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Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Estonia

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Supervisors:	Professor Katrin Õunap, MD, PhD, Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Estonia
	Lecturer Tiina Kahre, MD, PhD, Department of Clinical Genetics, Institute of Clinical Medicine, University of Tartu, Estonia
Reviewers:	Professor Vallo Volke, MD, PhD, Faculty of Medicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia
	Research Professor Lili Milani, PhD, Estonian Genome Center, Institute of Genomics, University of Tartu, Estonia
Opponent:	Professor Bernhard Horsthemke, PhD, University of Duisburg-Essen, Germany

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Paper II: participation in the study design; collecting, analyzing, and interpreting data; preparing figures and tables and writing the manuscript.

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Paper IV: collecting the data; preparing figures and tables and writing the manuscript.

ABBREVIATIONS

AHO	Albright hereditary osteodystrophy
AS	Angelman syndrome
BMI	body mass index
BWS	Beckwith-Wiedemann syndrome
cAMP	cyclic adenosine monophosphate
CI	confidence interval
CMA	chromosomal microarray
CNV	copy number variation
CPP	central precocious puberty
DD	developmental delay
DMR	differentially methylated region
DNA	deoxyribonucleic acid
ES	exome sequencing
FISH	fluorescence in situ hybridization
GOM	gain of methylation
$G_s\alpha$	the stimulatory G protein alpha-subunit
H2A	histone 2A
H2B	histone 2B
H3	histone 3
H4	histone 4
HP1	heterochromatin protein 1
IC	imprinting centre
IC1	imprinting centre 1
IC2	imprinting centre 2
ICR	imprinting control region
ID	intellectual disability
ImpDis	imprinting disorder
iPPSD	inactivating PTH/PTH-related protein signaling disorders
IUGR	intrauterine growth retardation
kb	kilobase
KOS	Kagami-Ogata syndrome
LCSH	long contiguous stretches of homozygosity
LLD	leg length discrepancy
lncRNA	long non-coding ribonucleic acid
LOM	loss of methylation
MALDI-TOF MS	matrix assisted laser desorption/ionization time-of-flight mass spectrometry
Mb	megabase
MDS	myoclonus-dystonia syndrome
MLID	multilocus imprinting disturbance
MLMD	multilocus methylation defect
MRI	magnetic resonance imaging

MRS	magnetic resonance spectroscopy
MS-MLPA	methylation-specific multiplex ligation-dependent probe amplification
MS-PCR	methylation-specific polymerase chain reaction
MS-SNuPE	methylation-specific single nucleotide primer extension
NGS	next-generation sequencing
NH-CSS	Netchine-Harbison clinical score system
PcG	polycomb-group proteins
PHP	pseudohypoparathyroidism
PHP1A	pseudohypoparathyroidism type 1A
PHP1B	pseudohypoparathyroidism type 1B
PHP1C	pseudohypoparathyroidism type 1C
PHP2	pseudohypoparathyroidism type 2
PPHP	pseudopseudohypoparathyroidism
PTH	parathyroid hormone
PWS	Prader-Willi syndrome
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RRBS	reduced representation bisulphate sequencing
SD	standard deviation
SGA	small for gestational age
snoRNA	small nucleolar ribonucleic acid
SNP	single nucleotide polymorphism
SRS	Silver-Russell syndrome
SYS	Schaaf-Yang syndrome
TNDM	transient neonatal diabetes mellitus
TS14	Temple syndrome
TSH	thyroid stimulating hormoone
TSO	TruSight One
UPD	uniparental disomy
UPD(7)	uniparental disomy of chromosome 7
UPD(14)mat	maternal uniparental disomy of chromosome 14
UPD(14)pat	paternal uniparental disomy of chromosome 14
UPD(15)	uniparental disomy of chromosome 15
UPD(20)mat	maternal uniparental disomy of chromosome 20
WGBS	whole genome bisulphate sequencing

Nomenclature of genes in text

APBA2	amyloid beta precursor protein binding family A member 2
ARID1B	AT-rich interaction domain 1B
ATP10A	ATPase, class V, type 10A
CDKN1C	cyclin-dependent kinase inhibitor 1C
CHD8	chromodomain helicase DNA binding protein 8
CTCF	CCCTC-binding factor

CTCFL	CCCTC-binding factor-like protein
CYFIP1	cytoplasmic FMR1-interacting protein 1
DIRAS3	DIRAS family GTPase 3
DLK1	delta, drosophila, homolog-like 1
GABRB3	gamma-aminobutyric acid type A receptor beta3 subunit
GCP5	tubulin gamma complex associated protein 5
GNAS	guanine nucleotide-binding proteiin, alpha-stimulating activity polypeptide 1
GRB10	growth factor receptor-bound protein 10
H19	imprinted maternally expressed transcript H19
HYMAI	hydatidiform mole-associated and imprinted transcript
IGF2	insulin-like growth factor 2
IGF2R	insulin-like growth factor 2 receptor
Igf2	insulin-like growth factor 2 (house mouse)
Igf2r	insulin-like growth factor 2 receptor (house mouse)
IPW	imprinted in Prader-Willi syndrome
KCNK9	potassium channel, subfamily K, member 9
KCNQ1	potassium channel, voltage-gated, KQT-like subfamily, member 1
KCNQ1OT1	KCNQ1-overlapping transcript 1
KHDC3L	KH domain containing 3 like, subcortical maternal complex member
L3MBTL1	lethal(3)malignant brain tumor-like protein 1
MAGEL2	MAGE-like protein 2
MEG3	maternally expressed gene 3
MEG8	maternally expressed gene 8
MEST	mesoderm specific transcript
MIR380	microRNA 380
MKRN3	makorin 3
NNAT	neuronatin
NDN	necdin
NIPA1	non-imprinted in Prader-Willi/Angelman syndrome 1
NIPA2	non-imprinted in Prader-Willi/Angelman syndrome 2
NLRP2	NLR family pyrin domain containing 2
NLRP5	NLR family pyrin domain containing 5
NLRP7	NLR family pyrin domain containing 7
NSD1	nuclear receptor binding SET domain protein 1
OCA2	oculocutaneous albinism II melanosomal transmembrane protein
PEG1	paternally expressed gene 1
PEG3	paternally expressed gene 3
PLAGL1	pleomorphic adenoma gene-like 1
RTL1	retrotransposon-like gene 1
SGCE	sarcoglycan, epsilon
SNRPN	small nuclear ribonucleoprotein polypeptide N

SNU13	small nuclear ribonucleoprotein 13
SNURF	SNRPN upstream reading frame
STX16	syntaxin 16
TRIM28	tripartite motif containing 28
TUBGCP5	tubulin gamma complex associated protein 5
UBE3A	ubiquitin protein ligase E3A
WRB	tryptophan rich basic protein
ZFP57	zinc finger protein 57
ZNF331	zinc finger protein 331

1. INTRODUCTION

Imprinting disorders (ImpDis) are a small but expanding group of rare congenital diseases caused by an aberrant expression of imprinted genes due to genetic or epigenetic abnormalities. Though the four classic and most common ImpDis – Prader-Willi syndrome (PWS; OMIM #176270), Angelman syndrome (AS; OMIM #105830), Beckwith-Wiedemann syndrome (BWS; OMIM #130650) and Silver-Russell syndrome (SRS; OMIM #180860) – were first clinically described as early as in the 50–60s of the 20th century [Prader *et al*., 1956; Angelman, 1965; B Beckwith, 1963; Russell, 1954; Silver *et al*., 1953; Wiedemann, 1964], the molecular mechanism of these disorders remained unclear for another two decades, until the mid–1980s.

In 1984, Davor Solter from the Wistar Institute (Philadelphia, USA) and, independently, Azim Surani from the AFRC Institute of Animal Physiology (Cambridge, UK), together with their colleagues, published the results of experiments with mouse embryos that contained either two sets of chromosomes inherited from the mother, or two sets of chromosomes inherited from the father [Barton *et al*., 1984; McGrath, and Solter, 1984; Surani *et al*., 1984]. These experiments demonstrated that the maternal set of chromosomes was not functionally equivalent to the set inherited from the father and one set of chromosomes from each parent was essential for normal development of embryos. It was assumed that some genes have parent-of-origin specific expression after fertilization and this phenomenon has been called genomic imprinting [Monk, 1987; Monk, 1988].

In 1991, the first three imprinted genes, *Igf2r*, *Igf2* and *H19*, were identified and mapped in the mouse genome [Barlow *et al*., 1991; DeChiara *et al*., 1991; Ferguson-Smith *et al*., 1991; Bartolomei *et al*., 1991]. One year later, monoallelic parental-specific expression was also confirmed in the human *H19* gene [Zhang, and Tycko, 1992]. Since that time, more than 100 imprinted genes have been discovered in the human genome. Moreover, there are about 100 more predicted but not confirmed imprinted human genes [Jirtle, 2018].

Over the years, molecular and clinical studies have shown that imprinted genes are essential not only for prenatal development, but also for many postnatal processes. Pathologic changes in expression of imprinted genes can significantly affect postnatal growth, brain function, behaviour, hormonal and metabolic systems, and cause a complex syndrome. Despite a large amount of discovered imprinted genes, the number of known congenital ImpDis is modest. At present, only 13 clinically recognized congenital ImpDis are known: PWS, AS, BWS, SRS, *GNAS*-gene-related ImpDis – pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism (PHP/PPHP; OMIM #103580, #603233, #612462, #612463), central precocious puberty (CPP; OMIM #615346), Temple syndrome (TS14; OMIM #616222), transient neonatal diabetes mellitus (TNDM; OMIM #601410), myoclonus-dystonia syndrome (MDS; OMIM #604149), Kagami-Ogata syndrome (KOS; OMIM #608149), maternal uniparental disomy of

chromosome 20 (UPD(20)mat; OMIM #617352), Schaaf-Yang syndrome (SYS; OMIM #615547) and Birk-Barel syndrome (OMIM #612292). Moreover, some uniparental disomies (UPD), like maternal UPD of chromosomes 6 and 16, can theoretically affect the function of imprinted genes, but the related phenotype is more likely caused by concomitant mosaic trisomy of the chromosomes occurring either in the placenta or in the body tissues.

The molecular etiology and the clinical presentation of ImpDis is highly variable, which makes diagnosis of these disorders difficult and sometimes challenging. It is assumed that a significant part of ImpDis cases remain undiagnosed. The clinical presentation and molecular mechanisms of ImpDis have been thoroughly studied and described in the literature, but only a limited amount of studies has explored the prevalence, incidence and other epidemiological data for these disorders. Almost all of them have focused on the epidemiology of PWS, AS and BWS. Furthermore, only a few of these studies have been performed during the last 10 years and the exact prevalence of ImpDis remains thus unclear.

In 2000–2004, the Estonian pediatric neurologist Eve Õiglane-Shlik studied the two most common ImpDis, PWS and AS. In her doctoral project, she first investigated the clinical phenotype, genetic etiology, possibilities of early recognition and diagnostics, live birth and population prevalence of these syndromes in Estonia. In her study, she succeeded in finding the prevalence of PWS and AS in Estonia during the period 1984–2004 [Õiglane-Shlik, 2007; Õiglane-Shlik *et al*., 2006a]. Her work motivated us to continue the research of ImpDis and provided the opportunity to make conclusions about changes in the prevalence and efficiency of clinical and molecular diagnosis of these two ImpDis in Estonia.

In 2014, at the beginning of our study, the number of patients with diagnosed ImpDis was almost 1.7 times lower compared to the number in 2018. Approximately two thirds of all ImpDis cases were, in 2014, patients with two classic and more common ImpDis, PWS and AS. There were no patients in Estonia with molecularly confirmed TNDM, CPP or MDS at the beginning of the study and most cases with other rare ImpDis, like PHP/PPHP, BWS and SRS, have been diagnosed during the study period.

The aim of this study was to find out as many patients with ImpDis as possible, to evaluate the prevalence of the most frequent ImpDis and all ImpDis together in Estonia, to compare the results with those of previously published studies, to describe unusual ImpDis cases, implement new molecular diagnostic methods and increase the awareness of physicians to improve diagnosis, treatment and care for people with ImpDis in Estonia.

2. LITERATURE REVIEW

2.1. Genomic imprinting

2.1.1. Overview

Humans, like other mammals, are diploid organisms, which means they have two matched sets of chromosomes in the cells, one inherited from the mother and one from the father. All nucleated nongametic human cells have 46 chromosomes, 23 from each parent. Each autosomal gene is therefore represented by two copies, or alleles, with one copy inherited from each parent at fertilization. In the vast majority of human genes, expression occurs from both alleles simultaneously. But there are also genes in the mammalian genome whose expression occurs from only one allele and the expression of the allele is dependent upon its parental origin. Some genes are expressed in this case only from the maternally inherited chromosomes and others from the paternally inherited chromosomes. This phenomenon got the name of genomic imprinting and functionally haploid genes with parent-of-origin dependent expression are called „imprinted“ [Barlow, and Bartolomei, 2014].

Genomic imprinting is a form of non-Mendelian inheritance. It affects both male and female offspring and is therefore a consequence of parental inheritance, not of sex. Imprinting mechanisms are usually gene-specific. For example, the imprinted *MKRN3* gene is normally active on a paternally inherited chromosome and will be active on the paternal chromosome and silent on the maternal chromosome in all males and females. Moreover, there is also a tissue-, isoform- and developmental stage-specific imprinting [Gregg, 2014]. For instance, the AS-causative *UBE3A* gene has imprinted maternal-specific expression only in the neurons and is biallelically expressed in all other cell types [Lopez *et al*., 2017]. It is also known that the imprint pattern in the placenta is drastically different from that of the embryo [Hanna *et al*., 2016; Monk, 2015].

2.1.2. Mechanisms of genomic imprinting

Genomic imprinting is an epigenetic process, meaning that it affects the way genes are expressed without changing the deoxyribonucleic acid (DNA) sequence itself. However, DNA sequence is important in the determination of the location of imprinted regions. The mechanisms for imprinting are very complex and still not completely understood. It is known that imprinting is defined by the effects of differentially methylated regions (DMRs) in the genome that direct the epigenetic regulation of imprinted domains. Nearly all imprinted genes have been associated with at least one DMR. DMRs are located at specific sites, called imprinting control regions (ICRs) [Barlow, and Bartolomei, 2014]. ICRs are *cis*-acting elements that regulate imprinting at

nearby genes, resulting in the formation of a single imprinted gene or, in most cases, an entire imprinted gene cluster. ICRs are often composed of repetitive DNA sequences and the removal of an ICR will usually result in a loss of imprinting. Maternal ICRs usually coincide with CpG island promoters located downstream of transcription start sites that are active during oocyte growth, while paternal ICRs have an intergenic location [Ferguson-Smith, and Bourc'his, 2018]. There are few possible epigenetic modifiers of gene expression: DNA methylation, histone modifications, expression of long non-coding ribonucleic acids (lncRNAs) and higher-order chromatin formation. Epigenetic modifiers can be identified by molecular analyses, and serve as markers of the parental origin of genomic regions. All the modifiers act within ICRs to establish and maintain the imprinted state [Gregg, 2014; Macdonald, 2012]. It is found that CCCTC-binding factor or CTCF, a transcription factor that in humans is encoded by the *CTCF* gene, has also an important role at some imprinted clusters, to regulate the expression of imprinted genes in a parental-origin-specific manner [Franco *et al*., 2014].

DNA methylation, a modification in mammals that covalently adds a methyl group ($-CH_3$) to the cytosine residue in the 5′-CpG-3′ dinucleotides, is the main epigenetic mechanism to be associated with genomic imprinting. DNA methylation in promoter or regulatory regions of imprinted genes results in the suppression of gene expression, whereas unmethylated genes remain active. Methyl groups directly prevent transcription factor binding and also lead to changes in chromatin structure that restrict access of transcription factors to the gene promoter. Methylation is acquired through the action of *de novo* methyltransferases (DNA methyltransferase 1, 3a and 3b), highly conserved enzymes that transfer methyl groups onto cytosine, and are maintained in situ each time the cell divides. DNA methylation is reversible and can be erased by demethyltransferases when it is time to establish a new mark [Li, and Zhang, 2014; Macdonald, 2012].

Histones are a family of basic proteins that bind tightly to DNA in the eukaryotic cell nucleus and help the DNA to condense into chromatin. Nuclear DNA is wrapped around nucleosomes, histone octamers composed of histones 2A (H2A), 2B (H2B), 3 (H3) and 4 (H4), forming the basic repeating unit of chromatin. Various epigenetic modifications of histones can affect chromatin conformation [Macdonald, 2012]. Histone acetylation, the addition of an acetyl group (CH_3CO-) to lysine residues in the N-terminal tail and on the surface of the nucelosome core of histone proteins, creates an accessible chromatin conformation (euchromatin) by changing the ionic charge of histone protein while histone deacetylation initiates a compressed chromatin state that promotes silencing and the formation of heterochromatin [Berger, 2002; Bannister, and Kouzarides, 2011].

Histone methylation, the addition of one, two, or three methyl groups ($-CH_3$) to histone lysine or arginine by histone methyltransferases, can either promote or repress gene expression, depending upon which amino acids of histone proteins are methylated. For instance, methylation of H3 at lysine 9, H4 at

lysine 20 and H3 at lysine 27 are silencing modifications, whereas methylation of H3 at lysine 4, H3 at lysine 48 and H3 at lysine 79 produces active chromatin [Cheung, and Lau, 2005; Greer, and Shi, 2012]. Regarding methylation of arginine residues, dimethylation of H3 at arginine 17 is an activation mark [Bauer *et al*., 2002], while dimethylation of H3 at arginine 2 is a signal for transcriptional silencing [Hyllus *et al*., 2007]. Unlike histone acetylation, methylation does not alter the charge of the histone protein, but rather functions as a docking site for the recruitment of specific chromodomain-containing proteins [Gayatri, and Bedford, 2014]. Besides the acetylation and methylation, other histone modifications, like phosphorylation (addition of a phosphate group), SUMOylation (addition of a small ubiquitin-like modifier protein) and ubiquitination (addition of a ubiquitin moiety), can also be involved in imprinting regulation [Barlow, and Bartolomei, 2014]. Histone modifications and DNA methylation are often intertwined, each can influence the other's recruitment and thus reinforce differential epigenetic states [Cedar, and Bergman, 2009].

RNA interference is a highly conserved post-transcriptional gene silencing mechanism in which double-stranded lncRNAs (>200 nucleotides), that is homologous in sequence to the silenced gene, neutralize complementary RNA transcripts through an RNA-induced silencing complex [Stanisławska, and Olszewski, 2005]. Based on their location, lncRNAs can be classified into intergenic, antisense, intronic and enhancer lncRNAs. All of them, with the exception of intronic lncRNAs, have been implicated in the expression of imprinted genes. Intergenic lncRNAs have been shown to modulate the levels of genomically neighboring or distal gene products through diverse molecular mechanisms. Enhancer lncRNAs regulate genomic imprinting by modulating replication timing and subnuclear positioning and antisense lncRNAs act as scaffolds to interact with and recruit chromatin-modifying machinery in a sequence-specific fashion [Marques, and Ponting, 2014; Kanduri, 2016]. Moreover, lncRNAs are found to be involved in the recruitment of DNA methyltransferases and other factors that facilitate higher-order chromatin structure [Zhao *et al*., 2016].

The transcriptional regulation of imprinted genes often involves the formation of a condensed higher-order chromatin structure, heterochromatin, that can spread in *cis* and generally impose transcriptional silencing by restricting the access of transcription factors and the transcriptional machinery to the DNA. Heterochromatic regions remain stable throughout development and are propagated through cell division. Heterochromatin protein 1 (HP1), a highly conserved non-histone chromatin protein, plays the central role in establishing and maintaining the heterochromatin state. HP1 is able to recruit other heterochromatic proteins and accessory factors, such as histone methyltransferases, to reinforce the structure of heterochromatin. HP1 can have activating as well as repressive function in gene expression [Grewal, and Elgin, 2002; Kellum, 2003; Macdonald, 2012]. Another molecular mechanism that appears to regulate the spreading of heterochromatin and thus the expression of imprinted genes are the Polycomb-group proteins (PcG). PcG repress gene expression and participate in

heterochromatin formation through methylation of histone H3 (H3 at lysine 27 and H3 at lysine 9) and ubiquitination of histone H2A [Golbabapour *et al*., 2013].

The establishment and maintenance of imprinted methylation marks is a complex process that involves a wide range of genetic factors. The parental allele-specific imprints are heritable to the daughter cells, but must be reset in each generation to establish parental specific epigenetic marks. It is now known that all imprinting marks are erased and reset in parental primordial germ cells. Imprints must then be reapplied during the following stages of gametogenesis and embryogenesis and subsequently maintained during the epigenetic reprogramming of the early embryo in a parent-of-origin-specific manner. Any error or defect in this process can result in the loss of imprinting. The differential imprinting in gametes correlates with differences in expression of the two alleles [Barlow, and Bartolomei, 2014]. Interestingly, in sperms methylation preferentially targets intergenic sequences and transposon repeats, whereas in oocytes methylation coincides with the body of actively transcribed genes, including intragenic CpG islands [Veselovska *et al*., 2015]. It is also known that epigenetic imprints may continue to evolve past 12 weeks of gestation and therefore can affect the results of prenatal diagnostics in the case of isolated methylation defect [Pozharny *et al*., 2010].

While parental imprinting pattern remains stable through somatic cell divisions, during gametogenesis and early embryo development, the pattern may be susceptible to the influence of environmental and *in vitro* conditions. It is considered that many environmental factors can affect the establishment of imprinting marks. So, maternal methyl-group donor-deficient or rich diet (decreased or increased consumption of methionine, folate, choline or betaine) during pregnancy can induce changes in the expression of some imprinted genes in the offspring. [Pauwels *et al*., 2017]. For example, the increased consumption of folic acid supplements by pregnant women after 12 weeks of gestation is linked to the increased methylation of the *IGF2* gene and decreased methylation of *PEG3*. However, the long-term effects of these expression changes on the health of offspring remain still unknown. [Haggarty *et al*., 2013]. Many studies have also found a link between impaired imprint acquisition and bad maternal habits (smoking, alcohol consumption), maternal food restriction and assisted reproductive technology [Kappil *et al*., 2015]. A positive association is found between the use of *in vitro* fertilization and the risk of some ImpDis, such as BWS, SRS, PWS and AS, in offspring [Okun *et al*., 2014; Johnson *et al*., 2018; Cortessis *et al*., 2018].

2.1.3. Imprinted genes

In humans, only 107 imprinted genes (<1% of the genome) and about 100 more genes predicted to be imprinted have now been discovered [Jirtle, 2018]. Most of these imprinted genes are grouped together in clusters, or domains, where

neighbouring maternally and paternally expressed genes are coordinately controlled by a single ICR, which possess parental-specific DNA methylation and/or histone modifications. In humans, a total of 25 ICRs have been identified, 22 of them are maternal ICRs and only three are paternal ICRs [Ferguson-Smith, and Bourc'his, 2018]. Imprinted clusters are distributed unevenly across the genome and usually contain 3–12 imprinted genes spanning over 80–3700 kilobases (kb) of genomic DNA. One imprinted cluster can contain both maternally and paternally expressed genes. The majority of genes in any one cluster are imprinted protein-coding messenger RNA genes; however, at least one is usually an imprinted lncRNA [Barlow, and Bartolomei, 2014].

The exact function of imprinted genes remains unknown. Although a majority of the known imprinted genes code for proteins, others code for untranslated RNA transcripts and can be important for the regulation of other genes. It has been found that most imprinted genes modulate fetal growth and resource acquisition. They are involved in organogenesis (including brain development), regulate the development of a normal, functioning placenta, affect both pre- and postnatal growth rate, and participate in energy homeostasis and resource allocation during pregnancy. Imprinted genes in the embryo and placenta influence maternal resource allocation by altering the transport of nutrients through the placenta, increasing or decreasing the intrinsic growth rate and signalling to the mother by the production of fetal or placental hormones that modify maternal behavior and metabolism [Cassidy, and Charalambous, 2018]. Paternally expressed imprinted genes function usually as growth promoters and show growth retardation in embryos deficient for expression of the genes. At the same time, many maternally expressed imprinted genes are growth repressors and cause a growth enhancement in embryos deficient for these genes. Thus, paternally expressed genes promote the extraction of maternal nutrients during pregnancy, whereas, maternally expressed genes try to limit it [Barlow, and Bartolomei, 2014].

2.1.4. Molecular basis of imprinting disorders

ImpDis disorders are conditions caused by genetic and epigenetic alterations resulting in aberrant expression or the dosage of imprinted genes. Certain ImpDis are associated with abnormalities on specific chromosomal loci, however, the same ImpDis can have multiple genetic and epigenetic etiologies, each with varying prevalence. Moreover, the phenotypic outcome depends on the parental allele affected by the alteration. The molecular causes of ImpDis are diverse. In general, four classes of molecular changes have been reported in the majority of ImpDis: copy number variations (CNVs), UPD, aberrant DNA methylation (epimutation) and genomic variants in imprinted genes (Figure 1). Isolated balanced chromosomal rearrangements can also rarely result in ImpDis. Furthermore, somatic and germline mosaicism of both genetic and epigenetic changes have been reported in some ImpDis. Somatic mosaicism can result in

atypical mild phenotype and is also associated with somatic asymmetry [Eggermann *et al*., 2015b; Grafodatskaya *et al*., 2017].

