacchia M, Capolongo G, Trepiccione F, Marion V. Impact of local and systemic factors on kidney dysfunction in Bardet-Biedl syndrome. *Kidney Blood Press Res*. 2017, 42(5):784–793.
848. Zager JS, Gastman BR, Leachman S, et al. Performance of a Prognostic 31-Gene Expression Profile in an Independent Cohort of 523 Cutaneous Melanoma Patients. *BMC Cancer* 2018, 18: 130.
849.Zaghloul NA and Katsanis N. Mechanistic insights into Bardet- Biedl syndrome, a model ciliopathy. *J Clin Inves*t 2009, 119(3): 428-37.
850. Zaki MS, Eid OM, Eid MM, Mohamed AM, Sayed IS, Abdel-Hamid MS, Abdel-Salam GM. Bilateral Calcification of Basal Ganglia in a Patient with Duplication of Both 11q13.1q22.1 and 4q35.2 with New Phenotypic Features. *Cytogenet. Genome Res.* 2019, 159: 130–136.
851. Zaki MS, Sattar S, Massoudi RA, Gleeson JG. Co-occurrence of distinct ciliopathy diseases in single families suggests genetic modifiers. *Am J Med Genet A.* 2011, 155(12):3042–3049.
852. Zarrei M, MacDonald J, Merico D, Scherer S. A copy number variation map of the human genome. *Nat. Rev. Genet.* 2015, 16: 172–183.
853. Zawerton A, Yao B, Yeager JP, et al. De novo SOX4 variants cause a neurodevelopmental disease associated with mild dysmorphism. Am J Hum Genet. 2019; 104(2):246-259.
854. Zelihić D, Hjardemaal FR, von der Lippe C. Caring for a child with Bardet-Biedl syndrome: a qualitative study of the parental experiences of daily coping and support. *Eur J Med Genet*. 2020, 63(4):103856.
855. Zhang C, He J, Qi L, Wan L, Wang W, Tu C, Li Z. Diagnostic and prognostic significance of dysregulated expression of circular RNAs in osteosarcoma. *Expert Rev. Mol. Diagn.* 2021, 21: 235–244.

856. Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, Zhu Z, Lin M, Liu Q, Tian Z, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: Clinical experience from 146 958 pregnancies. *Ultrasound Obstet. Gynecol.* 2015, 45: 530–538.
857. Zhang L, Shay JW. Multiple roles of APC and its therapeutic implications in colorectal cancer. *JNCI J. Natl. Cancer Inst.* 2017, 109(8).
858. Zhang Q, Yu D, Seo S, Stone EM, Sheffield VC. Intrinsic protein-protein interaction-mediated and chaperonin-assisted sequential assembly of stable Bardet-Biedl syndrome protein complex, the BBSome. *J. Biol. Chem.* 2012, 287, 20625–20635.
859. Zhang W, Zheng M, Kong S, Li X, Meng S, Wang X, Wang F, Tang C, Ju S. Circular RNA hsa_circ_0007507 May Serve as a Biomarker for the Diagnosis and Prognosis of Gastric Cancer. *Front. Oncol.* 2021, 11: 699625.
860. Zhang X, Luo S, Wu J, Zhang L, Wang W-H, Degan S, Erdmann D, Hall R, Zhang JY. KIND1 Loss Sensitizes Keratinocytes to UV-Induced Inflammatory Response and DNA Damage. *J. Investig. Dermatol.* 2017, 137: 475–483.
861. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. *Cell* 2014, 159: 134–147.
862. Zhang Y-L, Dai Y, Tu Z-G, Li Q-Y. 'Pure' Partial Trisomy 4q26?q35.2 Resulting from a Familial Unbalanced Translocation t(4;10)(q26;q26.3). *Cytogenet. Genome Res.* 2009, 127 : 67–72.
863. Zhao X, Zhang X, Zhang Z, Liu Z, Zhu J, Lyu S, Li L, Lang R, He Q. Comprehensive circular RNA expression profiling constructs a ceRNA network and identifies hsa_circ_0000673 as a novel oncogene in distal cholangiocarcinoma. *Aging* 2020, 12: 23251.
864. Zhao XX, Suzumori N, Ozaki Y, Sato T, Suzumori K. Examination of fetal cells and cell-free fetal DNA in maternal blood for fetal gender determination. *Gynecol Obstet Invest*. 2004, 58(1): 57-60.
865. Zhen N, Gu S, Ma J, Zhu J, Yin M, Xu M, Wang J, Huang N, Cui Z, Bian Z, et al. CircHMGCS1 promotes hepatoblastoma cell proliferation by regulating the IGF signaling pathway and glutaminolysis. *Theranostics* 2019, 9: 900.
866. Zhou MY, Yang JM, Xiong XD. The emerging landscape of circular RNA in cardiovascular diseases. *J. Mol. Cell. Cardiol.* 2018, 122: 134–139.
867. Zhou WY, Cai ZR, Liu J, Wang DS, Ju HQ, Xu RH. Circular RNA: Metabolism, functions and interactions with proteins. *Mol. Cancer* 2020, 19: 172.
868. Zhu X, Wang X, Wei S, Chen Y, Chen Y, Fan X, Han S, Wu G. hsa_circ_0013958: A circular RNA and potential novel biomarker for lung adenocarcinoma. *FEBS J.* 2017, 284: 2170–2182.
869. Zhu Y, Zhang W, Huo Z et al. A novel locus for maternally inherited human gingival fibromatosis at chromosome 11p15. Hum Genet. 2007; 121:113–123.
870. Zocco D, Bernardi S, Novelli M, et al. Isolation of Extracellular Vesicles Improves the Detection of Mutant DNA from Plasma of Metastatic Melanoma Patients. *Sci. Rep.* 2020, 10: 15745.
871. Zollino M, Zampino G, Torrioli G, Pomponi MG, Neri G. Further contribution to the description of phenotypes associated with partial 4q duplication. *Am. J. Med. Genet.* 1995, 57: 69–73.
872. Zuo Q, Liu J, Huang L, et al. AXL/AKT Axis Mediated-Resistance to BRAF Inhibitor Depends on PTEN Status in Melanoma. *Oncogene* 2018, 37: 3275–3289.