CNVs, deletions and duplications, involving imprinted regions either cause a loss of an expressed gene on that allele in the case of deletion or result in an overexpression of imprinted gene in the case of duplication. Small deletions of the ICR can also cause hypomethylation of an imprinted gene or cluster due to the removal of a negative cis-acting element, and therefore the loss of regulatory control of imprinting. CNVs can either occur *de novo*, or in some case they can be familial. In case of familial deletions or duplications, the imprinted monoallelic expression results in autosomal-dominant inheritance with a parent-of-origin-dependant phenotype [Eggermann *et al*., 2015b; Vals *et al*., 2015b]. CNVs that involve only the inactive, methylated allele of a single imprinted gene, as a rule, do not manifest phenotypically. However, the risk of ImpDis for the offspring can be up to 50% in this case and the risk depends on the sex of the CNV carrier. Imprinted clusters often contain both paternally and maternally expressed genes that are associated with several different ImpDis, and therefore larger deletions and duplications, which involve several imprinted genes or the whole imprinted cluster, can cause two clinically different ImpDis depending on their parental origin. For instance, paternal deletions of chromosomal region 15q11–q13 result in PWS, whereas maternal deletions of the same region result in AS. Thus, woman with deletion-caused PWS can theoretically give birth to a child with AS [Schulze *et al*., 2001]. In the case of large CNVs it is often difficult to precisely determine genes that cause the phenotype. In some cases, ImpDis are likely to be caused by polygenic gene dosage disruption [Cassidy, and Charalambous, 2018].

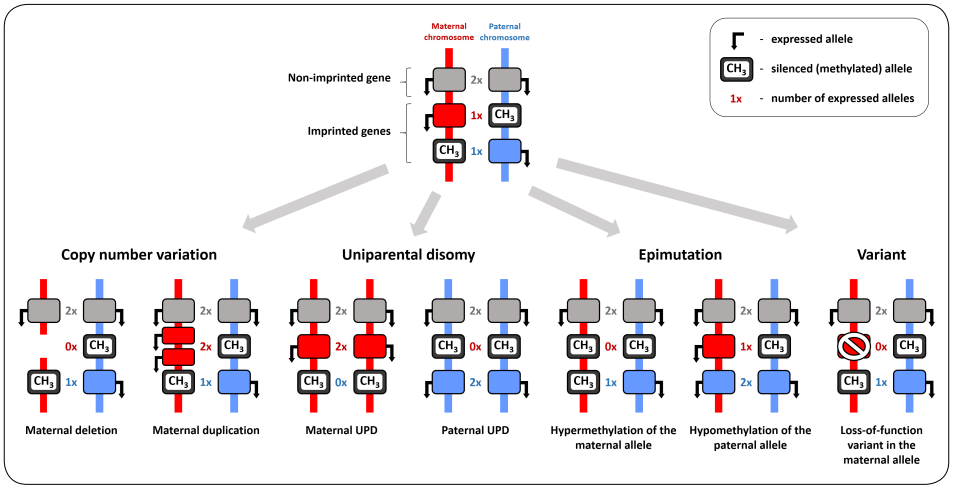

Figure 1: The four main classes of molecular changes, resulting in a disturbed expression of imprinted genes. Adapted from [Eggermann *et al*., 2015a].

UPD is the inheritance of both chromosomes in a pair from one parent with no functional copy from the other parent. Depending on parental origin, UPD can be paternal or maternal. The uniparentally inherited chromosomes can be identical (isodisomic UPD) or different (heterodisomic UPD) [Zilina *et al*., 2014a]. Isodisomic UPD is associated with the loss of heterozygosity and can, therefore, be accompanied by the risk of concomitant autosomal recessive disease. UPD can involve an entire chromosome or only one region of a chromosome (segmental UPD). In addition, uniparental chromosomes are frequently a mixture of isodisomic and heterodisomic segments due to the meiotic recombination. UPD can occur in combination with either chromosomally normal or abnormal cell lines [Yamazawa *et al*., 2010; Grafodatskaya *et al*., 2017]. When UPD occurs in the chromosomal region carrying an imprinted gene or cluster, the cells inherit either two active, expressed parental alleles or two silent, repressed parental alleles, leading to the abnormal dosage of the imprinted gene that can cause an ImpDis. UPDs have been reported in the majority of ImpDis. UPDs usually occur sporadically and the recurrence risk for offspring is generally low (<1%) with the exception of UPDs caused by familial Robertsonian translocations (usually chromosomes 14 and 15) [Eggermann *et al*., 2015b]. Interestingly, genome-wide UPDs, characterized by mosaic UPD involving several different chromosomes, have also been reported in a few individuals with complex and variable phenotypes [Inbar-Feigenberg *et al*., 2013; Kalish *et al*., 2013a].

An epimutation is an aberrant DNA methylation/histone modification pattern of a DMR without UPD or alteration of the same genomic DNA sequence. Epimutations account for approximately 50% of all the molecular changes in ImpDis. Epimutations include hypomethylation, loss of methylation (LOM) on the methylated allele, and hypermethylation or gain of methylation (GOM), an addition of methyl groups to the normally unmethylated allele of an imprinted gene. Hypo- or hypermethylation can affect several DMRs and thus influence the severity of some ImpDis [Eggermann *et al*., 2015b]. However, epimutations are typically isolated (primary epimutations) and have unclear etiology, there are also some molecular mechanisms that can cause an epimutation. Thus, small deletions or point variants in cis- or trans-acting ICRs or other regulatory domains, can result in the hypo- or hypermethylation of DMRs (secondary epimutation) [Finer *et al*., 2011; Czyz *et al*., 2012]. Primary epimutations often occur after fertilization during early embryogenesis and lead to somatic mosaicism. It has been estimated that primary epimutations are significantly more prevalent than somatic DNA variants and therefore their role in the pathogenesis of human diseases is probably underestimated. Primary epimutations usually imply very low risk of recurrence for both patient and parents, whereas secondary epimutations might have a 50% risk of recurrence [Horsthemke, 2006].

Genomic loss-of-function variants on the expressed allele of an imprinted gene directly affect the function, causing an ImpDis, whereas a variant in the silenced allele has no apparent effect. Point variants in protein-coding imprinted

genes have been reported only in some ImpDis. With the exception of PHP/PPHP, CPP, MDS and Birk–Barel syndrome, they account only for a small number of patients. Genomic variants are the only class of alterations in ImpDis which likely directly cause characteristic phenotypic features. Point variants can either occur *de novo* or be inherited from the parent, which could be affected or unaffected, depending which grandparent transmits the mutant allele. The risk of recurrence in the case of familial point variant is consistent with the imprinting status of the gene and might be 50% when transmitted from the parent contributing the expressed allele, otherwise, the recurrence risk is very small [Soellner *et al*., 2017].

In rare cases, ImpDis can be associated with microscopically visible balanced chromosomal rearrangements (translocations, insertions) that disrupt expression of imprinted genes but do not result in methylation alterations. Such balanced inversions and translocations of chromosomal region 11p15.5 have been described, for example, in some BWS cases. The mechanism by which these rearrangements generate the phenotype is unclear [Choufani *et al*., 2010]. Moreover, chromosomal translocations can predispose to both CMVs and UPDs. Thus, Robertsonian translocations involving acrocentric chromosomes and small supernumerary marker chromosomes can sometimes be observed in ImpDis cases caused by UPD [Liehr *et al*., 2011; Hoffmann, and Heller, 2011].

2.2. Imprinting disorders

To date, at least 13 ImpDis have been identified (Table 1) based on their distinct clinical presentation and association with molecular disturbances at specific imprinted loci. The majority of ImpDis show features belonging to common clinical groups: prenatal and/or postnatal growth retardation or overgrowth, hypo- or hyperglycemia, abnormal feeding behavior in early childhood and later, intellectual disability (ID), behavioral difficulties, precocious puberty. As the clinical features and molecular etiology of ImpDis are highly variable and overlapping, the diagnosis of these disorders is often difficult and sometimes challenging. It is assumed that a significant part of ImpDis cases remain undiagnosed. There are clinical scoring systems available for some ImpDis, which can be useful for diagnosis, but usually fail to detect patients with subtle or atypical clinical presentation. Furthermore, it is found that some patients meeting the clinical diagnostic criteria for a specific ImpDis carry a molecular alteration typically associated with another ImpDis [Soellner *et al*., 2017]. In addition, multilocus methylation defects (MLMDs), meaning a disturbed methylation at multiple imprinted loci, associated with variable phenotypes, have been reported in many ImpDis [Eggermann *et al*., 2011]. Several studies demonstrated that changes in the methylation of imprinted genes can also be associated with different types of cancer [Kim *et al*., 2015], autism [Loke *et al*., 2015], obesity [Soubry *et al*., 2015], polygenic diabetes [Mitchell, and Pollin, 2010] and Alzheimer's disease [Chaudhry *et al*., 2015].

Table 1: A list of known ImpDis, their acronyms, associated chromosomal regions and imprinted genes or clusters. Adapted from [Eggermann *et al*., 2011; Eggermann *et al*., 2015b; Eggermann *et al*., 2015a; Dagli *et al*., 2017; Driscoll *et al*., 2017; Rachad *et al*., 2017].

Imprinting disorder	Acronym	Chromosomal region	Imprinted gene or cluster
Prader-Willi syndrome	PWS	15q11–q13	*MKRN3, MAGEL2, NDN, SNURF-SNRPN, IPW,* snoRNA genes
Angelman syndrome	AS	15q11–q13	*UBE3A, ATP10A*
Beckwith-Wiedemann syndrome	BWS	11p15.5	IC1*(IGF2/H19),* IC2*(CDKN1C, KCNQ1OT1, KCNQ1)*
Silver-Russell syndrome	SRS	11p15.5, 7p12.1, 7q32.2	11p15.5: IC1 *(IGF2/H19),* IC2 *(CDKN1C, KCNQ1OT1, KCNQ1)* 7p12.1: *GRB10* 7q32.2: *PEG1/MEST*
Pseudohypoparathyroidism and pseudopseudo-hypoparathyroidism	PHP/PPHP	20q13.32	*GNAS*
Temple syndrome	TS14	14q32	*DLK1, MEG3, MEG8, RTL1*
Kagami-Ogata syndrome	KOS	14q32	*DLK1, MEG3, MEG8, RTL1*
Central precocious puberty	CPP	15q11.2	*MKRN3*
Transient neonatal diabetes mellitus	TNDM	6q24.2	*PLAGL1, HYMAI*
Myoclonus-dystonia syndrome	MDS	7q21.3	*SGCE*
Maternal uniparental disomy of chromosome 20	UPD(20)mat	chromosome 20	?
Schaaf-Yang syndrome	SYS	15q11.2	*MAGEL2*
Birk-Barel syndrome	–	8q24.3	*KCNK9*

SnoRNA – small nucleolar RNA; IC1 – imprinting centre 1; IC2 – imprinting centre 2

2.2.1. Prader-Willi syndrome

PWS was first clinically described in 1956 by Swiss doctors Andrea Prader, Alexis Labhart, and Heinrich Willi, as a result of their observations of nine patients with infantile muscular hypotonia, ID and obesity [Prader *et al*., 1956]. However, the main genetic cause of the syndrome was discovered only 25 years

later, in 1981, when Ledbetter and others used high resolution chromosome analysis to show that more than a half of the PWS patients they studied had an interstitial deletion in the chromosomal region 15q11–q13 [Ledbetter *et al*., 1981].

PWS is a complex neurodevelopmental genetic condition characterized by a range of mental and physical findings. Clinical presentation depends on the age of the patient. The clinical course of PWS has historically been divided into two distinct clinical stages (early failure-to-thrive and later childhood obesity). The first stage begins in pregnancy. The fetal PWS phenotype includes decreased fetal movement, polyhydramnion and an abnormal fetal position, which often requires an assisted delivery or a Cesarean section. Fetal size is generally within the normal range, though birth weight, length, and body mass index of infants with PWS are on average 15–20% less than in their unaffected siblings (although often still in the normal range) [Miller *et al*., 2011]. Severe hypotonia at birth, causing decreased movement, weak cry, poor reflexes, delayed psychomotor development, sucking and swallowing problems, is a nearly universal finding. Hypotonia is central in origin, and neuromuscular studies are generally normal. Infantile hypotonia and feeding difficulties result in failure to thrive in early infancy, often requiring naso-gastric feeding, gastrostomy tube placement or the use of special nipples for several weeks or months [Õiglane *et al*., 2002; Õiglane-Shlik *et al*., 2006b; Butler, 2011; Driscoll *et al*., 2017]. Hypotonia partially improves over time, but even adults remain mildly hypotonic with decreased muscle mass. Hypogonadism can also be noted during this stage. In both sexes, hypogonadism manifests as genital hypoplasia, incomplete pubertal development, and, in most cases, infertility [Driscoll *et al*., 2017].

The second clinical stage usually begins between the first and second years of age and is characterized by developmental delay (DD) and the onset of hyperphagia which leads to early-onset morbid obesity if not controlled. Obesity is a major factor influencing morbidity and mortality in PWS due to the absence of satiety, physical inactivity, a decreased metabolic rate, and an inability to vomit [Butler, 2011]. Delayed psychomotor development is present in almost all children with PWS. Early motor milestones are usually achieved at about double the normal age. Language development is typically delayed and speech articulation problems are also common. Most persons with PWS are mildly intellectually disabled, although some persons have low-normal intelligence or moderate ID. Most children and adults with PWS, regardless of their intellectual ability, have severe learning disabilities and personality problems (temper tantrums, depression, stubbornness, obsessive-compulsive disorder, intense preoccupation with food) [Whittington *et al*., 2004; Driscoll *et al*., 2017]. Characteristic dysmorphic facial features (a narrow forehead, almond-shaped eyes, a thin upper lip and down-turned mouth), as well as short stature, small hands and feet, are frequently observed. Other features of PWS include infertility, unmotivated sleepiness, decreased pain sensitivity, skin picking, periods of hypothermia, strabismus, hypopigmentation, scoliosis, sleep apnea, and dental anomalies [Butler, 2011]. Endocrine abnormalities such as hypothy-

roidism, growth and sex hormone deficiency, impaired glucose tolerance and central adrenal insufficiency are also reported in PWS [Heksch *et al*., 2017].

PWS arises from the lack of expression of paternally inherited genes (*MKRN3, MAGEL2, NDN, SNURF-SNRPN, IPW*) known to be imprinted and located in the 15q11–q13 region. There are three main classes of molecular abnormalities that lead to PWS: paternal 15q11–q13 deletion, maternal UPD of chromosome 15 (UPD(15)), and epimutations causing deficient expression of the paternally inherited imprinted genes on 15q11–q13. Approximately 65–75% of patients with PWS result from *de novo* interstitial deletion in the paternally derived chromosome 15q11–q13 region [Cheon, 2016]. Two common classes of deletions of the region have been described: larger type I deletion (40%), approximately 6.6 megabase (Mb) in size between breakpoint I and breakpoint III; and smaller type II deletion (60%), spanning 5.3 Mb between breakpoint II and breakpoint III [Butler *et al*., 2008]. There are four non-imprinted genes (*GCP5, CYFIP1, NIPA1, NIPA2*) located between breakpoint I and II that are affected by class I but not class II deletions, and individuals with type I deletions appear to have more behavioral problems and lower academic performance than individuals with the smaller type II deletions [Butler *et al*., 2004]. The second most frequent genetic finding in PWS is maternal UPD(15). It accounts for 20–30% of individuals with PWS. Most PWS patients have the heterodisomic form of UPD 15 [Angulo *et al*., 2015; Cheon, 2016]. UPD can also rarely be associated with small supernumerary chromosome 15 markers [Liehr *et al*., 2005]. The remaining PWS individuals (1–3%) result from imprinting defects, or epimutations. Approximately 15% of individuals with an epimutation have been found to have a small deletion (7.5 to >100 kb) in the PWS imprinting centre (IC) region located at the 5' end of the *SNRPN* gene and promoter [Buiting *et al*., 2003; Cheon, 2016]. In addition, several PWS cases caused by balanced chromosomal translocation involving chromosome 15 have been described in the literature [Sun *et al*., 1996; Conroy *et al*., 1997].

2.2.2. Angelman syndrome

AS is a sister syndrome to PWS, first described in 1965 by an English pediatrician, Dr. Harry Angelman, in three children with stiff, jerky gait, absent speech, excessive laughter and seizures [Angelman, 1965]. AS is a complex genetic disorder that primarily affects the nervous system. Newborns with AS typically have a normal phenotype, normal weight and head circumference. There is also usually no prenatal abnormalities or major birth defects in AS. However, some newborns with AS may have sucking difficulties and muscular hypotonia [Dagli *et al*., 2017]. One of the earliest distinctive features of AS may be persistent social smiling with excessive chortling or paroxysms of laughter beginning at 1–3 months. Mouthing behaviors, such as excessive chewing, drooling, tongue thrusting and protrusion, are also common in the AS children [Dagli *et al*., 2011].

DD in AS is usually evident by 6 to 12 months of age, with severely delayed attainment of gross motor, fine motor, receptive language, expressive language, and social skills. Most children with AS (>80%) also have delayed growth in head circumference, usually resulting in microcephaly by age of two years. Individuals with AS reach plateau at a developmental level between 24 and 30 months [Peters *et al*., 2004]. Cognitive and language development are usually severely delayed. Most individuals with AS lack speech entirely, however, rarely, some individuals are able to use single-words or phrases [Andersen *et al*., 2001]. Receptive language is typically superior to expressive language and the use of nonverbal communication systems (picture exchange cards, communication devices, modified sign language) is possible [Bird, 2014]. In addition, almost all patients with AS have behavioral abnormalities that include a happy demeanor, easily provoked laughter, short attention span, anxiety, hypermotoric and disruptive behavior, mouthing of objects, sleep disturbance with reduced need for sleep (sometimes as little as 5–6 hours per night) and abnormalities of the sleep–wake cycle, repetitive and stereotyped behaviour, and an affinity for water [Summers *et al*., 1995; Clarke, and Marston, 2000].

Seizures occur in 80–95% of children with AS and usually start before three years of age. Seizure types include myoclonic, generalized tonic–clonic, atypical absence, and atonic seizures. Many individuals exhibit multiple seizure types that require broad-spectrum anticonvulsants and often combination therapy [Pelc *et al*., 2008; Bird, 2014]. Electroencephalography typically demonstrates AS-specific combination of a very high amplitude rhythmic delta activity, diffuse high amplitude rhythmic theta activity, and posterior-predominant spike and sharp waves [Vendrame *et al*., 2012]. Movement disorders (jerkiness, ataxic gait, tremulous movement of limbs), abnormalities of tone (truncal hypotonia, distal extremity hypertonia or hyperreflexia), and impaired balance are also typical for AS [Bird, 2014].

However, children with AS are generally not dysmorphic as infants, some craniofacial features can develop with time, consisting of midface recession, prognathism, a wide mouth, wide-spaced teeth and a flat occiput. Patients with deletion-caused AS often have hypopigmentation of their skin, hair, and eyes. Other features of AS include strabismus, increased sensitivity to heat, abnormal food-related behaviors, obesity, scoliosis and constipation [Õiglane-Shlik *et al*., 2005; Williams *et al*., 2010; Dagli *et al*., 2011].

AS is caused by deficient expression of the maternal copy of the brain-only imprinted *UBE3A* gene located in the 15q11.2 region. AS can be caused due to one of four molecular etiologies: maternal deletion of the AS critical region on 15q11–q13, paternal UPD(15), epimutations causing lack of expression of the maternal copy of *UBE3A*, and variants in the maternally inherited copy of the *UBE3A* gene. A 5–7 Mb *de novo* deletions of maternal chromosome region 15q11–q13 accounts for approximately 70% of all AS cases [Bird, 2014; Eggermann *et al*., 2015a]. Typically, these deletions involve the same chromosomal region as in PWS and can be respectively divided into type I deletion (between breakpoint I and breakpoint III) and type II deletion (between breakpoint II and

breakpoint III). There is some suggestion that individuals with larger type I deletion (40% of deletion cases) may have more autistic traits, language and cognitive impairment than those with smaller type II deletion (50% of deletion cases) [Sahoo *et al*., 2006]. In rare cases (less than 10% of all AS deletions), individuals with AS syndrome can harbour larger deletions of up to 10.6 Mb, extending telomeric beyond breakpoint III [Sahoo *et al*., 2007]. There are also a few AS patients with complete or partial deletions of *UBE3A* described in the literature [Lawson-Yuen *et al*., 2006; Sato *et al*., 2007]. Around 1–7% of AS cases are attributable to paternal UPD(15) [Dagli *et al*., 2011; Buiting *et al*., 2016]. Individuals with paternal UPD tend to have a milder presentation with lower incidence of seizures [Lossie *et al*., 2001]. Epimutations causing deficient expression of the maternal copy of *UBE3A* are present in about 2–4% of AS individuals. Less than 10% of them have a secondary epimutation caused by small deletions that disrupt AS IC on maternal chromosome. But in the vast majority of patients, the imprinting defect represents a primary epimutation. Notably, over 40% of primary epimutations exhibit somatic mosaicism [Dagli *et al*., 2011; Buiting *et al*., 2016]. Variants in the *UBE3A* gene are found in about 10–15% of AS patients [Eggermann *et al*., 2015a]. The majority of *UBE3A* variants are premature stop variants, which in approximately 30% of cases are inherited from the mother and therefore associated with a 50% risk of recurrence [Buiting *et al*., 2016].

2.2.3. Beckwith-Wiedemann syndrome

BWS is the most common genetic overgrowth syndrome, described independently in 1963 and 1964 by Dr. J. Bruce Beckwith [B Beckwith, 1963], an American pediatric pathologist, and Dr. Hans-Rudolf Wiedemann [Wiedemann, 1964], a German geneticist. Since the findings in the 1990s of molecular alterations of chromosomal region 11p15.5 associated with BWS [Henry *et al*., 1991; Reik *et al*., 1995; Hatada *et al*., 1996], it has been recognized that various genetic and epigenetic changes in this region can result in very different clinical phenotypes. Therefore, BWS is considered a clinical spectrum, in which affected individuals may have many features or may have only one or two clinical features. Clinical phenotypes include classical BWS, isolated lateralized overgrowth and atypical BWS, which all are considered as part of BWS spectrum. As not all the patients with molecularly confirmed BWS display all usual phenotypic features of the disorder and some individuals with the classic clinical presentation of BWS do not have any molecular alteration in the 11p15.5 region, a consensus scoring system for the clinical diagnosis of BWS spectrum disorders was introduced by an international consensus of experts in 2018 [Brioude *et al*., 2018].

Clinical features of classical BWS include macroglossia, exomphalos, lateralized overgrowth, multifocal and/or bilateral Wilms tumour or nephroblastomatosis, prolonged hyperinsulinism (lasting >1 week and requiring

escalated treatment) and specific pathology findings (adrenal cortex cytomegaly, placental mesenchymal dysplasia, pancreatic adenomatosis), which all are cardinal features (2 points per feature) in the consensus scoring system. Suggestive features (1 points per feature) include a birth weight greater than 2 standard deviations (SD), facial naevus flammeus, polyhydramnios and/or placentomegaly, ear creases and/or pits, transient hypoglycaemia (lasting <1 week), typical embryonal tumours (neuroblastoma, rhabdomyosarcoma, unilateral Wilms tumour, hepatoblastoma, adrenocortical carcinoma, phaeochromocytoma), nephromegaly and/or hepatomegaly and umbilical hernias and/or diastasis recti. For a clinical diagnosis of classical BWS, a patient requires a score of ≥4. Patients with a score of ≥2 need genetic testing for investigation of BWS [Brioude *et al*., 2018].

However, prenatal and postnatal overgrowth was traditionally considered as one of the main features of BWS, it is now known that overgrowth occurs in only 43–65% of patients with molecular defect in the 11p15.5 region [Mussa *et al*., 2016b]. Therefore, overgrowth can only be a suggestive feature of BWS, otherwise it can lead to misdiagnosis in patients with normal anthropometric measurements. Embryonal tumours occur in ~8% of children with BWS spectrum [Mussa *et al*., 2016a] and the tumour risk correlates with the type of causative molecular abnormality [Cooper *et al*., 2005]. The overall tumour risk is the highest in the first two years of life, and the risk then declines progressively before puberty, approaching the cancer risk of the general population [Brioude *et al*., 2018]. Other symptoms like premature birth, long umbilical cord, cleft palate, polydactyly, supernumerary nipples, dysmorphic facial features (infraorbital folds, midfacial hypoplasia, prognathia), hemangiomas, renal medullary dysplasia, nephrocalcinosis, medullary sponge kidney, hypercalciuria, cardiomegaly, hypertrophic cardiomyopathy, hearing loss, hypothyroidism, hyperlipidemia, hypercholesterolemia, polycythemia, and subfertility in males are also observed in some individuals with BWS. Psychomotor and mental development is usually normal unless there is a concomitant chromosomal abnormality, brain malformation, or history of hypoxia or significant untreated hypoglycemia [Shuman *et al*., 2016; Cammarata-Scalisi *et al*., 2018].

Isolated lateralized overgrowth, previously called isolated hemihypertrophy or hemihyperplasia, is defined as a marked increase in the length and/or girth of most or all of one side of the body compared with its contralateral side. When it occurs with an 11p15.5 abnormality, it is considered a part of BWS spectrum. Lateralized overgrowth can generally be appreciated at birth, but may become more or less evident as the child grows. The most serious sign of the condition is leg length discrepancy (LLD) that may require orthopedic or surgical correction. Children with only isolated lateralized overgrowth still have a significantly increased risk of developing embryonal tumors and, therefore, need to be screened [Shuman *et al*., 2016; Brioude *et al*., 2018].

Patients with atypical BWS are individuals with BWS-specific genetic or epigenetic changes at the 11p15.5 locus who do not demonstrate an isolated lateralized overgrowth and have fewer cardinal and suggestive features than

those needed for a clinical diagnosis of BWS. Atypical BWS is a part of BWS spectrum and requires respective clinical management [Brioude *et al*., 2018].

BWS spectrum disorders are caused by a variety of genetic and epigenetic alterations that affect the expression of a cluster of imprinted genes located within the chromosome region 11p15.5. This locus is divided into two functionally independent domains: the centromeric (*CDKN1C, KCNQ1OT1*) and telomeric domains (*IGF2, H19*). Each domain harbours its own imprinting control region: *H19/IGF2*:IG DMR or imprinting centre 1 (IC1) in the telomeric domain, and *KCNQ1OT1*:TSS DMR or imprinting centre 2 (IC2) in the centromeric domain. Approximately 80% of patients with BWS spectrum disorders demonstrate a molecular defect affecting expression of imprinted genes in the BWS region, with epimutation being the most frequent aberration [Choufani *et al*., 2013]. LOM (hypomethylation) on the maternal IC2 allele is found in approximately 50% of patients and GOM (hypermethylation) on the maternal IC1 allele in 5–10% of patients. LOM at maternal IC2 occurs in most cases as an epigenetic change without an underlying genomic alteration, while GOM at maternal IC1 can occur in association with IC1 variant or CNV. Epimutations are often present in a mosaic form. Paternal UPD of 11p15.5 can be detected in 20% of BWS spectrum patients [Brioude *et al*., 2018]. UPD 11p15.5 usually encompasses both imprinted gene clusters, although the extent of UPD varies in different patients. UPD usually involve all or part of the short arm and can extend to the long arm of chromosome 11. The complete paternal UPD of chromosome 11 is very rare. Interestingly, the vast majority of BWS cases with segmental paternal UPD 11p15.5 demonstrate somatic mosaicism [Choufani *et al*., 2013]. Intragenic variants on the maternal allele of *CDKN1C* gene are detected in 5% of sporadic and 40% of familial BWS cases. *CDKN1C* variants reported in BWS are either missense variants or nonsense variants, both of which result in loss-of-function and increased cell proliferation due to the loss of cell cycle inhibition. In addition, chromosomal abnormalities (duplications of the paternally derived 11p15.5, translocations and inversions of the maternally derived chromosome 11) can be detected in <5% of patients. Molecular diagnosis is not reached in up to 20% of patients with symptoms of BWS spectrum disorder [Choufani *et al*., 2013; Eggermann *et al*., 2016; Brioude *et al*., 2018].

An important genotype-phenotype correlation has been established in BWS. Thus, paternal UPD of 11p15 and GOM at IC1 are associated with the highest risk of malignancies. Lateralized overgrowth is most commonly associated with the mosaic paternal UPD of 11p15 and aberrant methylation, but it is very rare in patients with a *CDKN1C* variant. Omphalocele is primarily associated with epimutations at IC2 and *CDKN1C* variants [Mussa *et al*., 2016c].

2.2.4. Silver-Russell syndrome

SRS is a rare growth disorder, originally described by Silver and colleagues in 1953 [Silver *et al*., 1953] and, soon afterwards, by Russell in 1954 [Russell, 1954], although the genetic etiology of the syndrome was not discovered until four decades after the first clinical description. The clinical presentation and molecular etiology of the syndrome is, as in the case of BWS, very variable and heterogeneous that makes the diagnosis difficult and often challenging. Therefore, a consensus statement for the diagnosis and management of SRS was developed by international expert consensus in 2017 [Wakeling *et al*., 2017].

SRS is characterized by severe intrauterine growth retardation (IUGR) (birth weight and/or birth length ≤−2 SD for gestational age), postnatal growth failure (height at 24 ± 1 months ≤−2 SD or height ≤−2 SD below mid-parental target height), relative macrocephaly at birth (head circumference at birth ≥1.5 SD above birth weight and/or length SD), protruding forehead (forehead projecting beyond the facial plane on a side view as a toddler), body asymmetry (LLD of ≥0.5 cm or arm asymmetry or LLD <0.5 cm with at least two other asymmetrical body parts), feeding difficulties and/or low body mass index (body mass index ≤−2 SD at 24 months or current use of a feeding tube or cyproheptadine for appetite stimulation). All these features are criterias of the revised Netchine-Harbison clinical score system (NH-CSS) [Netchine *et al*., 2007; Azzi *et al*., 2015] recommended by international consensus of experts for the clinical diagnosis of SRS. If all molecular tests are normal and differential diagnoses have been ruled out, patients scoring at least four of six criteria, including both prominent forehead and relative macrocephaly should be diagnosed as clinical SRS. Molecular testing for SRS is recommended in patients with a score of ≥3 [Wakeling *et al*., 2017].

Other clinical features of SRS include clinodactyly of the fifth finger, dysmorphic facial features (triangular face, down-turned corners of the mouth, micrognathia, blue tinge to the whites of the eyes in children, low-set and/or posteriorly rotated ears), low muscle mass, hypoglycemia, excessive sweating, delayed bone age, wide and late-closing fontanelle, brachydactyly, camptodactyly, second–third toe syndactyly, shoulder dimples, high pitched or squeaky voice, prominent heels, growth hormone deficiency, scoliosis, kyphosis, hearing loss, café au lait spots and other skin pigmentary changes [Saal *et al*., 2011; Wakeling *et al*., 2017]. Although most individuals with SRS have severe pre- and postnatal growth retardation, the growth is usually proportionate and growth velocity is normal. SRS patients do not experience the catch-up growth and the growth usually remains parallel to the growth chart curves but below the –2 SD line. Without growth hormone therapy, the average adult height is 151.2 cm (–7.8 SD) in males and 139.9 cm (–9 SD) in females [Wollmann *et al*., 1995]. Although psychomotor development and intelligence is normal in the majority of patients with SRS, there is an increased risk of motor and/or cognitive DD and learning disabilities [Noeker, and Wollmann, 2004]. Children with SRS have little subcutaneous fat, reduced body mass, and often have poor

appetites. Therefore, they are at risk of spontaneous or fasting hypoglycemia [Azcona, and Stanhope, 2005]. Gastrointestinal problems are also common and may include gastroesophageal reflux disease, esophagitis, food aversion, and constipation. Some individuals with SRS have birth defects like cleft palate, hypospadia and cryptorchidism [Saal *et al*., 2011].

The diagnosis of SRS is primarily based on the identification of consistent clinical features. However, it is known that similarly to other clinical scoring systems, the NH-CSS has high sensitivity (98%), but the specificity is low (36%) [Azzi *et al*., 2015], which could result in false-positive results when the diagnosis is just based on clinical findings. The identification of the precise molecular cause of SRS is also relevant for both appropriate clinical management as well as for genetic counselling. Although it is known that molecular testing confirm the diagnosis in only around 60% of patients with the clinical presentation of SRS [Netchine *et al*., 2007] and some persons with molecularly confirmed diagnosis lack typical SRS features or have a more subtle presentation [Eggermann *et al*., 2009].

SRS is the only ImpDis that is associated with abnormalities of imprinted clusters located in two different chromosomes, chromosomes 7 and 11. Both SRS and BWS share the same ICs, and imprinted centromeric (*CDKN1C, KCNQ1OT1*) and telomeric domains (*IGF2, H19*) on chromosomal region 11p15.5, but in SRS the expression of these genes is affected in an opposite manner. Most patients with the classical SRS phenotype carry molecular changes in 11p15.5 [Gicquel *et al*., 2005; Bartholdi *et al*., 2009]. LOM on the paternal IC1 allele accounts for 40–60% of patients with clinical SRS diagnosis. IC1 LOM results in reduced paternal *IGF2* expression and increased maternal *H19* expression, which leads to growth restriction. Most patients with IC1 LOM display mosaicism, consistent with a postzygotic event [Azzi *et al*., 2014]. About 1% of patients carry CNVs affecting the region 11p15.5. These CNVs are mostly maternal duplications involving the centromeric domain and the *CDKN1C* gene [Begemann *et al*., 2012b; Vals *et al*., 2015b]. Rare paternal deletions of enhancers in the telomeric domain, leading to lower levels of *IGF2* expression, have been also described [Gronskov *et al*., 2011]. The phenotype of patients with CNVs is influenced by the size and gene content of the aberration. Maternal UPD of chromosome 11, LOM at both IC1 and IC2, paternally inherited loss-of-function variants in the 11p15.5-encoded gene *IGF2* and maternally inherited gain-of-function variants in the imprinted gene *CDKN1C* have been described in only a few individuals [Bullman *et al*., 2008; Begemann *et al*., 2011; Begemann *et al*., 2015; Brioude *et al*., 2013]. Between 4% and 10% of SRS patients carry the complete or segmental maternal UPD of chromosome 7 (UPD(7)). In single cases chromosomal imbalances and epimutations affecting either the *GRB10* gene in 7p12.1 or the *MEST* gene in 7q32.2 have been reported [Kagami *et al*., 2007; Eggermann *et al*., 2012; Eggermann *et al*., 2014b; Carrera *et al*., 2016]. Moreover, in a considerable number of patients with clinically diagnosed SRS, alterations of chromosomes other than 7 and 11 may be detected. For instance, some SRS patients exhibit 14q32 abnormalities

[Geoffron *et al*., 2018] or maternal UPD of chromosomes 16 and 20, however, their clinical findings do not always fit the clinical scoring [Sachwitz *et al*., 2016; Kawashima *et al*., 2018a; Inoue *et al*., 2019]. SRS usually occurs sporadically, but some familial cases have been described [Õunap *et al*., 2004].

There is low genotype-phenotype correlation in SRS and different (epi)genotypes are generally clinically indistinguishable. However, it is known that patients with LOM at IC1 tend to have a lower birth length and weight, more frequent body asymmetry and congenital anomalies. Neurocognitive problems are more frequent in patients with UPD(7), and patients with CNVs affecting the region 11p15.5 usually do not have body asymmetry, but are at increased risk of DD and hearing loss [Wakeling *et al*., 2010; Azzi *et al*., 2015; Wakeling *et al*., 2017].

2.2.5. *GNAS*-gene-related imprinting disorders

In 1942, American endocrinologist, Dr. Fuller Albright, along with colleagues, described patients with combined presence of severe hypocalcemia and hyperphosphatemia, which are characteristic of hypoparathyroidism, in whom paradoxical elevation of serum parathyroid hormone (PTH) upon normal renal function have been found. This condition has been called PHP [Albright *et al*., 1942]. It was also noted that many patients with PHP had features of previously described Albright hereditary osteodystrophy (AHO), a complex of symptoms consisting of short stature, early-onset obesity, a round face, ectopic calcifications, subcutaneous ossifications, brachydactyly (short metacarpal and metatarsal bones) and sometimes neurodevelopmental abnormalities. Ten years later, in 1952, individuals with phenotypic appearance of PHP, but with unexpectedly normal levels of serum electrolytes and PTH, were described by Dr. Albright as patients with PPHP [Albright *et al*., 1952]. Decades later it was discovered that both PHP and PPHP are caused by genetic or epigenetic alterations in the complex imprinted *GNAS* locus, encoding the alpha-subunit of the stimulatory G protein ($G_s\alpha$) and several other transcripts [Cianferotti, and Brandi, 2018].

The classification of PHP is traditionally divided into PHP type 1A (PHP1A), PHP type 1B (PHP1B), PHP type 1C (PHP1C), PHP type 2 (PHP2) and PPHP according to the presence or absence of AHO, together with an *in vivo* response to PTH infusion and the measurement of $G_s\alpha$ protein activity in peripheral erythrocyte membranes *in vitro*. However, this classification is imprecise and fails to differentiate all patients with different and overlaping clinical and molecular findings, and, therefore, new nomenclature of PHP was proposed by the EuroPHP network. According to the new nomenclature, all disorders of the PTH receptor and its signaling pathway should be named „inactivating PTH/PTH-related protein signaling disorders“ (iPPSD) and can be divided into subtypes – iPPSD1 to iPPSD6, of which only iPPSD2 and iPPSD3 are associated with imprinted *GNAS* gene. iPPSD2 include disorders caused by

inactivating *GNAS* variants (PHP1A, PHP1C and PPHP) and iPPSD3 are caused by LOM at *GNAS* DMRs (PHP1B) [Turan, 2017].

2.2.5.1. Pseudohypoparathyroidism

PHP1A is characterized by the association of resistance to multiple hormones, including PTH and thyroid stimulating hormone (TSH), features of AHO and decreased $G_s\alpha$ activity in erythrocyte membranes. PTH resistance is defined as elevated functionally intact PTH with or without hypocalcemia and hyperphosphatemia. It is caused by a defect in the hormone-sensitive signal transduction pathway that activates adenylyl cyclase in renal proximal tubules. PTH resistance is usually absent at birth and develops during childhood (from 0.2 years to 22 years), while the resultant changes in serum levels of calcium and phosphorus occur later and develop gradually. Signs of severe hypocalcemia and hyperphosphatemia caused by PTH resistance may include muscle spasms or crams, tetany, lethargy, generalized seizures, rash, bone and joint pain. TSH resistance manifests as elevated serum TSH level in the presence of normal or slightly reduced thyroid hormone levels and is often present at birth that can lead to the misdiagnosis of congenital hypothyroidism. Resistance to gonadotropins, glucagon, adrenaline, calcitonin and growth hormone releasing hormone, whose receptors interact with Gs to stimulate adenylyl cyclase, is also possible. Obesity may develop already in early infancy. Intrauterine and postnatal growth retardation resulting in short final height is also a common finding in PHP1A. Brachydactyly usually develops over time and is characterized by variable shortening of the fifth, fourth and third metacarpals with shortened first and fourth distal phalanges. Metatarsals are often shortened as well. Only part of patients have subcutaneous ossifications, though it is a highly suggestive feature of PHP1A. Cognitive impairment presents in about 70% of patients with PHP1A and can be of very variable severity [Mouallem *et al*., 2008; Mantovani *et al*., 2018].

PHP1C has been defined as the association of all the features of PHP1A, but with normal $G_s\alpha$ activity in cell membranes *in vitro*. As the measurement of $G_s\alpha$ activity is not readily available, the distinction between PHP1A and PHP1C is not usually possible, and, therefore, PHP1C is often referred to as subgroup of PHP1A [Tafaj, and Juppner, 2017].

PHP1B was initially defined as isolated resistance to PTH, absence of AHO features and normal levels of $G_s\alpha$ activity. However, later, many studies demonstrated that some patients with PHP1B display features of AHO. Moreover, mildly decreased $G_s\alpha$ activity has been described in some PHP1B cases [Zazo *et al*., 2011]. As in PHP1A, PTH resistance might not be present at birth and develops only over time. Despite identical molecular changes, patients with PHP1B might show variable degrees of PTH-resistant hypocalcaemia or normocalcaemia. TSH resistance is also possible. It is found that TSH levels are at the high end of normal or mildly elevated in 30–100% of patients with

PHP1B [Molinaro *et al*., 2015; Mantovani *et al*., 2018]. Brachydactyly is present in 15–33% of PHP1B cases. Macrosomia, early-onset obesity and subcutaneous ossifications have also been described [de Nanclares *et al*., 2007; Mantovani *et al*., 2010; de Lange *et al*., 2016].

PHP2 is characterized by an increase in levels of cyclic adenosine monophosphate (cAMP) in response to exogenous PTH infusion but a deficient phosphaturic response. The exact molecular cause of this PHP variant is still unknown. It has been suspected that PHP2 could either be an acquired defect secondary to vitamin D deficiency or be due to defective signalling downstream of $G_s\alpha$ [Rao *et al*., 1985; Mantovani *et al*., 2018].

PHP is caused by alterations within or upstream of the *GNAS* locus on chromosome 20q13.32. *GNAS* is a complex imprinted locus that shows differential methylation at four DMRs: one paternally methylated DMR (*GNAS-NESP*:TSS-DMR) and three maternally methylated DMRs (*GNAS-AS1*:TSS-DMR, *GNAS-XL*:Ex1-DMR and *GNAS A/B*:TSS-DMR) [Monk *et al*., 2018]. The *GNAS* locus shows biallelic expression in most studied tissues, whereas primarily maternal expression is observed in renal proximal tubules, thyroid, pituitary and ovary tissues [Mantovani *et al*., 2002; Liu *et al*., 2003]. As the measurement of $G_s\alpha$ activity in erythrocyte membranes is not usually available in clinical practice and the clinical features of different PHP types are very variable and overlapping with each other and other health conditions, molecular genetic testing of the *GNAS* locus has become the gold standard for diagnosis and distinguishing of PHP variants. PHP1A is caused by inactivating genetic pathogenic variants (point variants or rare gene rearrangements) on the maternal allele of the *GNAS* gene within exons 1–13, which code for Gsα. Point variants can be either maternally inherited or *de novo*, with both types having similar incidences. Individuals with PHP1B show epigenetic alterations in the DMRs associated with the *GNAS* locus. A methylation defect can be classified as partial or complete and can affect one or multiple DMRs within *GNAS*. LOM at *GNAS A/B*:TSS-DMR is detected in all patients with PHP1B [Bastepe *et al*., 2001; de Sanctis *et al*., 2016]. Approximately 15–20% of all PHP1B cases are familial and inherited through the maternal line with an autosomal dominant pattern of inheritance. Most patients with familial PHP1B demonstrate LOM at *GNAS A/B*:TSS-DMR which is caused by a 3 kb microdeletion on the maternal allele of cis-acting control elements within *STX16* [Bastepe *et al*., 2003]. Sporadic PHP1B cases usually show epigenetic alterations in two or more DMRs, in addition to *GNAS A/B*:TSS-DMR. In 8–10% of these sporadic cases, the methylation defects are caused by the segmental or complete paternal UPD of chromosome 20 [Elli *et al*., 2016; Mantovani *et al*., 2018]. PHP1C can be caused by maternal loss-of-function variants in the carboxyl-terminus of *GNAS* or methylation defects in *GNAS* DMRs, thus molecularly mimicking both the PHP1A and PHP1B [Brix *et al*., 2014; Elli *et al*., 2016].

2.2.5.2. Pseudopseudohypoparathyroidism

Patients with PPHP have typical features of AHO and decreased $G_s\alpha$ activity in cell membranes but do not have PTH resistance. In some PPHP patients, however, mild resistance to PTH and TSH has been described [Turan *et al*., 2015]. Generally, clinical manifestation of PPHP differ from those of PHP1A only in absence of hormonal resistance and electrolyte abnormalities. As PHP1A, the PPHP is caused by inactivating variants (loss-of-function variants or deletions) in the *GNAS* gene within exons 1–13. But in the case of PPHP these alterations involve the paternal allele of the *GNAS* locus. Therefore, the same variant of *GNAS* often occurs within the same family, but manifests as PPHP in the case of paternal inheritance and as PHP1A if inherited from the mother. Both male and female offspring of a female with PPHP has a 50% risk of developing PHP1A [Mantovani *et al*., 2018].

2.2.6. Temple syndrome

TS14 is a rare ImpDis, first described in 1991 by Prof. Karen Temple, a British clinical geneticist and researcher, in a young male with a balanced Robertsonian translocation (13; 14) and maternal uniparental disomy of chromosome 14 [Temple *et al*., 1991]. TS14 is characterized by prenatal and postnatal growth retardation, congenital muscular hypotonia, feeding difficulties in the neonatal period, motor and mental DD, scoliosis, premature puberty, truncal obesity, short adult stature, small feet and hands as well as some nonspecific dysmorphic facial features such as almond-shaped eyes, broad nasal tip, micrognathia, high palate, short philtrum, and a high forehead [Ioannides *et al*., 2014; Severi *et al*., 2016]. Birth length, birth weight and postnatal height are below −2 SD in more than 80% of individuals with TS14. Premature birth and placental hypoplasia are common. Motor development is delayed in most patients that is consistent with a high frequency of marked muscular hypotonia. Intellect can be reduced or normal, although some patients with normal IQ demonstrate neurocognitive or emotional problems. ID is present in about 17% of patients with TS14 and is usually mild to moderate. Most patients (>70%) show gonadotropin-dependent central precocious puberty with rapid bone age progression. Mean age of menarche is approximately 10 years of age [Ioannides *et al*., 2014]. It is also notable that many TS14 patients demonstrate a SRS-like relative macrocephaly and prominent forehead in early childhood, but such appearance became obscure with age. Other features found in patients with TS14 include clinodactyly, simian crease, joint hypermobility, body asymmetry, undermasculinized male genitalia and type 2 diabetes mellitus [Kagami *et al*., 2017b].

TS14 is caused by abnormal expression of imprinted genes at the chromosomal locus 14q32. This region harbours the paternally expressed genes *DLK1* and *RTL1* as well as maternally expressed genes *MEG3*, *RTL1as*, *MEG8*, a small nucleolar RNA and microRNA gene cluster. This imprinted locus

includes three DMRs: *MEG3-DLK1*:IG-DMR and *MEG3*:TSS-DMR, that are methylated on the paternal allele and act as IC; and *MEG8*:Int2-DMR, that is methylated on the maternal allele [Beygo *et al*., 2017]. Maternal UPD of chromosome 14 (UPD(14)mat) is the leading molecular cause of TS14 found in about 70–78% of cases. UPD(14)mat may occur as isodisomy, heterodisomy, or as a mixture of both. Most patients with UPD(14)mat also have concomitant balanced Robertsonian translocation involving chromosome 14 or extra structurally abnormal chromosomes. Isolated epimutations (LOM at *MEG3* and *MEG8*; GOM at *DLK1* and *RTL1*) and paternal deletions of the imprinted locus 14q32 could be found in 12–20% and 10% of TS14 cases, respectively [Ioannides *et al*., 2014; Kagami *et al*., 2017b; Kagami *et al*., 2017b]. In several cases, a combination of UPD(14)mat and mosaic trisomy of chromosome 14 associated with atypical and unusually severe phenotype, has been reported [Yakoreva *et al*., 2018].

The absence of specific features makes TS14 underdiagnosed in clinical practice. Moreover, clinical presentation overlap considerably between TS14 and other ImpDis. Thus, UPD(14)mat is identified in some patients with clinical diagnosis of SRS [Goto *et al*., 2016; Luk, 2016] and in approximately 2–6% of patients with clinical suspicion of PWS [Hosoki *et al*., 2009; Lande *et al*., 2018]. The diagnosis of TS14 is now based primarily on genetic rather than clinical findings. Genetic testing for TS14 should therefore be considered for children with PWS- and SRS-like phenotype [Goto *et al*., 2016].

2.2.7. Kagami-Ogata syndrome

KOS, previously called Wang syndrome, is a rare ImpDis, first time reported in 1991 by Dr. Wang in a 9-year-old mentally retarded girl with multiple congenital anomalies and balanced Robertsonian translocation (13; 14) [Wang *et al*., 1991]. KOS is clinically characterized by placentomegaly, polyhydramnios, small bellshaped thorax with coat-hanger appearance of the ribs, abdominal wall defects, unique facial appearance and ID. Polyhydramnios with associated placentomegaly, fetal thoracic and abdominal abnormalities are present in most KOS cases, and can be found by ultrasound studies from 25 weeks of gestation. Premature birth and need for cesarean delivery are also common. Birth weight is often high, although birth length is usually normal. In contrast, postnatal growth failure is observed in about one-third of patients with KOS. Almost all patients demonstrate unusual small bell-shaped thorax, abdominal wall defects (omphalocele or diastasis recti) and specific facial dysmorphism consisting of full cheeks, protruding philtrum, depressed nasal bridge, micrognathia, and short webbed neck. Because of thoracic abnormalities, respiratory problems are common, being the main cause of infantile death. Child mortality can be as high as 30% [Ogata, and Kagami, 2016]. About one-third of infants with KOS need a mechanical ventilation and/or tracheostomy. DD and/or ID of variable degree is present in all patients. KOS is also associated with feeding difficulties (poor

sucking, impaired swallowing, gastric tube feeding), laryngomalacia and elevated risk of hepatoblastoma. Specific dysmorphic facial appearance and distinctive chest roentgenograms constitute the pathognomonic features of KOS through childhood and puberty [Kagami *et al*., 2015; Ogata, and Kagami, 2016].

Both KOS and TS14 are caused by abnormal expression of the same imprinted genes on chromosomes 14q32. The molecular causes of KOS include UPD of chromosome 14 (UPD(14)pat), isolated epimutations and maternal deletions affecting the chromosomal region 14q32. UPD(14)pat accounts for about 70% of KOS patients and can be associated with Robertsonian translocation (13; 14) or isochromosome (14q) [Ogata, and Kagami, 2016]. To date, mosaic UPD(14)pat has been reported in only one patient with mild KOS phenotype [Haug *et al*., 2018]. Both isolated epimutations (LOM at *DLK1* and *RTL1*; GOM at *MEG3* and *MEG8*) and maternal deletions of the imprinted locus 14q32 are found in about 15% of KOS patients. No KOS cases with a single gene variant or a duplication of the paternally derived 14q32.2 imprinted region have been reported [Ogata, and Kagami, 2016].

2.2.8. Central precocious puberty

MKRN3 gene-related CPP is a newly identified ImpDis, first time detected through whole-exome sequencing in 2013 by Dr. Abreu in 5 of 15 families with familial idiopathic CPP. CPP is defined as the development of secondary sex characteristics before eight years of age in girls and nine years of age in boys, due to premature reactivation of the hypothalamic-pituitary-gonadal axis. CPP is usually regarded to be idiopathic, although approximately 27.5% of all idiopathic CPP cases are familial [de Vries *et al*., 2004]. Alterations in the imprinted *MKRN3* gene were shown to be the main cause of familial idiopathic CPP. Different studies show that loss-of-function variants of the *MKRN3* gene can be found in about 3–10% of all female idiopathic CPP cases [Macedo *et al*., 2014; Lee *et al*., 2016; Nishioka *et al*., 2017] and in up to 40% of male idiopathic CPP cases [Bessa *et al*., 2017].

MKRN3 variants affect both genders equally. All patients with CPP due to *MKRN3* defects show the typical clinical and hormonal finding of CPP, including breast or testicular enlargement, advanced bone age, accelerated growth velocity, and stimulated luteinizing hormone levels elevation. Very few patients with *MKRN3* variants have mild nonspecific dysmorphic features [Abreu *et al*., 2015]. The median age of puberty onset is 6.0 years in girls (ranging from 3.0 to 7.5 years) and 8.25 years in boys (ranging from 5.9 to 9.0 years) [Abreu *et al*., 2013; de Vries *et al*., 2014; Schreiner *et al*., 2014; Settas *et al*., 2014], suggesting that *MKRN3* variants may affect girls more severely than boys. Two asymptomatic males with paternally inherited pathogenic *MKRN3* variants have also been described in the literature [Dimitrova-Mladenova *et al*., 2016], however, this result can be accounted for by poorly studied phenotype of

the patients [Brito, and Latronico, 2017]. CPP due to *MKRN3* variants is not known to cause developmental problems or other health issues, although the risk of breast cancer and cardiovascular disease can be elevated in untreated females due to effect of estrogen [Lakshman *et al*., 2009].

MKRN3 is a maternally imprinted gene, located at 15q11.2 in the PWS critical region. *MKRN3* encodes a makorin ring-finger protein 3, which is thought to be an upstream suppressor of the hypothalamic-pituitary-gonadal axis, and therefore *MKRN3* deficiency leads to the withdrawal of hypothalamic inhibition and gonadotropin-releasing hormone secretion, resulting in precocious puberty. *MKRN3* gene-related CPP is almost always caused solely by paternal loss-of-function missense and stop-codon variants in the *MKRN3* gene [Shin, 2016; Abreu *et al*., 2015]. Only one CPP patient with the heterozygous 4-nucleotide deletion in the *MKRN3* promoter region has been described in the literature [Macedo *et al*., 2018]. So far, it is not known why, despite the abnormal expression of *MKRN3*, paternal deletions of 15q11–q13 and maternal UPD(15) usually do not lead to CPP in PWS patients [Shin, 2016].

2.2.9. Transient neonatal diabetes mellitus

TNDM is a rare type of diabetes that appears during the first six months of life in a term infant and in most cases reverts spontaneously before five months of age. TNDM can results from a variety of genetic causes, but the majority of TNDM cases are caused by genetic or epigenetic alterations in an imprinted locus on chromosome 6q24. The link between TNDM and the imprinted locus 6q24 was not made until 1999, when Dr. Gardner, together with colleagues, using linkage analysis localized the TNDM gene to band 6q24.1–q24.3 [Gardner *et al*., 1999]. In 2000, an imprinted candidate gene for TNDM, *PLAGL1*, was identified in this region [Kamiya *et al*., 2000].

Main clinical features of 6q24-related TNDM are severe IUGR and hyperglycemia that begins in the first six weeks of life in a term infant and resolves by age 18 months. Signs of 6q24-related neonatal hyperglycemia include variable degrees of dehydration, failure to thrive, low plasma insulin concentration, absence of ketoacidosis and islet cell antibodies. The mean birth weight is about 2,000 g (–2.5 SD). In addition, the incidence of premature birth (<37 weeks of gestation) is approximately 30% that is significantly higher than the global incidence of 9.6% estimated by the World Health Organization [Docherty *et al*., 2013]. Diabetes mellitus usually starts within the first week of life and lasts on average for three months, but can last as long as for two years. TNDM usually requires initially treatment with exogenous insulin, but the need for insulin gradually declines over time. This is often accompanied by a significant weight gain and catch-up growth [Metz *et al*., 2002]. Intermittent episodes of hyperglycemia may occur later in childhood, particularly during intercurrent illnesses. There is also an elevated risk of diabetes mellitus in

adolescence and adulthood, especially during pregnancy [Temple, and Mackay, 2018].

Congenital abnormalities are common in children with the 6q24-related TNDM. Macroglossia and umbilical hernia are presented in approximately 40% and 20% of patients, respectively. Other associated less frequently reported congenital abnormalities include dysmorphic facial appearance, renal tract abnormalities (duplex kidneys, hydronephrosis, dilated renal pelvis and vesicoureteral reflux), cardiac anomalies (ductus arteriosus, tetralogy of Fallot, atrial-septal defects and persistent foramen ovale), hypothyroidism, clino-dactyly, polydactyly, nail and short finger abnormalities [Docherty *et al*., 2013]. Motor development and intelligence are usually normal, unless there is a concomitant autosomal recessive disorder, large CNV or hypomethylation at an additional imprinted loci. It is also notable that there are adult individuals with MODY-like diabetes, but without history of neonatal diabetes in whom TNDM-specific abnormality of 6q24 has been detected [Yorifuji *et al*., 2015].

TNDM is most frequently caused by overexpression of the paternally expressed genes *PLAGL1* and *HYMAI* on chromosome 6q24.2. This locus include only one DMR, *PLAGL1*:alt-TSS-DMR, that is located in the promoter region of *PLAGL1* and is normally methylated on the maternal allele. Three molecular disease-causing mechanisms have been described for the 6q24-related TNDM: paternal UPD of chromosome 6 (40% of cases), paternal duplications of 6q24 (32%) and maternal LOM at the *PLAGL1*:alt-TSS-DMR (28%) [Docherty *et al*., 2013]. Moreover, many patients with LOM at *PLAGL1*:alt-TSS-DMR may also have a MLMD presenting as LOM at additional imprinted loci in other chromosomes. In about half of these MLMD patients, recessive variants in the non-imprinted *ZFP57* gene (region 6p22.1), a transcription factor involved in the maintenance of methylation of the imprinted loci, have been identified [Bak *et al*., 2016]. LOM at multiple imprinted loci is characterized by more severe phenotype and frequent presence of congenital abnormalities and DD [Boonen *et al*., 2013].

2.2.10. Myoclonus-dystonia syndrome

The link between changes in imprinted gene *SGCE* and MDS, previosly called dystonia 11, was first identified in 2001 by Dr. Zimprich and colleagues through the positional cloning in patients with familial myoclonus and dystonia [Zimprich *et al*., 2001]. MDS is a rare ImpDis characterized by a combination of childhood-onset myoclonus mainly involving the upper body in combination with dystonic muscle contractions. This disorder usually occurs before age 10 whereas the onset after age 20 is unusual [Carecchio *et al*., 2013; Peall *et al*., 2014]. In most cases, the main and most disabling symptom is lightning-like myoclonic jerks, which may be isolated or associated with dystonia. Isolated dystonia is the initial manifestation in 15–30% of MDS cases. Myoclonus can affect all body regions, but generally predominates in the proximal segments of

the upper limbs and neck. Myoclonus is often mild at rest, but dramatically aggravated by action [Roze *et al*., 2018]. *SGCE* gene-related myoclonus has specific neurophysiological characteristics, which indicate a dysfunction of the cerebello-thalamic pathways [Roze *et al*., 2008]. When present, dystonia is mild to moderate, and usually involves the face, larynx, and trunk, although patients with early-onset MDS are more likely to develop lower limb dystonia [Peall *et al*., 2014]. Cervical dystonia and writer's cramp are the most common signs of dystonia. It is notable that most patients with MDS have an important motor improvement following alcohol ingestion with rebound worsening on alcohol withdrawal [Roze *et al*., 2018]. MDS is not associated with DD or ID. However, psychiatric problems, namely anxiety-related disorders and obsessive compulsive disorders, are common [Peall *et al*., 2016]. Severity and the rate of progression are unpredictable in MDS, ranging from severe motor disability in adolescence to mild, nonprogressive symptoms lasting decades [Roze *et al*., 2018].

SGCE is a paternally expressed and maternally imprinted gene located on chromosome 7q21.3. *SGCE* encodes a epsilon-sarcoglycan, protein with an unknown function, which is a part of the dystrophin-associated glycoprotein complex and is widely expressed in the brain. Familial or *de novo* loss-of-function variants in the paternal allele of *SGCE* are the leading genetic cause of MDS. Paternal intragenic or the whole gene deletions of *SGCE* are also detected in a notable proportion of patients [Grunewald *et al*., 2008; Huang *et al*., 2010; Peall *et al*., 2014]. However, changes in the *SGCE* gene are present in only about 50% of all patients with the typical MDS phenotype [Rachad *et al*., 2017; Roze *et al*., 2018]. In patients with contiguous gene syndrome because of a deletion encompassing the entire *SGCE* along with adjacent genes on chromosome 7, manifestations accompanying MDS frequently include ID, microcephaly, facial dysmorphism, and intrauterine and postnatal growth retardation [Asmus *et al*., 2007; Roze *et al*., 2018]. In rare cases, MDS may be associated with SRS due to maternal UPD(7) that causes biallelic silencing of *SGCE* [Guettard *et al*., 2008; Sheridan *et al*., 2013].

2.2.11. Maternal uniparental disomy of chromosome 20

UPD(20)mat, called also Mulchandani-Bhoj-Conlin syndrome, is a novel and very rare ImpDis. Only sixteen cases of isolated UPD(20)mat without concomitant mosaic trisomy 20 or marker chromosome have been described so far in the literature [Eggermann *et al*., 2001; Azzi *et al*., 2015; Mulchandani *et al*., 2016; Kawashima *et al*., 2018a; Kawashima *et al*., 2018b]. The features of UPD(20)mat are nonspecific and include mild IUGR, postnatal growth retardation, severe short stature with proportional head circumference, failure to thrive and profound feeding difficulties from birth requiring chronic gastric tube feeding or gastrostomy. Generally, the infants with UPD(20)mat do not show a normal hunger drive, do not wake to eat, and do not spontaneously cry to be fed. Hypoplastic placenta and oligohydramnios are described in most UPD(20)mat

pregnancies. Some patients also demonstrante triangular face, fifth-finger clinodactyly, mild abnormalities of skin pigmentation, delayed bone age and infantile hypotonia. The patients with isolated UPD(20)mat have neither any other significant dysmorfic facial features nor major congenital abnormalities. Psychomotor and mental development is usually normal. [Mulchandani *et al*., 2016]. UPD(20)mat can also be associated with *GNAS*-related increased sensitivity of PTH and/or TSH receptors, resulting in hypercalcemia with low or low-normal PTH levels and high-normal levels of thyroid hormones with decreased TSH levels. Because of low birth weight, postnatal growth delay, feeding difficulties and clinodactyly, patients with UPD(20)mat can be misdiagnosed as SRS. UPD(20)mat could account for approximately 5% of etiology-unknown SRS cases [Kawashima *et al*., 2018a].

Isolated UPD(20)mat is caused by aberrant expression of imprinted genes located on chromosome 20. However, so far, the causative imprinted gene/ genes are not known. The most promising locus is *GNAS* in the 20q region. Mouse models have shown that the loss of paternal *GNAS* alleles leads to a decreased adipose tissue, a higher metabolic rate, and poor sucking [Yu *et al*., 2000; Plagge *et al*., 2004; Xie *et al*., 2006]. In addition, there are other potentially important imprinted loci on chromosome 20, such as *L3MBTL1* and *NNAT*, which may also be implicated in pathogenesis of UPD(20)mat. All described UPD(20)mat patients demonstrate isodisomic and/or heterodisomic UPD of the whole chromosome 20 [Mulchandani *et al*., 2016].

2.2.12. Schaaf-Yang syndrome

SYS is a recently discovered ImpDis, for the first time identified in 2013 by Dr. Schaaf in four individuals with PWS-like phenotype and truncating variants in the paternal copy of the *MAGEL2* gene, located in the PWS critical region 15q11–q13 [Schaaf *et al*., 2013]. SYS is a neurodevelopmental disorder that has clinical overlap with PWS at the initial stages of life, but becomes increasingly distinct throughout childhood and adolescence. Patients with SYS are characterized by a wide phenotypic spectrum. The most frequent clinical features of SYS are neonatal hypotonia, infantile feeding problems, DD, ID, autism spectrum disorder, and distal joint contractures. PWS-like neonatal hypotonia and feeding problems requiring special feeding techniques are the main features of SYS in early infancy that are present in almost all patients. Gross motor and language milestones are usually achieved significantly later. Children with SYS, on average, start to walk independently at 50 months of age and learn to speak their first word at 36 months of age. The degree of ID can range from mild to profound. Autism spectrum disorders are common and present in >75% of children with SYS. The severity of joint contractures may range from affecting only interphalangeal joints to fetal akinesia with severe arthrogryposis. Additional symptoms of SYS include infantile respiratory distress, gastroesophageal reflux, chronic constipation, central and/or obstructive sleep apnea,

short stature, skeletal abnormalities (scoliosis, exaggerated kyphosis, small hands, small feet), hypogonadism and temperature instability. A spectrum of varying facial dysmorphic features including prognathia and malformations of the philtrum, nasal structure, ear position, frontal bossing and palpebral fissure length is present in the majority of individuals with SYS. Patients with SYS do not typically manifest the hyperphagia and severe obesity seen in children with PWS [Fountain *et al*., 2017; McCarthy *et al*., 2018].

SYS is almost always caused by truncating point variants of the paternal allele of *MAGEL2*, a maternally imprinted, paternally expressed gene located at 15q11.2. One individual with SYS caused by paternally inherited complex rearrangement interrupting the *MAGEL2* gene, consisting of a 22 kb inversion and 3 kb deletion that removes the last 852 bp and the 3' end of the gene, has also been reported [Jobling *et al*., 2018]. Interestingly, a paternally inherited deletions that includes whole *MAGEL2* gene, but not the PWS-related *SNURF-SNRPN* locus appear to have a milder phenotype, and do not cause SYS nor PWS [Kanber *et al*., 2009; Buiting *et al*., 2014]. It has been suggested that deletion of the entire paternal copy of *MAGEL2* could lead to leaky expression of the maternal copy of this gene [Matarazzo, and Muscatelli, 2013; Fountain, and Schaaf, 2016].

2.2.13. Birk-Barel syndrome

Birk-Barel syndrome, called also *KCNK9* imprinting syndrome, is a very rare ImpDis, first described in 2008 by Dr. Barel and Prof. Shalev in 15 individuals with ID and dysmorphic features, all members of the same Arab-Israeli family [Barel *et al*., 2008]. Since that time, only four more simplex cases of Birk-Barel syndrome have been reported [Graham *et al*., 2016]. This syndrome is characterized by congenital central hypotonia, persistent generalized weakness, infrequent joint contractures, transient neonatal hypoglycemia, severe feeding problems due to a poor suck and gastroesophageal reflux, variable clefting of the palate, wide alveolar ridges, mild retrognathia and/or micrognathia, normal findings on brain magnetic resonance imaging and electroencephalography, DD, ID of varying degree and occasionally diminished tearing upon crying. Distinctive facial dysmorphic features include dolichocephaly with bitemporal narrowing, short philtrum, tented upper lip, and medially flared eyebrows. With age, the face becomes myopathic and elongated, with a tapered chest and asthenic body build in most affected individuals [Barel *et al*., 2008; Graham *et al*., 2016; Zadeh, and Graham, 2017].

Birk-Barel syndrome is caused by point variants on the maternally derived allele of a paternally imprinted *KCNK9* gene located at 8q24.3. All described patients carry the same heterozygous missense variants c.706G>C, p.(Gly236Arg) (RefSeq NM_001282534.1) in the maternal copy of the *KCNK9* gene. Approximately 80% of affected individuals inherited this variant from a clinically unaffected mother, and 20% of patients have a *de novo* variant. To

date, it is unknown if other *KCNK9* pathogenic variants or maternal deletions of 8q24.3 can cause the same phenotype [Graham *et al*., 2016; Zadeh, and Graham, 2017].

2.2.14. Multilocus methylation defects

MLMDs, also known as multilocus imprinting disturbances (MLIDs), are methylation changes, often hypomethylation, at multiple imprinted loci. MLMDs are present in a significant number of patients with ImpDis due to primary epimutations and indicate a general disturbance of the establishment and/or maintenance of imprinting marks in the DMRs [Eggermann *et al*., 2011]. At least six ImpDis are associated with MLMDs. To date, MLMD has been reported predominantly in patients diagnosed with TNDM, BWS and SRS. The phenomenon is less frequently observed in PHP, AS and PWS, and have been described in other ImpDis only rarely. MLMDs have been linked to variants in three trans-acting factors – *ZFP57*, *NLRP2* and *NLRP5*. However, the molecular cause of MLMDs remains unclear in the majority of patients [Sanchez-Delgado *et al*., 2016].

Approximately half of TNDM patients with hypomethylation at *PLAGL1*:alt-TSS-DMR have also additional hypomethylation of other maternally methylated imprinted loci throughout the genome, including *GRB10*, *PEG3*, *PEG1/ MEST, KCNQ1OT1* and *GNAS-AS1*. This phenomenon is called maternal hypomethylation syndrome [Mackay *et al*., 2006]. Homozygous or compound heterozygous variants in *ZFP57*, a gene that plays a central role in the establishment of DNA methylation at maternally methylated DMRs, can be detected in about half of individuals with maternal hypomethylation syndrome [Bak *et al*., 2016]. At the phenotypic level, this syndrome is often indistinguishable from classic TNDM. However, additional features, such as heart defects, structural brain abnormalities, hypotonia, DD, learning difficulties, epilepsy and/or visual impairment, may be presented [Mackay *et al*., 2008; Boonen *et al*., 2013].

MLMDs are identified in up to 30% of patients with BWS caused by hypomethylation at IC2 [Court *et al*., 2013; Tee *et al*., 2013; Docherty *et al*., 2014] and also in some BWS cases with hypermethylation at IC1 [Maeda *et al*., 2014; Alders *et al*., 2014]. The MLMDs observed in BWS are different from those detected in TNDM, with both LOM and GOM observed at maternal and paternal DMRs. It seems that *PLAGL1*, *GRB10*, *MEST*, *GNAS*, *IGF2R*, and *ZNF331* are the DMRs most frequently disrupted in BWS with MLMD [Court *et al*., 2013; Tee *et al*., 2013; Docherty *et al*., 2014]. Some studies have also revealed LOM at IC1 and LOM at IC2 coexisting in the same patient that can, paradoxically, result in either BWS or SRS [Court *et al*., 2013; Azzi *et al*., 2009]. It is unknown why patients with similar patterns of LOM in these two loci demonstrate an opposite phenotype. Complex phenotypes may also be observed when loci other than IC1 and IC2 are involved. For example, a combination of BWS and PHP1B was described in a few patients with MLMDs

[Bakker *et al*., 2015; Sano *et al*., 2016]. Alternatively, one phenotype can dominate over another. For instance, an infant with LOM at both *PLAGL1* and IC2 presented neonatally with BWS and without neonatal diabetes, but later relapsed with adult diabetes [Sanchez-Delgado *et al*., 2016]. However, the majority of BWS cases with MLMDs do not demonstrate additional clinical features of other ImpDis [Bliek *et al*., 2009; Azzi *et al*., 2009; Court *et al*., 2013; Fontana *et al*., 2018]. The clinical presentation of MLMD may, theoretically, be affected by the severity of the mosaic methylation disturbances in different tissues [Sanchez-Delgado *et al*., 2016].

MLMDs has been described in approximately 15% of SRS cases with IC1 hypomethylation. Both paternally and maternally methylated DMRs can be affected. The most frequently disrupted DMRs found in SRS patients with MLMDs are *DIRAS3*, *PLAGL1*, *GRB10*, *MEST*, *MEG3-DLK1*:IG-DMR, *ZNF331*, *WRB*, and *SNU13* DMRs [Azzi *et al*., 2009; Begemann *et al*., 2011; Court *et al*., 2013; Docherty *et al*., 2014]. Interestingly, the hypomethylation observed in SRS patients with MLMD is often less severe when compared with other ImpDis with MLMD. The observation can probably be associated with high frequency of mosaic methylation defects in SRS. In most cases, the SRS phenotype is indistinguishable between isolated LOM at IC1 and MLMD [Azzi *et al*., 2009; Court *et al*., 2013]. 27,45]. However, it was found that SRS patients with MLMD may have less severe growth phenotype and increased prevalence of DD and congenital abnormalities [Poole *et al*., 2013].

MLMDs have been reported in approximately 8–10% of patients with PHP1B. MLMDs in PHP1B are often mild and affect isolated additional DMRs [Perez-Nanclares *et al*., 2012; Maupetit-Mehouas *et al*., 2013; Rochtus *et al*., 2016; Kagami *et al*., 2017a]. These additional methylation defects do not seem to affect the clinical presentation of PHP1B. Interestingly, that methylation defects at the *GNAS* locus are frequently observed in MLMD patients with BWS and with normal hormonal levels, whereas patients with PHP1B rarely have MLMDs [Sanchez-Delgado *et al*., 2016].

To date, little is known about the frequency of MLMDs in patients with PWS, AS and TS14, mainly because only a small proportion of these patients present with epigenetic anomalies. MLMDs have been detected in only few patients with molecular diagnosis of AS [Baple *et al*., 2011; Bens *et al*., 2016] and TS14 [Bens *et al*., 2016; Kagami *et al*., 2017a]. No cases of MLMD have been so far reported for KOS [Kagami *et al*., 2017a].

The exact etiology of MLMDs is not definitely established. There are several causative genes for MLMDs (*ZFP57*, *NLRP2*, and *NLRP5*), as well as candidate genes for MLMDs (*NLRP7*, *KHDC3L*, and *TRIM28*), have been identified in a few patients with MLMDs [Court *et al*., 2013; Docherty *et al*., 2015]. These genes participate in the establishment and maintenance of imprinting marks at DMRs, and their abnormalities may therefore result in hypomethylation and/or hypermethylation at multiple imprinted loci. However, the mechanisms underlying the regulation of imprinting marks across the genome remain to be elucidated [Mackay *et al*., 2015; Sanchez-Delgado *et al*., 2016; Kagami *et al*., 2017a].

2.3. Epidemiology of imprinting disorders

Due to the high clinical and molecular heterogeneity of ImpDis and lack of awareness of ImpDis among medical health professionals, patients frequently experience a delay in diagnosis, or remain without diagnosis. Moreover, the frequent presence of nonspecific features and the phenotypic overlap between ImpDis and other genetic disorders challenges the accurate diagnosis of ImpDis. As a result, the exact incidence and prevalence of ImpDis are currently unknown and can be only roughly estimated. Understanding the epidemiology of these disorders is valuable and helps to improve the diagnostic yield of ImpDis, to observe changes in the diagnostic yield over time and to provide appropriate health care services.

Previously, a limited number of studies have explored the prevalence, incidence and other epidemiological data related to ImpDis (Table 2) [Clayton-Smith, and Pembrey, 1992; Clayton-Smith, 1993; Kyllerman, 1995; Petersen *et al*., 1995; Steffenburg *et al*., 1996; Buckley *et al*., 1998; Strømme, 2000; Thomson *et al*., 2006a; Õiglane-Shlik *et al*., 2006a; Mertz *et al*., 2013; AlSalloum *et al*., 2015; Luk, and Lo, 2016; Burd *et al*., 1990; Åkefeldt *et al*., 1991; Ehara *et al*., 1995; Whittington *et al*., 2001; Smith *et al*., 2003; Vogels *et al*., 2004; Thomson *et al*., 2006b; Lionti *et al*., 2015; Richard-De Ceaurriz *et al*., 2017; Bar *et al*., 2017; Thorburn *et al*., 1970; Higurashi *et al*., 1980; Higurashi *et al*., 1985; Higurashi *et al*., 1990; Wiedemann, 1997; Arroyo Carrera *et al*., 1999; Bianchi, 2002; Halliday *et al*., 2004; Mussa *et al*., 2013; Barisic *et al*., 2018]. Almost all of these studies have focused on the epidemiology of PWS, AS and BWS, and only a minority of them have been performed during the last 10 years. The prevalence of PWS, AS and BWS demonstrates variation of up to seven-fold between countries without clear explanation. To the best of our knowledge, there have been no studies that have looked into the actual prevalence or incidence of SRS or other less common ImpDis, with only theoretical estimates of the prevalence available [Wakeling *et al*., 2017; Eggermann *et al*., 2015b].

The first attempts to determine the prevalence of the most frequent ImpDis, PWS, were made in 1990 by Burd *et al*., who studied a population of the state of North Dakota, USA, and determined the population prevalence of PWS to be 1/16,062 in the age range 9–30 years. However, this study was based on a clinical phenotype of PWS only. The subsequent epidemiological studies of PWS performed by Åkefeldt *et al*. (1991), Ehara *et al*. (1995) and Whittington *et al*. (2001) included the patients with both clinical and molecularly confirmed diagnosis of PWS. In these studies, the difference between population prevalences of PWS was up to six-fold, from 1/8,333 in Sweden (0–25 years old) to 1/52,000 in UK (all ages) (Table 2). The majority of the following epidemiological studies included only individuals with molecularly confirmed diagnosis of PWS. Smith *et al*. (2003), Vogels *et al*. (2004) and Thomson *et al*. (2006b) demonstrated that the birth incidence of PWS could be about 1/25,000–30,000. However, the most recent studies performed by Lionti *et al*. (2015),

Richard-De Ceaurriz *et al*. (2017) and Bar *et al*. (2017) revealed the birth incidence of PWS to be somewhat higher, approximately 1/10,000–20,000 (Table 2). In Estonia, a 21-year-study (1984–2004) of both PWS and AS was previously performed by Õiglane-Shlik *et al*. (2006). This study showed that the birth prevalence of PWS in Estonia is 1/30,439 and population prevalence 1/30,606 in the age range 0–20 years. However, in 2004, at the end of this study, a significantly higher PWS prevalence of 1/12,547 was observed [Õiglane-Shlik *et al*., 2006a].

The first epidemiological study on AS performed in 1992 by Clayton-Smith and Pembrey estimated the incidence of AS to be around 1/20,000. Later, Clayton-Smith (1993) suggested the minimum population prevalence of AS in the UK being 1/62,000, mostly considering referrals to genetic consultations (all ages). Kyllerman (1995) estimated the minimum prevalence of AS to be 1/12,000 amongst 6–13 years old Swedish children with ID and epilepsy, however, only half of AS patients identified had a genetically confirmed 15q11–q13 deletion. The majority of further epidemiological studies performed during the last two decades [Buckley *et al*., 1998; Thomson *et al*., 2006a; Mertz *et al*., 2013; AlSalloum *et al*., 2015; Luk, and Lo, 2016] revealed the birth incidence of AS to be approximately 1/20,000–40,000 (Table 2), however, only a part of studied patients had molecularly proven diagnosis of AS. Previous Estonian study demonstrated the lowest birth prevalence of AS obtained (1/52,181 in the period of 1984–2004), however the birth prevalence of 1/23,640 was received at the end of this study, in 2004 [Õiglane-Shlik *et al*., 2006a], suggesting a low diagnostic rate of AS at the beginning of the study as a cause of low birth prevalence found.

Table 2: Studies on the prevalence of ImpDis. Adapted from [Õiglane-Shlik *et al.*, 2006a].

Prader-Willi syndrome	**Estimated character**	**Study population**	**Period**	**Data**
Burd *et al.*, 1990	Population prevalence	9–30 years old		1/16,062
Åkefeldt *et al.*, 1991	Population prevalence	0–25 years old		1/8,333
Ehara *et al.*, 1995	Birth incidence		1980–1989	1/15,060
	Population prevalence	0–15 years old		1/17,482
Stromme *et al.*, 2000	Prevalence	Norway	1980–1985	1/10,000
Whittington *et al.*, 2001	Population prevalence	One UK Health Region, all ages		1/52,000
	Birth incidence			1/29,000
Smith *et al.*, 2003	Birth prevalence	Australia	1998–2000	1/25,000
Vogels *et al.*, 2004	Population prevalence	Flandria, all ages		1/76,574
	Birth incidence		1993–2000	1/26,676
Thomson *et al.*, 2006b	Birth prevalence	Disability Services Commission	1953–2003	1/29,500
Õiglane-Shlik *et al.*, 2006	Birth prevalence	Estonia	2000–2004	1/30,439
	Population prevalence	0–20 years old, Estonia		1/30,606
Lionti *et al.*, 2015	Birth prevalence	Australia	2003–2012	1/15,830
Richard-De Ceaurriz *et al.*, 2017	Birth incidence	France		1/7,937
Bar *et al.*, 2017	Birth incidence	France	2013	1/21,000
Angelman syndrome	**Estimated character**	**Study population**	**Period**	**Data**
Clayton-Smith and Pembrey, 1992	Incidence	Mainly referrals to genetic consultations, all ages	unknown	1/20,000
Clayton-Smith, 1993	Population prevalence			1/62,000
Kyllerman, 1995	Prevalence rate	6–13 years old, ID, epilepsy	1975–1986	1/12,000
Petersen *et al.*, 1995	Prevalence rate	Neuropaediatric clinic	1983–1991	1/10,000
Steffenburg *et al.*, 1996	Prevalence			1/12,000
Buckley *et al.*, 1998	Incidence	Institutionalized, severe ID	1998	1/20,000
Stromme *et al.*, 2000	Prevalence	Norway	1980–1985	1/15,000

Thomson *et al*., 2006a	Birth prevalence	Western Australia	1953–2003	1/40,000
Õiglane-Šlik *et al*., 2006	Birth prevalence	Estonia	2000–2004	1/52,181
	Population prevalence	0–20 years old, Estonia		1/56,112
Mertz *et al*., 2013	Birth incidence	Denmark		1/24,580
Al Salloum *et al*., 2015	Birth prevalence	Saudi Arabia	2004–2005	1/45,682
Luk *et al*., 2016	Birth incidence	Hong Kong China	1995–2015	1/22,305
Beckwith-Wiedemann syndrome	**Estimated character**	**Study population**	**Period**	**Data**
Thorburn *et al*. 1970	Birth prevalence	Jamaica, Victoria Jubilee Hospital and University Hospital of the West Indies	1966–1968	1/13,700
Higurashi *et al*., 1980	Birth prevalence	Japan, one maternity hospital in Tokyo	1972–1985	1/14,430
Higurashi *et al*., 1985				1/22,063
Higurashi *et al*., 1990				1/27,472
Wiedemann, 1997	Birth prevalence	Germany	1978–1991	1/11,969
Arroyo Carrera *et al*., 1999	Birth prevalence	Spain	1976–1997	1/79,520
Bianchi *et al*., 2002	Birth prevalence	Italy	1992–1999	1/65,486
Halliday *et al*., 2004	Birth prevalence	Australia, State of Victoria	1983–2003	1/35,580
Mussa *et al*., 2013	Birth prevalence	Italy, Piedmont region	1996–2009	1/10,569
Barisic *et al*., 2018	Birth prevalence	16 European countries	1990–2015	1/26,000
Pseudohypoparathyroidism	**Estimated character**	**Study population**	**Period**	**Data**
Nakamura *et al*., 2000	Population prevalence	Japan	1997	1/294,000
Underbjerg *et al*., 2016	Population prevalence	Denmark		1/90,900

ID – intellectual disability

Epidemiological studies of BWS are rare. In 1970, Thorburn *et al*. described six infants with exomphalos-macroglossia-gigantism syndrome, now called BWS, and calculated the birth prevalence of this syndrome in Jamaica as 1/13,700. However, molecular testing of 11p15.5 was not possible at this time, and the diagnosis of BWS was clinical. Higurashi *et al*. estimated the birth prevalence of BWS in Japan to be around 1/14,430 in 1980, 1/22,063 in 1985 and 1/27,472 in 1990, however the study was restricted to one maternity hospital in Tokyo and there was only one patient with BWS during the whole study period. Until 2018, a population-based prevalence data of BWS in Europe was limited to four studies confined to a specific region or country [Wiedemann, 1997; Arroyo Carrera *et al*., 1999; Bianchi, 2002; Mussa *et al*., 2013]. The prevalence of BWS in Europe found in these studies ranged greatly from 1/10,569 in Italy [Mussa *et al*., 2013] to 1/79,520 in Spain [Arroyo Carrera *et al*., 1999]. In 2018, Barisic with colleagues performed a EUROCAT (European Surveillance of Congenital Anomalies) registries-based epidemiological study of BWS that covered the population of 16 European countries. This study demonstrated that the estimated mean birth prevalence of classic BWS in Europe is approximately 1/26,000 or 3.8/100,000 births.

To date, there are no epidemiological studies on SRS and the birth prevalence of SRS is only roughly estimated as 1/75,000–1/100,000 births [Eggermann *et al*., 2015a]. The exact prevalence of PHP and PPHP is also unknown. Studies published in 2000 and 2016 estimated the population prevalence of uncpecified PHP to be 1/294,000 or 0.34/100,000 in Japan [Nakamura *et al*., 2000] and 1/90,900 or 1.1/100,000 in Denmark [Underbjerg *et al*., 2016]. An important limitation of both studies is that the clinical diagnosis of PHP was not confirmed by a molecular analysis in the majority of patients. The estimated prevalence of unspecified TNDM is approximately 1/300,000 [Polak, and Cave, 2007]. The prevalence of other ImpDis and the total prevalence of all ImpDis are unknown due to the rarity of these disorders and the absence of systematic studies. A epidemiological study of all UPDs was recently performed [Nakka *et al*., 2019]. Interestingly, this study revealed that the incidence rate of all UPDs in the general population is unexpectedly high at 1/2,000 births. However, the information is difficult to correlate directly with the prevalence of ImpDis as the majority of UPDs do not result in ImpDis.

2.4. Molecular diagnostic methods for imprinting disorders

As the molecular etiology of ImpDis is very variable and heterogeneous, there is no single molecular analysis technique for the diagnosis of all ImpDis. Although the identification of the molecular cause of ImpDis is required for a precise molecular diagnosis and a well-directed genetic counseling. A broad range of molecular tests is now available for ImpDis. However, because of their different sensitivities, levels of coverage, complexity and costs, only some of these molecular tests are used routinely in clinical diagnostic laboratories (Table 3).

Table 3: Four main molecular diagnostic methods used for molecular diagnosis of ImpDis. Adapted from [Yakoreva *et al*., 2017].

Molecular diagnostic method	Detectable alterations	Undetectable alterations
MS-MLPA	• Epimutations • CNVs • UPD[1]	• Variants[2] • Balanced chromosomal rearrangements
CMA	• CNVs (>50–100 Kb)[3] • Isodisomic UPD[4] • Heterodisomic UPD[5]	• Epimutations • Variants[6] • Balanced chromosomal rearrangements
Sanger sequencing, large NGS panels, ES	• Variants	• Epimutations • Balanced chromosomal rearrangements • UPD[7] • CNVs[7]
Chromosome analysis	• Balanced chromosomal rearrangements • Large CNVs (>5–10 Mb)	• Epimutations • UPD • Variants • CNVs (<5–10 Mb)

MS-MLPA – methylation-specific multiplex ligation-dependent probe amplification; CMA – chromosomal microarray; NGS – next-generation sequencing; ES – exome sequencing; CNV – copy number variation; UPD – uniparental disomy

[1] discrimination between isodisomic UPD and heterodisomic UPD is not possible
[2] some MS-MLPA kits contain probes designed to detect certain variants
[3] CMA resolution depends on density of probes, can be as good as <10 Kb
[4] discrimination between isodisomic UPD and regions of homozygosity, and identification of parental origin of UPD are possible only in the case of parent/parents-proband analysis
[5] detection of heterodisomic UPD is possible only in the case of parent/parents-proband analysis
[6] some CMA platforms also include probes for disease-causing variants
[7] detection of CNVs (>50 kb) and UPD is sometimes possible using large NGS panels/ES and special CNV or UPD detection algorithms

2.4.1. DNA methylation analysis

Many molecular alterations observed in ImpDis, including CNVs, epimutations, and UPDs, result in abnormal DNA methylation patterns at DMRs. DNA methylation analysis is therefore used as a first-tier molecular test for the majority of ImpDis. There are a large variety of molecular techniques that can be used for DNA methylation testing, although many of them are too outdated or labor intensive to be used nowadays in clinical diagnostic laboratories [Grafodatskaya *et al*., 2017].

DNA methylation analysis techniques can be based either on restriction enzyme digestion or sodium bisulfite conversion. Some restriction enzymes are sensitive to methylation of cytosine in CpG dinucleotides within the restriction site. It provides an opportunity to use these restriction sites for analysis of methylation status, since unmethylated DNA will be cut by restriction enzymes at this site, whereas methylated DNA remains undigested. Methylation non-sensitive restriction enzymes cutting at the same sequence is often used as a control in these analyses. The pattern of restriction can be further analyzed by downstream techniques such as methylation-sensitive Southern blot hybridization or methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). MS-MLPA is a semiquantitative technique based on hybridization of two adjacent locus-specific probes, ligation and digestion with methylation-sensitive enzymes and further amplification of target sequences using universal primers, the fragment length of which is further analyzed by using an automated sequencer. If the probes are not ligated due to the deletion of target sequences or the digestion of umethylated CpG sites, no amplification product is formed. Thus, this method can detect both methylation defects and CNVs [Grafodatskaya *et al*., 2017; Moelans *et al*., 2018].

Sodium bisulfite conversion methods are based on chemical conversion of unmethylated cytosine to uracil, since methylated cytosine is resistant to this conversion. The ratio of DNA molecules with methylated cytosine to unmethylated cytosine can then be assessed in qualitative and quantitative assays including methylation-specific polymerase chain reaction (MS-PCR), methylation-specific single nucleotide primer extension (MS-SNuPE), matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), sequencing methods including Sanger, methylation-specific pyro-sequencing, methylation-specific next-generation sequencing (NGS), reduced representation bisulphate sequencing (RRBS), whole genome bisulphate sequencing (WGBS), and methylation BeadChip microarrays using Infinium chemistry [Ammerpohl *et al*., 2009; Soellner *et al*., 2015].

Some of these techniques, for example Southern blot and MS-PCR, target only one imprinted locus, whereas MS-MLPA, MS-SNuPE and MALDI-TOF MS can analyze multiple imprinted genes or loci simultaneously. Massively parallel non-Sanger-based high-throughput DNA sequencing technologies, including methylation-specific NGS, RRBS, and WGBS, allow to analyze DNA methylation in the whole genome at once, but are relatively expensive and require advanced bioinformatics infrastructure and knowledge [Soellner *et al*., 2015]. Until the last decade, Southern blot and MS-PCR were the most frequently used techniques in diagnostic laboratories. However, as they are labor intensive and analyze only one imprinted site in one assay, their use has greatly decreased [Grafodatskaya *et al*., 2017]. On the other hand, MS-MLPA can target up to 46 imprinted sites, and allow to detect and discriminate both epimutations and CNVs, and is often provided as ready-to-use kits. All these advantages have made MS-MLPA a method of the first choice in diagnostics of such ImpDis, as PWS, AS, BWS, SRS and PHP/PPHP [Soellner *et al*., 2015].

2.4.2. Copy number variation analysis

CNVs can be detected by a variety of molecular methods including quantitative polymerase chain reaction (qPCR), chromosomal microarray (CMA), MLPA, and fluorescence in situ hybridization (FISH). FISH is only suitable for identification of relatively large deletions (>50 kb). Other methods can identify both large CNVs and microdeletions or microduplicatons within ICs if the probes/primers are located within this region. In clinical practice, CNV analysis is often performed as a part of MS-MLPA analysis. However, MLPA allow to detect only the presence or absence of certain DNA probes and, therefore, is often unable to determine the exact size and range of bigger CNVs. In this case, the use of additional confirmatory molecular tests, such as CMA, is usually necessary [Grafodatskaya *et al*., 2017]. Currently, CMA is a first-tier diagnostic test for patients with DD, ID, autism spectrum disorders, and/or congenital multiple congenital anomalies in Estonia [Zilina *et al*., 2014b]. Using CMA, individuals with AS and PWS caused by deletions can thus be identified before the onset of all usual clinical features when a disorder-specific methylation-based diagnostic test can be considered. However, the important disadvantage of CMA is inability to detect small variants (usually <50 kb) and to define the parental origin of CNV [Grafodatskaya *et al*., 2017]. Large NGS panels and ES can also be sometimes used for detection of CNVs bigger than 50 kb, however this method is challenging due to many limitations and has not been widely used yet [Wang *et al*., 2014; Yao *et al*., 2017]. Still, when using specific variant callers it is possible to detect CNVs on large NGS panels and ES data [Pajusalu *et al*., 2018].

2.4.3. Uniparental disomy analysis

Testing for UPD is usually recommended in the case of structural or numerical chromosome abnormalities involving imprinted regions and methylation defects involving several DMRs located on the same chromosome. UPD testing can be performed by genotyping of microsatellite markers distributed along the length of the chromosome in the patient and both parents. Microsatellite markers analysis is often not able to detect segmental UPD and low-level of UPD mosaicism. The majority of currently used CMAs contain probes for single nucleotide polymorphisms (SNP). SNP-based CMA can identify a long contiguous stretches of homozygosity (LCSH) that are suggestive of either consanguinity if distributed throughout the genome or could be a hallmark of isodisomic UPD if found within a single chromosome. LCSH, however, are not diagnostic for UPD and follow-up testing should be performed [Grafodatskaya *et al*., 2017]. UPD analysis is often carried out by comparative analysis of the SNPs using the CMA results of proband and at least one of his/her parents. The important advantage of this method is the ability to detect UPD of any chromosome, to distinguish isodisomy from heterodisomy and precisely determine the location of a segmental UPD. SNP-based CMA has also been

shown to allow detecting UPD mosaicism as low as 5% [Conlin *et al*., 2010; Kalish *et al*., 2013b]. Alternatively, UPD can be detected directly from genotypes using the data of parent-offspring trio ES, by searching for a genotype that are only compatible with uniparental inheritance [King *et al*., 2014].

2.4.4. Sequence analysis

Point variants are the main disease-causing mechanism in CPP, PHP1A/PPHP, MDS, SYS and Birk-Barel syndrome, in which sequence analysis is usually performed as first-tier diagnostic test. Moreover, point variants have also been found in a notable proportion of patients with AS, BWS and MLMD. Sequence analysis can be performed using Sanger sequencing, large NGS panels or ES. There are also some ready-to-use MS-MLPA kits that contain probes for certain point variants. Sanger sequencing can only sequence short pieces of DNA (up to 300–1000 base pairs). Therefore, it is used only for sequencing of single genes or certain DNA fragments. Contemporary sequencing technologies such as large NGS panels and ES are capable of sequencing millions of short sequences simultaneously which allows to study hundreds and thousands genes at once. Thus, large NGS panels and ES have higher sensitivity, resolution and ability to identify novel variants, but require advanced bioinformatics skills and knowledge [Neuheuser *et al*., 2019].

2.4.5. Cytogenetic analysis

Rare cases of ImpDis are associated with microscopically visible chromosomal abnormalities, including balanced rearrangements disrupting expression of imprinted genes, but not resulting in methylation anomalies and Robertsonian translocations or small supernumerary marker chromosomes resulting in UPD. Cytogenetic analysis is usually performed by karyotyping on metaphase chromosomes using routine G-banding. In the case of mosaicism, other body tissues (lymphocytes, skin fibroblasts, uroepithelium, buccal epithelium) can be additionally tested. There is no consensus regarding the need for routine karyotype analysis for diagnosis of ImpDis as structural chromosomal abnormalities are rarely observed in ImpDis, and these analyses are costly and labor intensive. However, cytogenetic analyse can be important in the case of familial chromosomal rearrangements that is associated with high risk of ImpDis in future offspring [Grafodatskaya *et al*., 2017].

2.5. Summary of the literature

To date, much is unknown about causes, molecular pathogenic mechanisms, clinical manifestation, inheritance, treatment and epidemiology of ImpDis. More than 100 imprinted genes have been identified in humans. Despite the fact that only a minority of these genes has been found to be associated with ImpDis, the number of described ImpDis has grown significantly in the past two decades. In addition to classic ImpDis such as PWS, AS, BWS and SRS, and less common PHP/PPHP, TS14 and KOS, a very rare and lesser known CPP, 6q24-related TNDM, MDS, UPD(20)mat, SYS, Birk-Barel syndrome and MLMDs have been also discovered. It has been found that the clinical presentation of ImpDis is very heterogeneous and may range in severity from asymptomatic to incompatible with life. Moreover, clinical features of ImpDis are often unspecific and overlap with symptoms of both other ImpDis and genetic disorders not related to imprinted genes. The same ImpDis can be caused by a variety of molecular alterations that may affect every level of genome structure ranging from chromosomal aberrations and large CNVs to point variants and methylation alterations. Therefore, presently there is no molecular diagnostic method that could completely exclude any ImpDis. All these factors make the diagnostics of ImpDis complex and challenging. As ImpDis are rare and many cases of ImpDis remain probably un- or misdiagnosed, there is a limited number of epidemiological studies of ImpDis and little is known about the birth incidence and population prevalence of ImpDis. The study was started due to an increased interest in this growing group of genetic disorders, considering the advantage which the small population of Estonia could give to a population-based epidemiological study of ImpDis.

3. AIMS OF THE PRESENT STUDY

The aims of the present study were:

1. To study the frequency of genetic and methylation abnormalities among Estonian patients selected by the previously published clinical diagnostic scoring systems for SRS and BWS (Paper I);
2. To evaluate the nationwide prevalence of the most common ImpDis in Estonia and time-trend changes in the live birth prevalence of these disorders (Paper II);
3. To establish new molecular diagnostic tests for ImpDis and evaluate their effectiveness in Estonia (Paper I and II);
4. To increase the awareness of ImpDis among Estonian doctors (Paper II and IV) in order to detect as many ImpDis as possible, and to characterize new rare ImpDis in Estonia (Paper III).

4. MATERIAL AND METHODS

4.1. Study subjects

4.1.1. Cohort for epidemiological study of imprinting disorders

Estonia is a small country with a total population of 1,319,133 people, of whom 275,399 are 0–19 years old (data of Statistics Estonia on January 1, 2018 [Statistics Estonia, 2018]). There are two tertiary care children's hospitals in the country: in Tallinn (Tallinn Children's Hospital) for northern Estonia and in Tartu (Children's Clinic, Tartu University Hospital) for southern Estonia. The size of this country and nationalized healthcare system make it possible to perform an epidemiological study that covers the whole population and detects the maximum possible number of patients with ImpDis.

The study was conducted in the Department of Clinical Genetics of Tartu University Hospital (including a branch in Tallinn), the only genetic referral centre in Estonia that provides comprehensive genetic counselling and molecular diagnostics of ImpDis for all patients living in Estonia. From 2014 onward, we conducted a retrospective and prospective study to find all cases of both clinically and molecularly diagnosed ImpDis among patients referred to a medical geneticist or other medical specialists.

We retrospectively reviewed the clinical and laboratory data of all Estonian patients with molecularly diagnosed ImpDis from 1998–2014. Prospective data are available from 2014–2018. Relevant information was obtained from the database of molecular diagnostics laboratory of the Department of Clinical Genetics. We created a registry of all molecularly confirmed ImpDis patients, including their personal data, laboratory findings, their age at diagnosis, life/death status and inheritance.

All individuals from this group who met the clinical diagnostic criteria for BWS (Table 4) [Brioude *et al*., 2018] and SRS (Table 5) [Wakeling *et al*., 2017] were clinically classified as BWS or SRS, respectively.

Additionally, several lectures to raise awareness of the clinical presentation, diagnosis, and principles of supervision and treatment of ImpDis were given at various departments at our institution (including neurology, neonatology, endocrinology and general pediatrics) and other hospitals. Information about this study and descriptions of ImpDis were repeatedly provided at national medical meetings in 2014–2018. Physicians were also given the opportunity to request free genetic testing for their patients with a clinical suspicion of ImpDis using a special sample submission form.

Table 4: Consensus clinical diagnostic criteria for BWS. A patient requires a score of ≥4 for a clinical diagnosis of classical BWS. Patients with a score of ≥2 merit genetic testing for the investigation and diagnosis of BWS. Adapted from [Brioude *et al*., 2018].

Cardinal features (2 points per feature)	Suggestive features (1 point per feature)
Macroglossia	Birthweight >2 SD above the mean
Exomphalos	Facial naevus simplex
Lateralized overgrowth	Polyhydramnios and/or placentomegaly
Multifocal and/or bilateral Wilms tumour or nephroblastomatosis	Ear creases and/or pits
Prolonged hyperinsulinism (lasting >1 week and requiring escalated treatment)	Transient hypoglycaemia (lasting <1 week)
Pathology findings: adrenal cortex cytomegaly, placental mesenchymal dysplasia or pancreatic adenomatosis	Typical embryonal tumours (neuroblastoma, rhabdomyosarcoma, unilateral Wilms tumour, hepatoblastoma, adrenocortical carcinoma or phaeochromocytoma)
BWS – Beckwith-Wiedemann syndrome; SD – standard deviation	Nephromegaly and/or hepatomegaly
	Umbilical hernia and/or diastasis recti

Table 5: Revised Netchine-Harbison clinical scoring system for SRS. If all molecular tests are normal and differential diagnoses have been ruled out, patients scoring at least four of six criteria, including both protruding forehead and relative macrocephaly should be diagnosed as clinical SRS. Adapted from [Netchine *et al*., 2007; Azzi *et al*., 2015].

Clinical criteria	Definition
SGA (birth weight and/or birth length)	≤−2 SD for gestational age
Postnatal growth failure	Height at 24 ± 1 months ≤−2 SD or height ≤−2 SD below mid-parental target height
Relative macrocephaly at birth*	Head circumference at birth ≥1.5 SD above birth weight and/or length SD
Protruding forehead*	Forehead projecting beyond the facial plane on a side view as a toddler (1–3 years)
Body asymmetry	LLD of ≥0.5 cm or arm asymmetry or LLD <0.5 cm with at least two other asymmetrical body parts (one non-face)
Feeding difficulties and/or low BMI	BMI ≤−2 SD at 24 months or current use of a feeding tube or cyproheptadine for appetite stimulation

SGA – small for gestational age; SD – standard deviation; LLD – leg length discrepancy; BMI – body mass index

* obligatory criteria for clinical diagnosis of SRS

An additional search for patients with possible BWS was also conducted using our hospital electronic health record system. The clinical data of patients with ICD-10 codes Q87.3 (Congenital malformation syndromes involving early overgrowth, including BWS), Q38.2 (Macroglossia), C64 (Malignant neoplasm of kidney, except renal pelvis, only Wilms' tumor), C22.2 (Hepatoblastoma), Q79.2 (Exomphalos) and K42 (Umbilical hernia, only in the case of surgery) were thoroughly examined in search of other BWS symptoms. Persons who got ≥2 points in the clinical scoring system of BWS [Brioude *et al*., 2018] and in whom genetic testing for ImpDis was not performed were invited to the medical genetics clinic, and a molecular genetic analysis of BWS was provided for all interested patients.

4.1.2. Study group of patients selected by the previously published clinical diagnostic scoring systems for SRS and BWS

Altogether, 48 patients with clinical suspicion of SRS or BWS were included in the study group. The patients were selected among the patients investigated in the Children's Clinic and the Department of Clinical Genetics of Tartu University Hospital as well as Tallinn Children's Hospital by clinical geneticists or pediatricians.

For selecting patients with the suspicion of SRS, a slightly modified Bartholdi *et al*. (2009) scoring system (birth weight and length ≤10th centile, relative macrocephaly, postnatal height ≤3rd centile, normal head circumference, normal cognitive development, asymmetry of face, body, and/or limbs, distinctive facial features, and other features such as brachymesophalangy, syndactyly of toes, inguinal hernia, and pigmentary changes) was used. Patients were clinically classified as SRS when the total score was ≥8 points. The difference between this scoring and the Bartholdi *et al*. scoring was that this scoring system gave the patients with facial, body, and/or limb asymmetry 0–3 points, depending on how many body parts were affected, instead of 0 or 3 points.

For choosing patients with the suspicion of BWS, a referral form with Weksberg's major (abdominal wall defect, macroglossia, macrosomia, ear creases or pits, visceromegaly, embryonal tumor, hemihyperplasia, cytomegaly of adrenal fetal cortex, renal abnormalities, positive family history of BWS, and cleft palate) and minor criteria (pregnancy-related findings, neonatal hypoglycemia, nevus flammeus, cardiac anomalies, characteristic facies, diastasis recti, and advanced bone age) [Weksberg *et al*., 2010] was used. Patients were clinically classified as BWS when at least three major findings or two major findings, and one minor finding were present. Some patients were referred as incomplete BWS with one or two positive criteria.

In addition to clinical data obtained from referral forms, the hospital electronic database was used to get additional clinical information about the patients to correct and/or add the data. Fenton's intrauterine growth curves [Fenton, 2003] and Estonian age- and gender-specific growth curves [Uibo *et al*., 2010] were

used to evaluate and correct growth parameters at birth and later. The age and growth parameters were corrected until two years of age in all children born <32 gestational weeks.

In all 48 patients with clinical suspicion of SRS or BWS, a MS-MLPA analysis of 11p15.5 region was performed at first. To the majority of patients with typical phenotype for SRS and BWS, who met the diagnostic criteria according to the above-mentioned scoring systems for SRS and BWS, a MS-SNuPE assay and/or MS-MLPA analysis of imprinted loci in chromosomes 6, 7, and 14 were performed. The aim of these analyses was to exclude MLMDs or single alterations in other imprinted regions, as well as UPD(7) or other chromosomes among these patients.

In addition, the parents of patients with abnormal methylation status were also studied for methylation defects in the 11p15.5 region. Ten healthy and normal-stature individuals as references were tested for the 11p15-imprinted region by MS-MLPA.

4.2. Molecular methods

4.2.1. Methylation-specific polymerase chain reaction

From 1998 to 2012 all patients with clinical suspicion of PWS or AS were tested using MS-PCR. The analysis was performed with the primers SNRPN-common (5′-CTC CAA AAC AAA AAA CTT TAA AAC CCA AAT TCC-3′), SNRPN-Mat (5′-TAT TGC GGT AAA TAA GTA CGT TTG CGC GGT C-3′) and SNRPN-Pat (5′-GTG AGT TTG GTG TAG AGT GGA GTG GTT GTT G-3′). PCR products were analyzed using 2.5% agarose gel electrophoresis with TBE buffer.

4.2.2. Cytogenetic and fluorescence in situ hybridization analyses

Patients with a positive MS-PCR reaction were additionally studied using a FISH DNA probe for the PWS/AS region at 15q11–13 (D15S63, SNRPN/imprinting center) and a 15q telomere-specific control probe (154P1) (Cytocell Ltd., Oxfordshire, UK). Translocations involving chromosomes 14 and 15 were tested using standard GTG-banding of peripheral blood chromosomes and FISH analysis with probes specific for regions 14cen (D14Z1/D22Z1), 15p11.2 (D15Z1 classical satellite), 15cen (D15Z alphoid DNA), 15q11.2–q12 (SNRPN), 15q22 (PML), 15q13.1 (spanning from 25,725,185 to 25,892,792 Mb) and 15q26 (D15S207). At least 15 metaphases were usually investigated for each case per analysis.

4.2.3. Chromosomal microarray

CMA was introduced into clinical practise in Estonia in 2010 as a first-tier diagnostic genetic test for patients with unclear DD and/or congenital anomalies [Zilina *et al*., 2014b]. As a secondary test, CMA was performed in patients with a high suspicion of specific ImpDis in whom the first-tier genetic testing was negative or with a specific suspicion of copy number variation. The analysis was carried out using a 300,000-SNP HumanCytoSNP-12 v2.1 BeadChip (Illumina, Inc., San Diego, CA, USA) and analyzed using GenomeStudio software (Illumina, Inc.). The cnvPartition plugin (Illumina Inc.) for GenomeStudio was used to detect long contiguous stretches of homozygosity and isodisomic chromosomal regions, with the minimum region size set to 5 Mb.

4.2.4. Uniparental disomy analysis

Until 2010, the test for UPD(15) was performed by microsatellite markers analysis using a PCR with fluorescently labelled oligonucleotides and the following set of microsatellites from outside of the PWS/AS critical region: D15S123, D15S153, D15S125, D15S131, D15S100, D15S211. Since 2010 UPD analysis was, if needed, carried out by comparative analysis of the SNPs using the CMA (described above) results of the patient and at least one of his/her parents.

4.2.5. MS-MLPA analysis of PWS/AS, BWS/SRS, UPD(6, 7, 14) and *GNAS* loci

MS-MLPA analysis of the region 11p15.5 has been a golden standard in the molecular diagnostics of BWS and SRS in Estonia since 2010. Most of the patients with suspected BWS or SRS born in 1998–2017, in whom only methylation-specific single nucleotide primer extension (MS-MLPA) analysis of chromosomal region 11p15.5 was performed and returned negative, were additionally tested for methylation defects and CNVs in imprinted regions 6q24, 7p12, 7q32 and 14q32 (UPD(6, 7, 14) MS-MLPA test). The analysis was done using patients' DNA archived in the molecular diagnostics laboratory. Only patients without alternative molecular diagnoses were involved in the testing. The PWS/AS MS-MLPA was implemented in the molecular diagnostics laboratory of Tartu University Hospital in 2012, UPD (6, 7, 14) MS-MLPA in 2015 and the *GNAS* locus MS-MLPA in 2017. These analyses were performed using SALSA® MS-MLPA® probemixes ME030 BWS/SRS, ME028 PWS/AS, ME032 UPD7-UPD14 and ME031 GNAS (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's instructions. PCR products were analyzed on capillary sequencer using the Genescan software (ABI 3130XL Genetic Analyzer; Applied Biosystems, Darmstadt, Germany). MLPA data

analysis was performed with the Coffalyser software (MRC-Holland). Expected normalized values for copy number analysis were 1.0 (range 0.85–1.15) in absence of any change and 0.5 or 1.5 in case of a heterozygous deletion or duplication, respectively. For methylation status analysis, the expected methylation index for normal individuals was 0.5.

4.2.6. Methylation-specific single nucleotide primer extension assay

The MS-SNuPE assay was performed in 18 patients with different growth disorders and dysmorphic features in suspicion of ImpDis. This assay is based on the ABI PRISM® SNaPshot® Multiplex Kit (Applied Biosystems, Darmstadt, Germany). PCR products were analyzed on capillary sequencer using the GeneMapper® software (AB 3130 Genetic Analyzer; Applied Biosystems, Darmstadt, Germany). The MS-SNuPE assay allowed the simultaneous characterization of 10 imprinted loci in five chromosomes (*PLAGL1*, 6q24.2; *IGF2R*, 6q25.3; *GRB10*, 7p12.1; *MEST*, 7q32.2; *H19*, *KCNQ1OT1*, and *IGF2* in 11p15.5; *MEG3* and *MEG3-DLK1*:IG-DMR in 14q32.2; *SNRPN*, 15q11.2) [Gonzalgo, and Liang, 2007; Begemann *et al*., 2012a].

4.2.7. *CDKN1C* gene sequencing

Sequencing of *CDKN1C* was carried out in patients with clinical suspicion of BWS and in some patients with features of SRS in whom MS-MLPA and MS-SNuPE returned with normal results. Primers for the amplification of *CDKN1C* gene were designed with the help of the Primer 3 software (available from URL: http://bioinfo.ut.ee/primer3/). Exon 1 and exon 2 of the *CDKN1C* gene were amplified by PCR and sequenced directly using standard methods.

4.2.8. Next generation sequencing and whole exome sequencing analyses

NGS panel analysis was performed using the TruSight One (TSO) panel (Illumina Inc., San Diego, California) in patients with a clinical suspicion of ImpDis caused by a pathogenic variant in a specific gene or in cases of other diagnostic hypotheses. The TSO panel includes 125,396 probes aimed to capture 11,946,514-bp targeted exon regions. These regions consist of 4,813 genes, which according to Online Mendelian Inheritance in Man database (OMIM, http://www.omim.org/), are associated with known genetic disorders or clinical phenotypes. A trio ES on an Illumina HiSeq platform and later data reanalysis by Genomics Platform at the Broad Institute of Harvard and MIT (Broad Institute, Cambridge, MA, USA) was performed in one patient. Confirmation and segregation of variants detected by the TSO panel or ES was performed by routine Sanger sequencing in the proband and his/her parents.

4.2.9. Statistical analysis

The live birth prevalence of PWS, AS, BWS, SRS and PHP/PPHP was calculated by dividing the number of live births in Estonia during the period 2004–2016 by the total number of patients with these ImpDis born within the same period. We excluded all the cases born before 2004 and in the last two years (2017–2018) from the prevalence calculations as some of the patients born in these years may have had the diagnosis of ImpDis made at a later age. According to Statistics Estonia, there were 190,387 live births from 2004–2016. Live birth prevalence was estimated via the Generalized Linear Model Analysis using GENMOD procedure of the SAS system, Release 8.2 (SAS Institute, 1999). The distribution of the prevalence cases was assumed to be binomial, and the default logit link function was used. The only variable factor in the model was the observation year. The mean (expected) prevalence rate for a given year and a corresponding 95% confidence interval were predicted with the OUTPUT statement of the GENMOD procedure. Differences were considered statistically significant if the p-value was less than 0.05. The analysis included individuals diagnosed with PWS, AS, BWS, SRS and PHP/PPHP.

4.3. Ethics

All procedures performed were in accordance with the ethical standards of Tartu University Hospital and Research Ethics Committee of the University of Tartu (approval date 03/16/2015 (246/T-13), 12/19/2016 (265/M16) and 11/20/2017 (275/M-11_4)). Written informed consent was obtained from the eligible patients, parents or legal guardians of the children.

5. RESULTS AND DISCUSSION

5.1. The frequency of genetic and methylation abnormalities among Estonian patients selected by the previously published clinical diagnostic scoring systems for SRS and BWS (Paper I)

In this study, we evaluated the frequency of genetic and methylation abnormalities at the chromosomal region 11p15.5 among Estonian patients who were selected according to the previously published clinical diagnostic scoring systems for SRS [Bartholdi *et al*., 2009] and BWS [Weksberg *et al*., 2010]. We did not have any preselection and we studied all the patients referred with clinical suspicion of SRS or BWS, as there exists a recommendation for both syndromes to look for molecular abnormalities in every patient with a suspicion of SRS or BWS, even if the clinical picture is incomplete.

5.1.1. Patients with clinical suspicion of SRS

Out of 48 patients in the study group, 20 patients (15 girls, 5 boys) were referred with clinical suspicion of SRS. The most frequent findings in these patients were poor postnatal growth (18/20, 90%), low birth weight (17/20, 85%) and height (16/20, 80%), prominent forehead (16/20, 80%), clinodactyly of fifth finger (13/20, 65%), and triangular face (13/20, 65%). Thirteen of the patients had at least eight positive features according to the Bartholdi *et al*. (2009) scoring system and therefore met the criteria required for the clinical diagnosis of SRS. The score ranged from 8 to 14 in individuals with clinical SRS and from 6 to 14 in all patients from this study group (maximal score in this scoring system=15). All patients with clinical diagnosis of SRS demonstrated poor postnatal growth and prominent forehead, 92% (12/13) had low birth weight and height, 85% (11/13) clinodactyly of fifth finger, 77% (10/13) triangular face, 69% (9/13) facial, body or limb asymmetry, 54% (7/13) relative macrocephaly, and 31% (4/13) genital abnormalities. Cognitive DD was reported in 38% (5/13) of patients with clinical SRS, and in 40% (8/20) of all patients from the SRS group.

All referred 20 patients and 10 controls were blind-tested by MS-MLPA of 11p15.5 at least twice. Molecular diagnostics confirmed the SRS diagnosis in 5 of 13 patients (38%) clinically scored as SRS patients. Four patients, including two sisters described previously [Õunap *et al*., 2004], had LOM at IC1, and one patient had a familial 1.3 Mb duplication in 11p15.5–p15.4 involving both IC1 and IC2 [Vals *et al*., 2015a]. None of the patients with the clinical suspicion of SRS (including four patients with LOM at IC1), to whom MS-SNuPE or UPD (6, 7, 14) MS-MLPA analyses were done, showde UPD(7), MLMD, and/or single alterations in other imprinted regions. The clinical score of molecularly

confirmed SRS cases ranged from 9 to 14 points. None of the patients with a score <8 points had an alteration in 11p15.5.

All 10 control samples were tested repeatedly (n=45) and gave all reproducible results with all the probes both for copy number and methylation analysis, except the probe 08745–L08765, which was noninformative (mentioned also by the manufacturer). The mean methylation indices for the normal reference samples were 0.56 (SD=0.03; range 0.48–0.60) for four MS-MLPA probes at IC1 and 0.61 (SD=0.04; range 0.57–0.68) for four MS-MLPA probes at IC2. Compared to the control group, all four SRS patients with LOM at IC1 showed methylation between 0.11 and 0.40 in all four analyzed MS-MLPA probes for IC1, whereas the methylation ratios for IC2 were in the normal range, similar to controls. MS-MLPA CNV analyses of these patients were normal.

The investigation of the SRS patient with a 1.3 Mb duplication in 11p15.5 revealed an increase in CNV ratios in altogether 26 MLPA probes in the whole analyzed 11p15.5 region. The mean ratio of these probes was 1.38 (SD=0.13, probe ratios >1.3 are regarded as indicative of a heterozygous duplication). This result was confirmed by CMA that revealed a 1.3 Mb duplication on 11p15.5–p15.4 (hg19: 1,849,354–3,116,073).

We also studied the parents of four SRS patients. All the parents, except one, had normal CNV and methylation analysis. The mother and maternal grandfather of a patient with duplication on 11p15.5–p15.4 and SRS syndrome have also the same duplication, but with opposite methylation pattern and clinical diagnosis of BWS [Vals *et al*., 2015a].

There are at least six different clinical diagnostic scoring systems for SRS published in the literature [Lai *et al*., 1994; Price *et al*., 1999; Netchine *et al*., 2007; Bartholdi *et al*., 2009; Dias *et al*., 2013; Azzi *et al*., 2015]. All these scoring systems display some similarities, but are based on different clinical criteria which are also weighted differently. The comparison between studies using different clinical scoring systems is therefore problematic. In our study group, 38% of SRS patients with positive Bartholdi *et al*. (2009) scoring had abnormal methylation in chromosome 11p15.5. This result is very similar to those obtained by Bartholdi *et al*. (2009) herself, who found abnormal methylation at 11p15.5 in 39% (41/106) of patients with clinical diagnosis of SRS. However, the total diagnostic rate was higher (45%) in the Bartholdi *et al*. study because yet 7% (7/106) of her patients with clinical SRS showed a maternal UPD(7). In 2007, Netchine *et al*. proposed a new scoring system with fewer subjective criteria that was revised and modified in 2015 by Azzi *et al*. This new scoring system, called NH-CSS (Table 5), demonstrated significantly higher positive predictive value. In the NH-CSS cohorts, the frequency of patients with LOM at IC1 or maternal UPD(7) is >75% [Azzi *et al*., 2015]. During our study, we did not use the NH-CSS as the clinical diagnostic scoring system had not been developed and approved yet by international expert consensus [Wakeling *et al*., 2017] at the time of this study.

Reanalysis of our inital SRS group demonstrated that only 54% (7/13) of the patients with positive Bartholdi *et al*. (2009) scoring, including four patients with molecular alteration at 11p15.5, met the criteria for clinical diagnosis of SRS using NH-CSS. The detection rate in this case is significantly higher (4/7, 57%). However, in our cohort of patients with clinical diagnosis of SRS relative macrocephaly at birth, an obligatory criterion for clinical diagnosis of SRS in NH-CSS, was not very common (7/13, 54%). Although relative macrocephaly was presented only in patients with positive NH-CSS criteria, one of five patients with molecularly confirmed SRS (LOM at IC1) did not have this criterion and would be classified as non-SRS if a NH-CSS was used in our study. Moreover, one patient from the SRS group without relative macrocephaly at birth and with negative Bartholdi *et al*. scoring (score of 6), who was not therefore tested by MS-SNuPE or UPD (6, 7, 14) MS-MLPA during this study, demonstrated a maternal UPD(7) in the retrospective study of the further epidemiological research. Generally, it seems that among our cohort, NH-CSS is more effective comparing with previously published clinical diagnostic scoring systems, however, false negative results are also possible. Hence it can be concluded that the NH-CSS should be preferred for the clinical diagnosis of SRS, however, it must be combined with molecular diagnostic methods.

5.1.2. Patients with clinical suspicion of BWS

Totally, 28 patients (16 girls, 12 boys) were referred with clinical suspicion of BWS. The most frequent major findings were macrosomia (13/28, 46%), hemihyperplasia (9/28, 32%), distinctive ear features (9/28, 32%), and macroglossia (7/28, 25%). The most frequent minor finding was characteristic facies (13/28, 46%). There were no individuals with embryonal cancer in this group. Interestingly, most of our BWS group patients were referred because of macrosomia, but some of them did not have previously defined overgrowth and their actual clinical score did not meet the required minimum. Also, some patients had weight, but not height >97th centile that refers to simple overweight or obesity.

After the correction of data, only 12 of the referred patients met the Weksberg's criteria [Weksberg *et al*., 2010] required for clinical diagnosis of BWS. The number of major and minor criteria in individuals with clinical BWS ranged from two major plus one minor criteria to five major plus five minor criteria (maximal possible in this scoring system is 11 major plus 7 minor criteria). The most frequent clinical findings in patients who met the Weksberg's criteria were macrosomia (9/12, 75%), macroglossia (7/12, 58%), characteristic facies (7/12, 58%), distinctive ear features (5/12, 42%), and visceromegaly (5/12, 42%).

All 28 patients referred with clinical suspicion of BWS and 10 controls were blind-tested by MS-MLPA of 11p15.5 at least twice. Only one patient had IC2 hypomethylation in chromosome 11p15.5 (1/12, 8%) with methylation indices

between 0.13 and 0.25. BWS was clinically diagnosed in a male patient at the age of 1.5 and molecularly confirmed at the age of 7. He had two major findings (macroglossia and macrosomia) and three minor findings (neonatal hypoglycemia, congenital heart anomaly, and characteristic facies). Birth parameters were within normal limits. Both parents of the patient had normal CNV and methylation analyses at 11p15.5.

MS-SNuPE analysis revealed an unexpected hypomethylation of the *PLAGL1* (6q24) and *IGF2R* (6q25) genes in the patient with the highest BWS scoring (five major plus five minor criteria). The same result was obtained using UPD (6, 7, 14) MS-MLPA and DNA from the patient's blood, fibroblasts and buccal swab. It was assumed that the patient has paternal heterodisomy of chromosome 6, but comparative analysis of the SNPs using her mother's CMA results, excluded any UPD. Hypomethylation of *PLAGL1* should result in 6q24-related TNDM [Docherty *et al*., 2013; Temple *et al*., 2015]. Although the patient did not have diabetes in the neonatal periood, it is known that TNDM can first manifest later in life as insulin resistance or gestational diabetes [Boonen *et al*., 2013]. Moreover, some symptoms of TNDM such as macroglossia and omphalocele overlap with symptoms of BWS and this circumstance can explain several BWS-associated phenotypic features of this patient. MS-SNuPE and UPD (6, 7, 14) MS-MLPA analyses did not show any CNV or methylation alterations in other patients from the BWS group, although a Coffin–Siris syndrome caused by *ARID1B* gene variant was later diagnosed by ES analysis in one patient with obesity, macrocephaly, hepatomegaly and hyperinsulinism [Vals *et al*., 2014].

Compared to other studies [Gaston *et al*., 2001; Bliek *et al*., 2009; Calvello *et al*., 2013; Mussa *et al*., 2013; Ibrahim *et al*., 2014], our detection rate of epigenetic alterations in chromosome 11p15 was considerably lower in the BWS group. In our BWS group, of all the patients who met the clinical criteria, only one patient was diagnosed with IC2 hypomethylation (1/12, 8%), whereas in other studies, the detection rate for molecular abnormalities at 11p15.5 has been 28–72% [Gaston *et al*., 2001; Calvello *et al*., 2013; Mussa *et al*., 2013; Baskin *et al*., 2014; Eggermann *et al*., 2014a].

As in the case of SRS, multiple clinical diagnostic scoring systems have been proposed for BWS. Six different BWS scoring systems have been previously published in the literature [Elliott, and Maher, 1994; DeBaun, and Tucker, 1998; Gaston *et al*., 2001; Zarate *et al*., 2009; Weksberg *et al*., 2010; Ibrahim *et al*., 2014]. In 2018, a new clinical diagnostic scoring system was developed by an international consensus statement (Table 4) [Brioude *et al*., 2018]. The goal of this scoring system was to recognize that BWS falls into a clinical spectrum and that some features that have long been considered to be classical parts of BWS are not present in every patient.

Clinical reanalysis of our BWS group showed that only 42% (5/12) of the patients with clinical BWS according to Weksberg's criteria, including the only patient with molecularly confirmed BWS, met the criteria for clinical diagnosis of classical BWS using the new diagnostic scoring system (score of ≥4).

However, the detection rate remains still low (1/5, 20%). This result is also in contradiction with the statement that the positive predictive value of the new scoring system is 80.4% [Brioude *et al*., 2018]. Interestingly, the only BWS patient with LOM at IC2 had a score of only four in the new diagnostic scoring system. At the same time, according to this scoring system 38% (6/16) of those patients who did not meet the Weksberg's criteria merit genetic testing for investigation and diagnosis of BWS (score of ≥2) and in the case of negative genetic testing should be considered to a BWS expert for further evaluation. So it seems that there is currently no ideal clinical scoring system for BWS that has high detection rate, sensitivity and specificity, although this conclusion could be influenced by the low number of molecularly confirmed BWS cases in our study group.

5.2. The prevalence of the most common imprinting disorders in Estonia (Paper II)

From 1998 to the end of the study period, a total of 984 patients were tested for ImpDis in Estonia. Among them, 656 patients were tested for PWS/AS, 233 for BWS/SRS and 95 for other ImpDis. Genetic or epigenetic alterations were identified in 6.4% of all the performed PWS/AS tests and in 7.7% of BWS/SRS tests. Altogether, 7.9% of all the tested patients were diagnosed with molecularly confirmed ImpDis.

Eighty seven individuals with ImpDis were identified: 27 (31%) of them had PWS, 15 (17%) AS, 15 (17%) SRS, 12 (14%) BWS, 10 (11%) PHP or PPHP, 4 (5%) CPP, 2 (2%) TS14, 1 (1%) TNDM and 1 (1%) MDS. No cases of KOS, UPD(20)mat or other rare ImpDis have been found (Table 6).

The age at diagnosis varied from the prenatal period to 83 years of age. Prenatal diagnosis was made in one case of *GNAS* variant-related familial PHP and in one case of UPD-caused PWS. An 83-year-old individual with BWS was a grandfather of a child with SRS and familial 1.3 Mb duplication on 11p15.5–p15.4 found in the previous study [Vals *et al*., 2015b]. Of all symptoms of BWS, this patient had probably only macrosomia and characteristic facies, and was identified due to segregation analysis of this duplication [Vals *et al*., 2015a]. The mean age at which a molecular diagnosis of the most common and classic ImpDis was made has decreased over time. In our case, the difference between the mean age of diagnosis of PWS for patients born in the periods 1998–2008 (1.31 years, range: 30 days to 8 years, N=12) and 2009–2018 (0.06 years or 22 days, range: prenatally to 30 days, N=9) was clinically important and notable, but not statistically significant, likely owing to the small sample size (P=0.2641).

Table 6: ImpDis diagnosed in Estonia since 1998 up to the end of this study. Adapted from [Yakoreva *et al*., 2019].

Imprinting disorder	Acronym	Number of patients	% of all ImpDis
Prader-Willi syndrome	PWS	27	31
Angelman syndrome	AS	15	17
Silver-Russell syndrome	SRS	15	17
Beckwith-Wiedemann syndrome	BWS	12	14
Pseudo- and pseudopseudo-hypoparathyroidism	PHP/PPHP	10	11
Central precocious puberty	CPP	4	5
Temple syndrome	TS14	2	2
Transient neonatal diabetes mellitus	TNDM	1	1
Myoclonus-dystonia syndrome	MDS	1	1
Kagami-Ogata syndrome	KOS	0	0
Maternal uniparental disomy of chromosome 20	UPD(20)mat	0	0
Schaaf-Yang syndrome	SYS	0	0
Birk-Barel syndrome	–	0	0
ImpDis – imprinting disorders	Total	87	

Only patients with molecularly confirmed diagnosis were included in our PWS and AS group. Out of 27 patients with PWS, deletion of a paternal chromosomal region 15q11–q13 was present in 11 (41%), maternal UPD(15) in 9 (33%) and Robertsonian translocation involving chromosome 15 in one patient (4%). The remaining six patients (22%) had a positive MS-PCR test but tested negative for deletion of 15q11–q13 by FISH and were assumed to have either maternal UPD(15) or other less common alterations. Unfortunately, parental DNA was not available for further investigations in these cases. The leading molecular cause of AS was a maternal 15q11–q13 deletion found in 11 patients (73%). Two (13%) patients with atypical AS had an isolated methylation defect in the region, one patient had paternal UPD(15) and one pathogenic heterozygous variant c.281C>G, p.(Ser94*) in the maternal copy of the *UBE3A* gene (RefSeq NM_000462.3).

One third of all SRS (5/15) and BWS (4/12) patients fulfilled the new clinical diagnostic criteria for these disorders approved by international expert consensus [Brioude *et al*., 2018; Wakeling *et al*., 2017] but were negative for genetic abnormalities. Among the remaining 10 molecularly confirmed SRS patients, hypomethylation of IC1 was found in seven (70%), UPD(7) in two

patients and duplication of the maternal 11p15.4–p15.5 region in one patient. Hypomethylation of IC2 was detected in three (37.5%) BWS patients. The mosaic paternal UPD of chromosome 11 had the same frequency of 37.5% (3 cases) and duplication of paternal 11p15.4–p15.5 region was found in two (25%) BWS cases.

Out of a total 10 PHP/PPHP patients, the molecular cause for five patients with PHP and three patients with PPHP was a pathogenic missense variant in *GNAS* – c.103C>T, p.(Gln35*) (RefSeq NM_000516.5), c.2229A>C, p.(Lys743Asn) (RefSeq NM_080425.3) or c.2234C>T, p.(Ala745Val) (RefSeq NM_080425.3). The molecular cause of the two remaining PHP patients was abnormal methylation of DMRs at the *GNAS* locus. One of them had an isolated GOM at *GNAS-NESP*:TSS-DMR (methylation index 1.0) and LOM at *GNAS-AS1*:TSS-DMR, *GNAS-XL*:Ex1-DMR and *GNAS A/B*:TSS-DMR (methylation indices between 0.1–0.2). Another PHP patient had secondary LOM at *GNAS A/B*:TSS-DMR caused by a small heterozygous deletion in the *STX16* gene (exons 5 and 6).

All four patients with CPP were the members of a single large family and had the same heterozygous paternally inherited variant c.326G>A, p.(Cys109Tyr) in *MKRN3* (RefSeq NM_005664.3). Both cases of TS14 had a concurrent trisomy – one patient with triple X syndrome and the other with mosaic trisomy 14 – in addition to maternal UPD(14) [Yakoreva *et al*., 2018]. The only patient with TNDM had atypical clinical presentation and isolated hypomethylation of the *PLAGL1* and *IGF2R* genes [Vals *et al*., 2015b]. The only case of MDS was caused by a paternal heterozygous nonsense variant – c.21G>A, p.(Trp7*) – in *SGCE* (RefSeq NM_003919.2).

The total number of molecularly diagnosed ImpDis cases per year in Estonia is very variable (0–8 cases per year), but the general upward tendency is evident (Figure 2). All patients with PHP/PPHP, CPP, TS14, TNDM, MDS and most patients with SRS and BWS were diagnosed in the last six years. The percentage of these rare ImpDis dramatically increased during the last few years (Figure 3). Figure 3 illustrates the change of proportion of diagnosed ImpDis during 1998–2016, 1998–2017 and 1998–2018, respectively. All these changes, indicating an increase in diagnostic rate of ImpDis, are due to improved diagnostic methods and increased awareness of physicians.

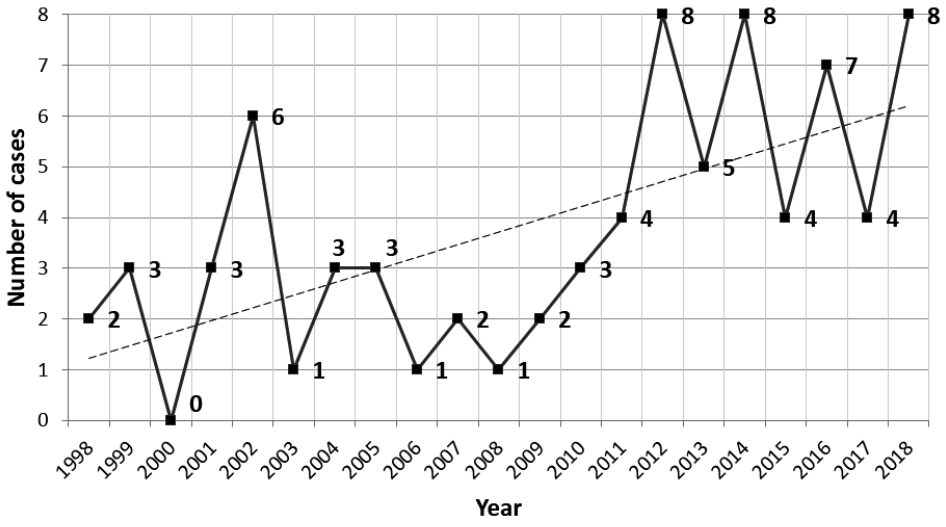

Figure 2: Number of molecularly diagnosed cases of ImpDis by year in Estonia from 1998 to 2018. The steady line shows the number of ImpDis cases and the dashed line the general tendency.

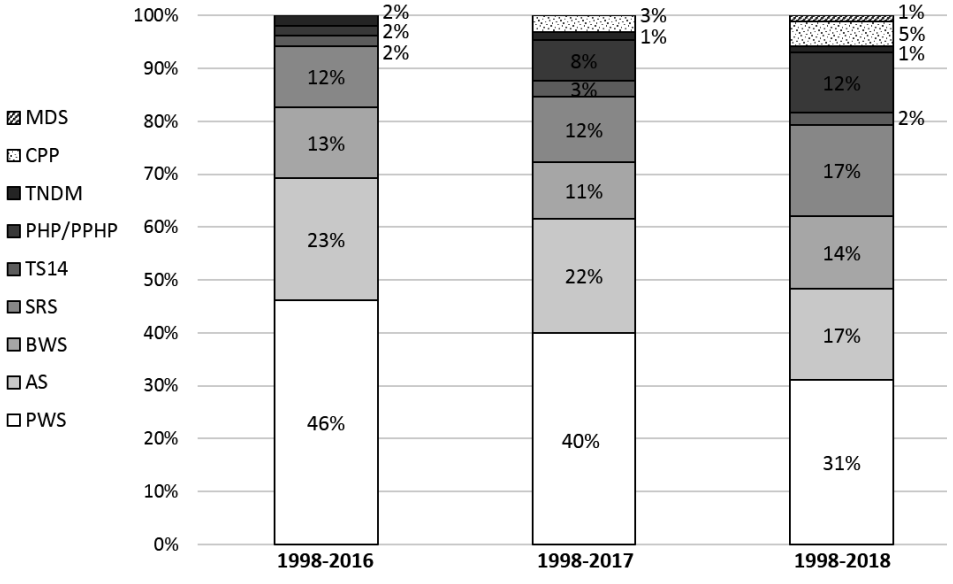

Figure 3: Diagrams showing the percentage of all molecularly and clinically diagnosed ImpDis in Estonia during the periods 1998–2016, 1998–2017 and 1998–2018. Note the important increase in percentage of rare ImpDis (PHP/PPHP, CPP, TS14, TNDM, MDS) during the last few years.

Seventy-six individuals with ImpDis were alive as of January 1, 2018, indicating the total prevalence of ImpDis in Estonia is 5.8/100,000 (95% confidence intervall (CI) 4.6/100,000–7.2/100,000). The live birth prevalence of all ImpDis in Estonia in 2004–2016 was 1/3,462 (95% CI 1/2,660–1/4,505), PWS 1/13,599 (95% CI 1/8,101–1/22,828), AS 1/27,198 (95% CI 1/13,175–1/56,147), BWS 1/21,154 (95% CI 1/11,130–40,207), SRS 1/15,866 (95% CI 1/9,076–1/27,734), and PHP/PPHP 1/27,198 (95% CI 1/13,175–1/56,147) (Table 7).

Table 7: The minimum live birth prevalence of the five most common ImpDis and all ImpDis together in Estonia in 2004–2016.

Imprinting disorder	Live birth prevalence	95% confidence interval
Prader-Willi syndrome	1/13,599	1/8,101–1/22,828
Angelman syndrome	1/27,198	1/13,175–1/56,147
Beckwith-Wiedemann syndrome	1/21,154	1/11,130–40,207
Silver-Russell syndrome	1/15,866	1/9,076–1/27,734
Pseudo- and pseudopseudohypoparathyroidism	1/27,198	1/13,175–1/56,147
All imprinting disorders	1/3,462	1/2,660–1/4,505

There was a statistically significant increase in the live birth prevalence of all ImpDis in Estonia during the years 1998–2016 (from 14.95/100,000 live births or 1/6,690 in 1998 to 38.22/100,000 or 1/2,616 in 2016, P=0.027) (Figure 4A). There was no statistically significant increase in the live birth prevalence of PWS (from 7.43/100,000 live births or 1/13,462 in 1998 to 7.57/100,000 or 1/13,217 in 2016, P=0.9806) (Figure 4B), AS (from 2.29/100,000 live births or 1/43,691 in 1998 to 4.68/100,000 or 1/21,386 in 2016, P=0.5331) (Figure 4C) and PHP/PPHP (from 2.51/100,000 live births or 1/39,809 in 1998 to 3.52/100,000 or 1/28,433 in 2016, P=0.7792) (Figure 4F) during the last 19 years. However, there was a significant increase in the live birth prevalence of BWS (from 0.52/100,000 live births or 1/92,456 in 1998 to 11.56/100,000 or 1/8,654 in 2016, P=0.0206) (Figure 4D) in Estonia during the years 1998–2016. The live birth prevalence of SRS in Estonia also increased during this period (from 2.84/100,000 live births or 1/35,157 in 1998 to 9.52/100,000 or 1/10,505 in 2016), but this trend was not statistically significant (P=0.1851) (Figure 4E).

We have determined the prevalence of all ImpDis in a single, nationwide population. To the best of our knowledge, this is the first epidemiological study to estimate the birth prevalence and the population prevalence of all ImpDis in the same population at the same time. Despite the fact that the total prevalence of all ImpDis is unknown due to the rarity of these disorders and the absence of systematic studies, it is thought that ImpDis are very rare disorders. However, we found the overall prevalence of ImpDis in Estonia to be 5.8/100,000 (1/17,132).

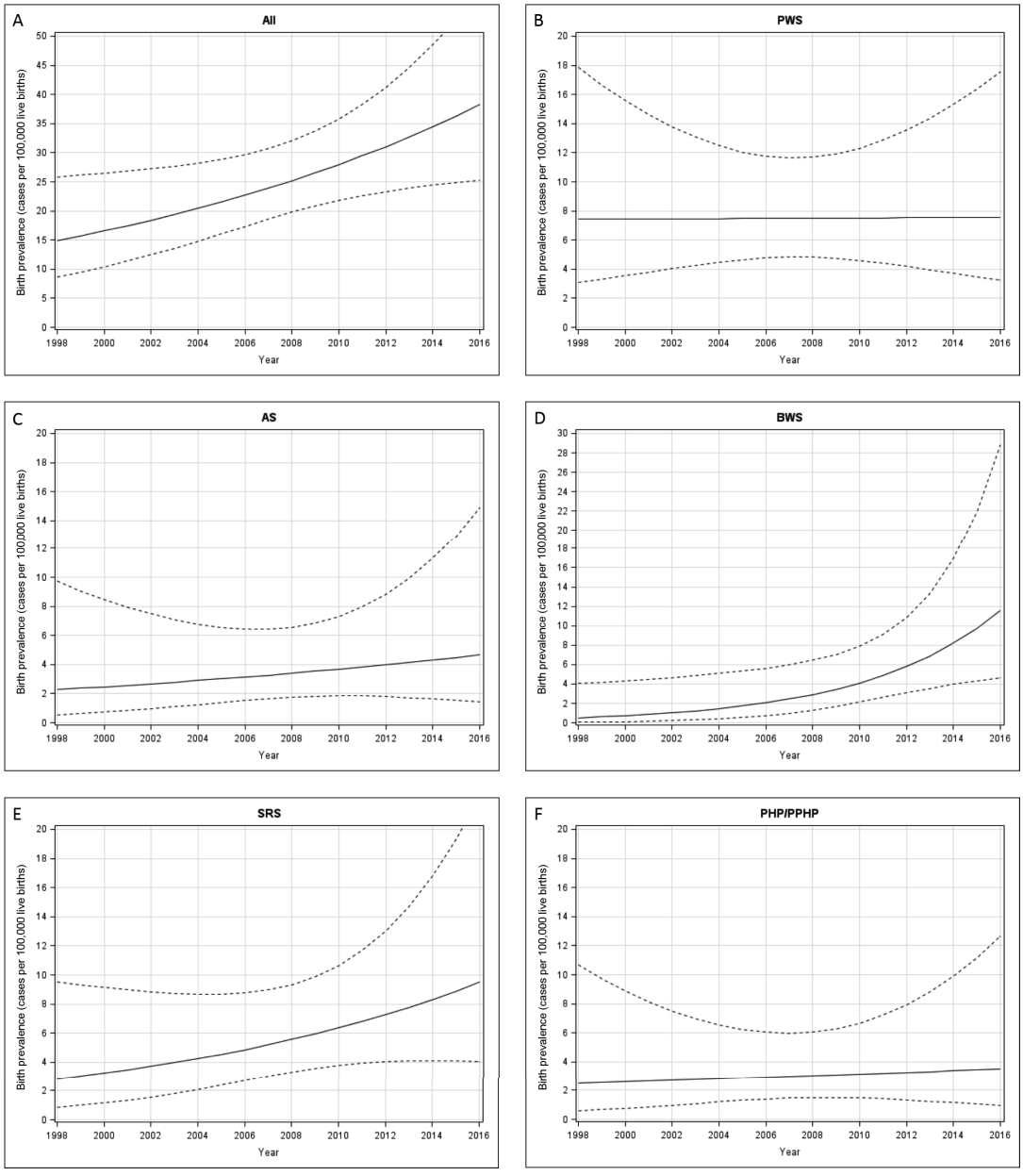

Figure 4: Distribution of live birth prevalence of all ImpDis (A), PWS (B), AS (C), BWS (D), SRS (E) and PHP/PPHP (F) in Estonia from 1998 to 2016 by statistical logit analysis. The solid line represents birth prevalence and the dashed lines the 95% confidence interval. Note a statistically significant increase in the live birth prevalence of all ImpDis (P=0.027) and BWS (P=0.0206). There was no statistically significant increase in the live birth prevalence of PWS (P=0.9806), AS (P=0.5331), SRS (P=0.1851) and PHP/PPHP (P=0.7792) during this period.

Compared to the prevalence of the most common metabolic disorders, such as mitochondrial disease (1/5,000) [Parikh *et al*., 2017] or phenylketonuria (1/10,000) [van Wegberg *et al*., 2017], ImpDis are only 2–3 times less prevalent. Taking into account the birth prevalence of all ImpDis in Estonia in the last 13 years (1/3,462 during the period 2004–2016) it can be concluded that while each ImpDis, taken separately, is very rare, all ImpDis together are relatively common. As there was also a significant increase in the live birth prevalence of all ImpDis in Estonia in the last 19 years (Figure 4A), the total prevalence of all ImpDis in Estonia may further increase in the future.

The birth prevalence of PWS found in earlier publications is variable (1/7,937–1/30,439) (Table 2) and the prevalence of PWS found in this study (1/13,599) is one of the highest. However, our result is similar to that found by the most recent epidemiological studies of PWS performed by Lionti *et al*. (2015), Richard-De Ceaurriz *et al*. (2017) and Bar *et al*. (2017), who revealed the birth incidence of PWS to be approximately 1/10,000–20,000 (Table 2). This result also corresponds to a previously estimated worldwide prevalence of PWS 1/10,000–1/25,000 [Eggermann *et al*., 2015a]. Compared to our previous 21-year-study (1984–2004) of PWS in Estonia [Õiglane-Shlik *et al*., 2006a], the birth prevalence of PWS has more than doubled: from 1/30,439 to 1/13,599. However, in 2004, at the end of the previous study a significantly higher and very similar PWS prevalence of 1/12,547 was observed [Õiglane-Shlik *et al*., 2006a]. The statistical analysis also did not reveal any noticeable increase in the live birth prevalence of PWS in Estonia during the years 1998–2016 (Figure 4B). It can be assumed that the molecular diagnostic methods used in the last two decades allowed efficient detection of almost all patients with PWS and the true birth prevalence of the disorder in Estonia is approximately 1/13,000 or 7.7/100,000. Previous studies have demonstrated that the mean age of molecular diagnosis of PWS significantly decreased during the last decade [Thomson *et al*., 2006b; Lionti *et al*., 2015]. For example, Lionti *et al*. (2015) found that in Australia the mean age of molecular diagnosis of PWS decreased from 1.3 years in 1973–1981 to 0.16 years (60 days) in 2003–2012. In our study we saw a similar tendency. For those born between 1998 and 2008, the mean age of molecular diagnosis of PWS was 1.31 years compared to those who were born between 2009 and 2018, where the mean age was 0.06 years (22 days), but this increase was not statistically significant (P=0.2641) due to a small sample size.

We have also shown that the birth prevalence of AS in Estonia is 1/27,198, which is lower than the estimated prevalence of 1/12,000–1/20,000 [Eggermann *et al*., 2015a]. However, it is known that the estimated prevalence of AS is based mostly on studies that included patients with both molecularly confirmed and molecularly unproven diagnosis of AS. The estimation of AS prevalence can therefore be imprecise. Our results are in line with studies by Thomson *et al*. (2006a) and Mertz *et al*. (2013) who found the prevalence of AS to be 1:40,000 in Western Australia and 1:24,580 in Denmark, respectively. Moreover, the obtained result is similar to that received at the end of the previous Estonian study (1/23,640) [Õiglane-Shlik *et al*., 2006a], although the

total AS birth prevalence for the whole period of 1984–2004 was almost two times lower (1/52,181). Our statistical analysis showed an increase in the live birth prevalence of AS during the years 1998–2016 (Figure 4C). However, this trend was not statistically significant. This increase can be explained by the implementation of new diagnostic methods that allowed detection of methylation defects of region 15q11–q13, UPD(15) and the *UBE3A* gene variants in addition to copy number anomalies. The birth prevalence of AS in Estonia is about 1/25,000 or 4/100,000, therefore, as it was shown in the previous Estonian survey [Õiglane-Shlik *et al*., 2006a], our study also does not confirm the general opinion that PWS and AS are equally represented disorders, as the livebirth prevalence of AS was found to be 1.9 times less than that of PWS. The reason for this difference is not clear at present. Some studies have shown that paternal *de novo* variants and CNVs are more prevalent than the maternal ones, and can be explained by high number of mitoses occurring in the sperm cells during male gametogenesis [Acuna-Hidalgo *et al*., 2016; Sibbons *et al*., 2012]. This possibly explains the higher prevalence of paternal *de novo* deletions of chromosomal region 15q11–q13 in the cases of PWS if compared to maternal deletions in AS.

The live birth prevalence of BWS revealed in this study (1/21,154) is about 1.4 times lower than the expected prevalence of 1/15,000 [Eggermann *et al*., 2015a]. However, the prevalence of BWS found in earlier studies varies greatly from 1/10,569 [Mussa *et al*., 2013] to 1/79,520 [Arroyo Carrera *et al*., 1999] and our result falls in the middle of this range. Our study includes patients with both clinical and molecular diagnosis of BWS and the low prevalence of BWS found in Estonia may be in some way associated with the usage of the new scoring system [Brioude *et al*., 2018] that contains more precise and less subjective criteria for the clinical diagnosis of BWS. Our result is also close to the prevalence of 1/26,000 found in the latest population-based study of BWS covering a population of 16 European countries [Barisic *et al*., 2018]. As the prevalence of BWS was unexpectedly low at the beginning of our study, an advanced search for patients with the clinical symptoms of BWS was performed. We assume that we have now likely identified the majority of BWS cases in Estonia and therefore the true live birth prevalence of BWS is 1/21,000 or 4.8/100,000. We have also shown a significant increase in the live birth prevalence of BWS in Estonia from 1998–2016 (Figure 3D), most likely due to both improved diagnostic methods as well as increased awareness of physicians of BWS. Based on our previous experience with PWS, we can assume that the prevalence reached at the end of this study is close to the true prevalence of BWS in Estonia. Interestingly, a noticeable proportion of our patients with molecularly confirmed diagnosis of BWS had unusually mild presentation of the syndrome. Only 63% (5/8) of BWS patients with molecular alterations at 11p15.5 met the new clinical diagnostic criteria for BWS [Brioude *et al*., 2018], and the maximum score in this group of patients was only 5 out of 20 possible points.

We have found an unexpectedly high live birth prevalence of SRS in Estonia (1/15,866), which differs greatly from the proposed SRS prevalence of 1/75,000–1/100,000 [Eggermann *et al*., 2015a]. Even when considering only patients with molecularly confirmed SRS diagnosis, the prevalence is 1/27,198: about three times higher than estimated. To the best of our knowledge, there are no epidemiological studies on the prevalence of SRS and therefore our results are difficult to compare to those of other countries. We have shown an increase in the live birth prevalence of SRS in Estonia during the last 19 years (Figure 4E), but this trend was not statistically significant. The cause of the high prevalence of SRS in Estonia is unclear. We propose that the true SRS prevalence is about 1/16,000, which is higher than the prevalence of both BWS and AS. Notably, as in the case of BWS, only a part of patients (70%, 7/10) with genetically confirmed SRS met the clinical diagnostic criteria of NH-CSS. The maximum score in this group was 6 out of 6 possible. Three other SRS patients lacked relative macrocephaly at birth, an obligatory criterion in NH-CSS, and therefore did not meet requirements for the clinical diagnosis of SRS. However, atypical clinical presentation can possibly be caused by unusual molecular alterations found in these patients – one of them had maternal UPD(7) and two low-level mosaicism (<50%) for LOM at IC1. In general, it seems that the clinical diagnostic criteria for SRS are more sensitive and have higher diagnostic rate compared with the clinical criteria for BWS.

The live birth prevalence of *GNAS* gene-associated ImpDis (PHP and PPHP) found in our study was, similarly to SRS, unexpectedly high. Our prevalence of 1/27,198 is 10 times higher than the prevalence of unspecified PHP 1/294,000 found in Japan [Nakamura *et al*., 2000] and more than three times higher than the prevalence of 1/90,900 in Denmark [Underbjerg *et al*., 2016]. However, it is known that both previous studies included patients with both the clinical diagnosis of PHP and the molecularly confirmed PHP, and the prevalence found in these studies may be inconsistent due to the use of different diagnostic criteria and PHP nomenclature systems. The prevalence of PHP and PPHP in Estonia is the same as the prevalence of AS. There was no statistically significant increase in the live birth prevalence of PHP/PPHP in Estonia during the years 1998–2016 (Figure 4F). As minimal data are available regarding the epidemiology of PHP/PPHP, and as our results may be influenced by the fact that most of our cases are familial (7 patients out of 9 are from two families), the exact prevalence of PHP/PPHP remains unknown. Although we assume that the true prevalence of PHP/PPHP is also higher than estimated. We did not calculate the prevalence of other less common ImpDis due to the small number of patients identified.

To the best of our knowledge, this is the first epidemiological study to estimate the birth prevalence of all ImpDis in a single population. We have demonstrated that the true prevalence of some ImpDis, like SRS and PHP/PPHP, is significantly higher than previously estimated, and that the live birth prevalence of all ImpDis together (1/3,462) can be compared with the prevalence of other relatively common genetic disorders. From the results

obtained in our epidemiological study, we can conclude that there is a statistically important increase in the diagnostic rate of some ImpDis during the last years. Generally, there are three ways to improve the diagnostics of ImpDis: gathering new knowledge about ImpDis, implementing new diagnostic methods, and increasing awareness of ImpDis among medical health professionals. This gives reason for an assumption that the possibilities in the diagnostics of ImpDis are not yet exhausted and the prevalence of many ImpDis may further increase in the future.

5.3. New molecular diagnostic tests for imprinting disorders and their effectiveness in Estonia (Paper I and II)

A broad range of molecular methods and diagnostic tests is now available for ImpDis. Until the last decade, molecular diagnostic tests, such as methylation-sensitive Southern blot hybridization and MS-PCR, were generally restricted to single disease-specific loci. As there is a considerable phenotypic overlap between different ImpDis, the use of a single-locus test can preclude the diagnosis of molecular defects and therefore leave a patient without diagnosis or lead to misdiagnosis. Moreover, many individuals with ImpDis demonstrate MLMDs that can manifest as a broad clinical spectrum and the phenotype can be ambiguous or even atypical of one of the known ImpDis. In the last years, several molecular methods that cover the parallel detection of variants and epimutations at different imprinted loci were therefore implemented in the laboratory practice. These methods include, for example, MS-MLPA, MS-SNuPE, CMA and NGS [Soellner *et al*., 2015]. However, only some of these new molecular methods can be used routinely in clinical diagnostic practice because of their different diagnostic effectiveness, cost-effectiveness and complexity.

In 2014, at the beginning of this study a CMA (300,000-SNP HumanCytoSNP-12 v2.1 BeadChip; Illumina, Inc.) and MS-MLPA of PWS/AS and BWS/SRS loci (SALSA® MS-MLPA® probemixes ME030 BWS/SRS and ME028 PWS/AS; MRC-Holland) have already been in practical use in the molecular laboratory of our department. The BWS/SRS MS-MLPA was implemented in our laboratory in 2010 and PWS/AS MS-MLPA in 2012. Initially there was an idea to introduce a MS-SNuPE analysis based on ABI PRISM® SNaPshot® Multiplex Kit (Applied Biosystems) in our laboratory. This method allow to detect methylation defects and CNVs at multiple different imprinted loci at once (Table 8). However, it turned out that the implementation of this method is complex, labor intensive and not cost-effective due to a low number of patients for whom this genetic test may be indicated in Estonia. Moreover, the resolution of MS-SNuPE analysis is limited to ten specific CpG sites and only the indirect discrimination of CNVs and epimutations is possible by this method [Soellner *et al*., 2015]. Therefore it was decided to use different MS-MLPA kits instead of MS-SNuPE.

The UPD (6, 7, 14) MS-MLPA (SALSA® MS-MLPA® probemix ME032 UPD7-UPD14; MRC-Holland) was implemented in our molecular diagnostics laboratory in 2015 and the *GNAS* locus MS-MLPA (SALSA® MS-MLPA® probemix ME031 *GNAS*; MRC-Holland) in 2017. ME032 UPD7-UPD14 probemix contains probes that target the following imprinted regions: *PLAGL1*, 6q24; *GRB10*, 7p12; *MEST*, 7q32; *DLK1*, *MEG3*, *RTL1*, and *MIR380* in 14q32 (MRC-Holland, product description version A1–01; 16 February 2018). ME031 *GNAS* probemix contains multiple probes for different parts of the *GNAS* complex locus: *STX16*, *GNAS-NESP*:TSS-DMR, *GNAS-AS1*:TSS-DMR, *GNAS-XL*:Ex1-DMR, *GNAS A/B*:TSS-DMR, *GNAS* exons (MRC-Holland, product description version 19; 07 June 2017). It can been seen that these MS-MLPA kits together with previously implemented PWS/AS and BWS/SRS MS-MLPA cover almost all MS-SNuPE sites (Table 8). Furthermore, MS-MLPA analysis is significantly more precise because, unlike the MS-SNuPE, it contains multiple probes for each imprinted gene or locus and in some cases allow to reveal a molecular cause of secondary epimutations.

First diagnostic ES analyses were performed in our department in 2014. In 2015, Illumina's TSO NGS panel (4,813 OMIM genes) was also introduced in our molecular diagnostics laboratory. Since that time, both methods were actively used in clinical diagnostics by medical geneticists, as well as by pediatricians and other medical specialists. These diagnostic methods gave the opportunity to sequence almost all the genes, in which pathogenic variants are implicated in ImpDis. Thus, all variants of *MKRN3*, *GNAS*, *UBE3A* and *SGCE* were found in probands using these two methods. Causative genes for secondary epimutations (*ZFP57*, *NLRP2*, and *NLRP5*) were additionally analyzed using the TSO NGS panel in several patients with methylation defects, however the results were negative. In some patients with clinical suspicion of ImpDis, the TSO NGS panel or ES revealed the diagnosis of other clinically similar but non-imprinting disorder. As the patients with suspicion of ImpDis constituted only a very small part of all the individuals tested by the TSO NGS panel or ES in our laboratory, and these analyses were usually performed only in individuals with a strong suspicion of a specific ImpDis, the exact rate of effectiveness of these methods in the diagnostics of ImpDis is impossible to estimate accurately. Despite this, large NGS panels and ES are undoubtedly very useful and should be further used as diagnostic methods of some ImpDis.

Table 8: Comparison of the imprinted loci covered by MS-SNuPE analysis and different MS-MLPA kits used in our molecular diagnostics laboratory (MRC-Holland, product description 2017–2019).

ImpDis	Molecular analysis / Gene/DMR	MS-SNuPE	BWS/SRS MS-MLPA	PWS/AS MS-MLPA	UPD (6, 7, 14) MS-MLPA	*GNAS* locus MS-MLPA
TNDM	6q24.2: *PLAGL1*	**X**			**X**	
TNDM	6q25.3: *IGF2R*	**X**				
SRS	7p12.1: *GRB10*	**X**			**X**	
SRS	7q32.2: *MEST*	**X**			**X**	
BWS/SRS	11p15.5: *H19*	**X**	**X**			
BWS/SRS	11p15.5: *IGF2*	**X**	**X**			
BWS/SRS	11p15.5: *KCNQ1OT1*	**X**	**X**			
BWS/SRS	5q35, 11p15.5: *NSD1, KCNQ1, CDKN1C*		**X**			
TS14/KOS	14q32.2: *MEG3*	**X**			**X**	
TS14/KOS	14q32.2: *MEG3-DLK1*:IG-DMR	**X**			**X**	
TS14/KOS	14q32: *RTL1, MIR380*				**X**	
PWS	15q11.2: *SNRPN*	**X**		**X**		
PWS/AS	15q11–13: *NIPA1, TUBGCP5, MKRN3, MAGEL2, NDN, SNURF-SNRPN, UBE3A, ATP10A, GABRB3, OCA2, APBA2*			**X**		
PHP/PPHP UPD(20)mat	20q13.32: *GNAS* complex locus					**X**

In our study, PWS/AS MS-PCR and PWS/AS MS-MLPA analyses together revealed genetic or epigenetic alterations in 6.4% (42/656) of all performed PWS/AS tests. This number is significantly lower than those found by Buchholz *et al*., who detected molecular alterations in 26% (30/115) of individuals referred with suspicion of PWS and in 20% (28/143) of individuals with suspicion of AS [Buchholz *et al*., 1998]. Analogically, Varela *et al*. molecularly confirmed the diagnosis in 53% (38/72) of all the patients with the clinical diagnoses of PWS and AS [Varela *et al*., 2002]. At the same time, Davies and Ogilvie found deletion of 15q11–q13 only in 3.7% (11/298) of patients referred with suspicion of AS, though only FISH analysis was performed in this study [Davies, and Ogilvie, 2007]. Other similar studies with less number of tested individuals [Christianson *et al*., 1998; Santa María *et al*., 2001; Poyatos *et al*., 2009; Liu *et al*., 2009; Acs *et al*., 2018] also showed that the detection rate of molecular tests in patients with clinical suspicion of PWS and AS is higher than 20%. However, it is known that the majority of patients in these studies were positive for the clinical diagnostic criteria for PWS or AS. While in our molecular diagnostics laboratory, all patients referred to genetic testing for PWS or AS were molecularly investigated without regard to their clinical presentation. Thus, many referred individuals had only one or few PWS or AS symptoms, for example neonatal hypotonia, obesity, epilepsia or microcephaly. Therefore, the results of studies with carefully selected patients is difficult to extrapolate to daily clinical practice in genetic department. The detection rate of PWS/AS tests in our study (6.4%) is closer to a detection rate of PWS of 10.7% (7/65) and 12% (6/50) found by Tuysuz *et al*. and Richer *et al*., respectively, in children with infantile hypotonia [Richer *et al*., 2001; Tuysuz *et al*., 2014].

The detection rate of BWS/SRS MS-MLPA and UPD(7) MS-MLPA analyses found in this study (7.7%, 18/233) is somewhat higher than those of PWS/AS MS-PCR and PWS/AS MS-MLPA analyses. However, this detection rate is several times lower than those reported in the literature. Thus, Bartholdi *et al*. detected molecular alterations in 30% (60/201) [Bartholdi *et al*., 2009], and Azzi *et al*. (47/69) in 68% [Azzi *et al*., 2015] of all referred patients with suspected SRS who were not selected using diagnostic scoring systems for SRS. However, the detection rate of molecular analyses is lower in the routine clinical practice. For example, in the study by Eggermann *et al*., the general detection rate for molecular disturbances in the patients routinely referred as BWS was 28.6% (40/140) and in patients referred as SRS 19.9% (114/571) [Eggermann *et al*., 2014a]. At the same time, the efficacy of molecular diagnostic tests in the cohort of patients selected by clinical scoring systems for SRS or BWS may be higher than 70% [Azzi *et al*., 2015; Dias *et al*., 2013]. As in the case of PWS and AS, many patients in our study group referred to genetic testing for SRS or BWS had only few features of these disorders, such as SGA, body asymmetry or macrosomia. Therefore, a new clinical diagnostic scoring systems could be used to avoid low detection rates, but they might miss patients with atypical or mild phenotypes not fulfilling the diagnostic scoring criteria. Thus, in our study group only 63% of BWS patients and 70% of SRS patients

with molecularly confirmed diagnosis met the new clinical diagnostic criteria for these disorders [Brioude *et al*., 2018; Azzi *et al*., 2015].

The effectiveness of UPD (6, 7, 14) MS-MLPA and the *GNAS* locus MS-MLPA remains unknown because of a low number of patients tested by these methods in our laboratory at the moment, and the fact that the majority of individuals with detected abnormalities of methylation at imprinted loci in chromosomes 6, 7, 14 or 20 have been found using other molecular methods, or were initially tested in laboratories abroad.

In conclusion, it is obvious that the implementation of new advanced molecular diagnostic tests and methods provide the opportunity to diagnose rare and atypical ImpDis. In addition, the continuing development of molecular technologies gives reason to suppose that even more new ImpDis will be discovered in the future.

5.4. The awareness of imprinting disorders among doctors in Estonia (Paper II and IV)

As ImpDis are very rare disorders, the awareness of them amongst doctors and other medical specialists is not high. During this PhD study, we tried to raise interest in and awareness of ImpDis in Estonia. Thus, several lectures about clinical features, diagnostic methods, and principles of supervision and treatment of ImpDis were given at departments of pediatric neurology, neonatology, endocrinology and general pediatrics in Tartu University Hospital and Tallinn Children's Hospital. Additionally, information about this doctoral study and descriptions of ImpDis were repeatedly provided at national medical meetings in 2014–2018, such as Congress of Estonian Pediatric Society, Gene Forum, Scientific Conference of the Faculty of Medicine of University of Tartu and other. An overview article in Estonian about ImpDis was also published in the local medical journal Estonian Medical Journal (Paper IV). Moreover, lectures about ImpDis were introduced into the curricula of medical students of University of Tartu and resident doctors of Tartu University Hospital.

It is not possible to objectively evaluate changes in awareness of ImpDis amongst Estonian medical specialists achieved as a result of this PhD study, as we did not survey this directly. It can be seen that the number of MS-MLPA tests for the four most prevalent ImpDis ordered from the molecular laboratory of our department by doctors has been constantly rising during the last five years (Figure 5). The detection rate of molecular tests and number of molecularly diagnosed ImpDis cases also increased during this period (Figure 2). It was also noted that some physicians phoned or emailed to consult about their patients with unusual symptoms after the introductory lectures about ImpDis. Moreover, after these lectures, more patients were referred to genetic counselling with suspicion of ImpDis. All these changes probably indicate increased interest in and awareness of ImpDis in Estonia in the last years.

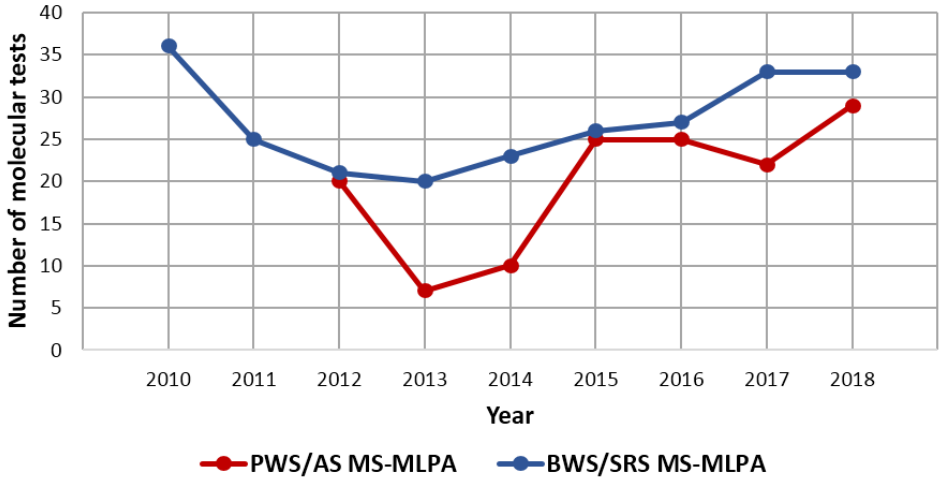

Figure 5: The number of PWS/AS MS-MLPA (red line) and BWS/SRS MS-MLPA (blue line) tests performed in the molecular laboratory of the Department of Clinical Genetics of Tartu University Hospital from 2010 to 2018.

5.5. New rare imprinting disorders in Estonia (Paper III)

5.5.1. Patient with a combination of Temple syndrome and mosaic trisomy 14

The proband, a girl, was originally referred to genetic counseling because of a DD, congenital heart defect, and dysmorphic features. Her family history was unremarkable. Pregnancy was complicated by IUGR and oligohydramnios. An amniocentesis was performed at the 20th week of pregnancy because of fetal hypotrophy and a positive second-trimester screening test for aneuploidies. The fetal karyotype was normal, no trisomic cells or other chromosomal abnormalities were detected in 20 analyzed metaphases. She was born prematurely at the 35th week of gestation by emergency cesarean section because of pathological Doppler sonography. Her birth weight was 1,832 g (–1.5 SD), length 42 cm (–2 SD) and Apgar scores 7 and 8 at 1 and 5 min, respectively [Yakoreva *et al*., 2018].

After birth, an atrioventricular septal defect with congestive heart insufficiency was diagnosed. Pulmonary artery banding was performed at the age of five months. At the same age, a right-sided hemihypertrophy, LLD and increasing linear pigmentation on the arms and legs were noted (Figure 6). During the first years of life, the child had feeding and sucking difficulties and needed partial nasogastric tube feeding until the age of six months. After birth, her height, weight, and head circumference have always been below –2 SD. She was diagnosed with muscular hypotonia, severe psychomotor and cognitive DD, and progressive neuromuscular kyphoscoliosis. At the corrected age of one year and two months, her motor skills were at the 6-month-old level; mental and social

skills were less affected. She began to walk independently at 2.5 years of age. Neither brain magnetic resonance imaging (MRI)/magnetic resonance spectroscopy (MRS) scan nor electroneuromyography revealed any abnormalities. Later, hyperopia and strabismus were also observed [Yakoreva *et al*., 2018].

Neuropsychological testing (NEPSY-II tests), performed at the age of four years and eight months, showed that her cognitive development is at least one year behind and sensorimotor functions, short-term and long-term memory as well as receptive/expressive communication and visual-spatial abilities are below the expected level. At the age of 6.0 years, her height was 93.3 cm (–5 SD), weight 15.6 kg (–2 SD), and head circumference 48 cm (–2.5 SD). She was overweight (BMI >85th centile) and the difference in leg length and thigh circumference was approximately two cm. The girl had some dysmorphic features, such as upslanting palpebral fissures, short philtrum, asymmetrical face, a short neck, bilateral simian crease, small hands and feet as well as genu valgum (Figure 6). X-ray of the left hand revealed a broad thumb, short middle phalanx of the little finger, delayed bone age, and significantly decreased bone mineralization [Yakoreva *et al*., 2018].

The patient was initially tested by CMA and BWS/SRS MS-MLPA analyses using DNA extracted from peripheral blood. Neither of these analyses revealed any definitely pathogenic genetic or epigenetic alteration. ES analysis performed next detected compound heterozygous variants of uncertain clinical significance, paternal variant c.1408C>T, p.(Arg470Cys) and maternal variant c.1573C>G. p.(Gln525Glu), in the *CTCFL* gene (RefSeq NM_080618). As this gene has been found to be associated with the regulation of several ICRs [Skaar *et al*., 2012], and the patient has growth retardation and body asymmetry typical for many ImpDis, UPD (6, 7, 14) MS-MLPA analysis was additionally performed on blood DNA. This analysis revealed complete LOM at the *MEG3* gene in the region 14q32.2. The same result was obtained using DNA extracted from buccal swab, urine, hyperpigmented and normal skin fibroblasts. Comparative analysis of the SNPs using the CMA results of the patient and her mother confirmed the diagnosis of maternal heterodisomic UPD of the entire chromosome 14, and TS14 was diagnosed. The revealed compound heterozygous variants in the *CTCFL* gene are thus an incidental finding and not associated with the UPD(14)mat [Yakoreva *et al*., 2018].

Later, because of unusually severe clinical presentation, congenital heart malformations and skin pigmentary anomalies, a possibility of mosaic trisomy 14 was suspected. Routine chromosomal analysis of blood lymphocytes revealed trisomy 14 in 4% of 50 analyzed metaphases (47,XX,+14[2]/46,XX[48]), and in about 7% of 150 analyzed interphase nuclei detected by FISH analysis (nuc ish 14q32×3[11]/14q32×2[139]). Neither routine chromosomal analysis nor FISH analysis revealed trisomy 14 in 50 metaphases and 150 interphases analyzed in both fibroblast cultures from normal and hyperpigmented skin biopsies. Although in hyperpigmented skin, one cell with trisomy 14 was detected by FISH, this was not reported because of the low reliability of the result [Yakoreva *et al*., 2018].

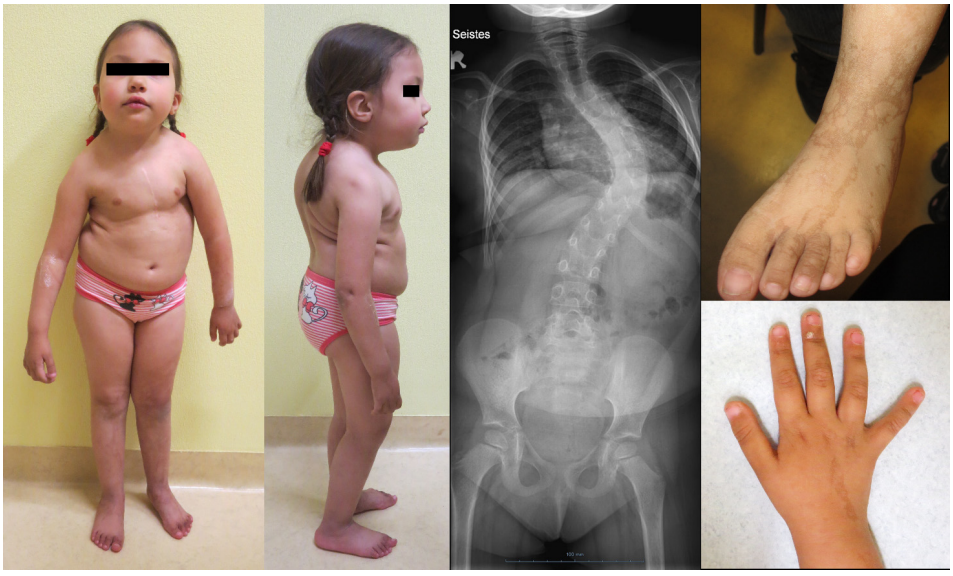

Figure 6: Clinical features of the proband at 4.5 years of age. Note the right-sided hemihypertrophy, LLD, linear hyperpigmentation on the arms and legs, dysmorphic facial features, short neck, small hands and feet, genu valgum, and severe neuro-muscular kyphoscoliosis.

The coexistence of UPD(14)mat and mosaic trisomy 14 can be explained by the formation of both anomalies in the result of trisomy 14 rescue in the zygote. Despite the common mechanism of formation, the combination of these anomalies is very rare. To the best of our knowledge, this patient is the ninth described case of concomitant UPD(14)mat-associated TS14 and mosaic trisomy 14. The clinical phenotype of the patient expand the knowledge about both the TS14 and mosaic trisomy 14.

5.5.2. Patient with a dual diagnosis of *MKRN3* gene-related central precocious puberty and *CHD8* gene-related autism spectrum disorder

The proband is a girl first referred to genetic counselling at 2.5 years of age due to global DD, autistic behaviour, macrocephaly, dysmorphic features and a small atrial septal defect. She was born at the 39th week of gestation. Her birth weight was 4220 g (+1.5 SD) and length 56 cm (+2 SD). Her motor and speech development was delayed. She started to walk independently at two years of age. At the age of two years and five months her speech was evaluated to be at one year and six months level. Developmental assessments by Griffiths scale at the age revealed cognitive delay of one year. At 2.5 years of age, her head circumference was above +2 SD, weight and height were on +1.5 SD. At the age of four years and ten months, electroencephalogram revealed focal epileptic activity and antiepileptic treatment was started. Mild ID was diagnosed at the

age of six years. Her head circumference and height were always above +2 SD. The girl also had some dysmorphic features, such as epicanthal folds, prominent nasal bridge, partial syndactyly of the second and third toes and wide gap between the first and second toes.

At the age of one year and one month, the breast enlargement was first noted in the patient. However, there were no other signs of puberty and the breast enlargement partially regressed spontaneously over the next six months. Slight breast enlargement began again at the age of five years. Further, the development of secondary sexual characteristics was very quick and menarche occurred at 6.5 years of age. Hormonal assessment showed remarkable elevation of estradiol and luteinizing hormone, and CPP was diagnosed. The pelvic ultrasound revealed uterine and ovarian changes typical for postmenarchial girls. Compared to her chronologic age, the bone age was advanced by three years. The patient's brain MRI initially revealed a ~5 mm pituitary microadenoma of uncertain clinical significance that did not need neurosurgical intervention. However, two years later, the result of hypophysis MRI was normal. The treatment with triptorelin, a synthetic agonist analog of gonadotropin releasing hormone, was effective in suppressing the pubertal development of the patient.

Four-year-old brother (Figure 7, V:2) of the patient (Figure 7, V:1) had similar motor and mental DD, autistic features (stereotypic movements, absence of speech and eye contact), low muscle tone, small atrial septal defect, bilateral inguinal hernias, macrocephaly and facial dysmorphism. There were no signs of precocious puberty in the brother of the proband. Her father (Figure 7, IV:1) had inguinal hernia, learning difficulties and social communication problems during childhood. The information about the age of her father's puberty was incomplete.

Initially, the patient was genetically investigated because of DD, mild ID, autistic behaviour, macrocephaly and dysmorphic features. The patient was tested by Illumina's TSO panel (4,813 OMIM genes). This analysis revealed a heterozygous variant c.2423_2424del, p.(Arg808Lysfs*12) in the *CHD8* gene (RefSeq NM_001170629.1). Since variants of the *CHD8* gene have been described previously in individuals with autism, ID, macrocephaly and dysmorphic facial features [Yasin *et al*., 2019; Wang *et al*., 2018; Merner *et al*., 2016], it was reported as the cause of the patient's developmental and behavioural problems. The *CHD8* gene variant appeared *de novo* in father and was also detected in the brother.

As CPP is not typical for the *CHD8* gene variants, reanalysis of TSO panel data was later additionally performed and a novel heterozygous variant c.326G>A, p.(Cys109Tyr) of the *MKRN3* gene (RefSeq NM_005664.3) was found. Although this variant has not been previously reported in the databases of the normal human genome and databases of pathogenic variants, *in silico* analysis identified this variant as disease-related. Familial segregation analysis showed that both proband and her father inherited the *MKRN3* gene variant from their fathers (Figure 7, IV:1 and III:1). The variant was not detected in the brother. Later, the same novel variant of the *MKRN3* gene was also found in

another Estonian case of familial CPP (Figure 7, V:5 and IV:4). Precise analysis of pedigree data demonstrated the presence of familial relationship between our case and the family (Figure 7).

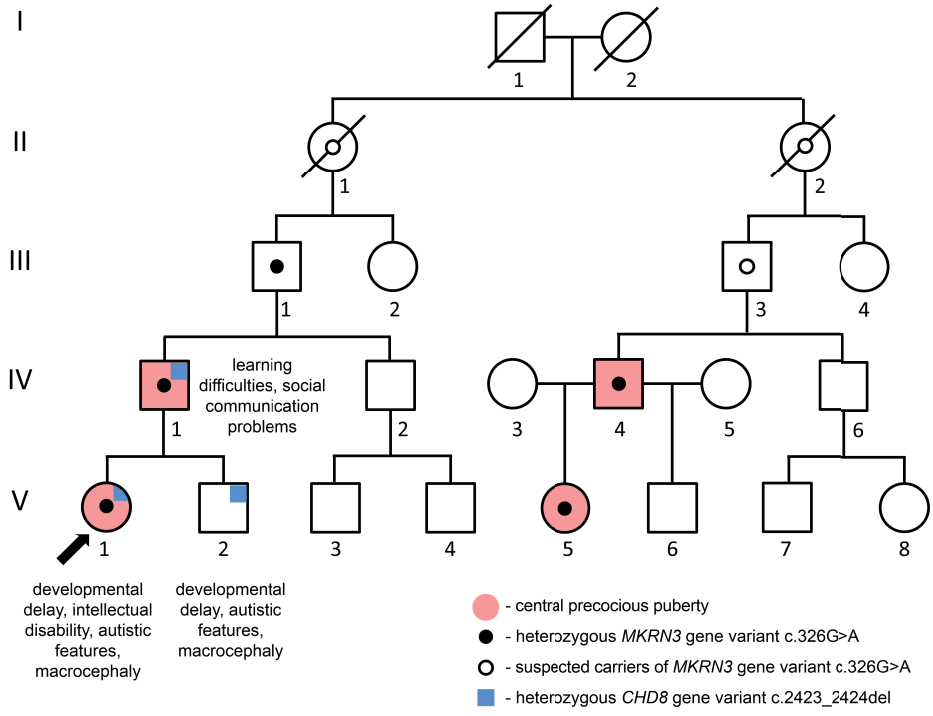

Figure 7: Family pedigree of the patient (V:1).

The findings in this patient demonstrate that ImpDis can be combined with another genetic disorder resulting in complex phenotype. Therefore, in the case of atypical clinical presentation, additional molecular testing or data reanalysis should be performed to test for double diagnosis.

5.5.3. Patient with a pseudopseudohypoparathyroidism

The proband is a girl repeatedly investigated by a medical geneticist because of a complex phenotype. She was the first child in the family and her family history was unremarkable. The pregnancy was complicated by IUGR. She was born from stimulated vaginal delivery at the 40th week of gestation with a birth weight of 2475 g (–2 SD), body length of 46 cm (–2 SD), head circumference of 32 cm (–2 SD), and Apgar scores of 7, 8 and 9 at 1, 5 and 10 min, respectively. Small placental infarcts and an unusually thin umbilical cord were discovered after the birth. Because of IUGR, chromosomal analysis was initially performed, and the karyotype was found to be normal (46,XX).

In the first two days of life, the girl had episodes of hypoglycemia that were successfully treated with intravenous glucose. She had feeding problems and poor appetite during the first year of life. Her weight and height have always been below –2 SD, although her head circumference has been in the normal range. Motor development was slightly delayed. She started to walk at the age of one year and four months. Some dysmorphic features, such as blue scleras, frontal bossing, hypertelorism, epicantal folds, almond-shaped eyes, thin upper lip, prominent smooth philtrum, micrognatia, brachydactyly, unilateral simian crease, hypoplastic nails, 4th toe clinodactyly, and wide gap between first and second toes were noted in the patient.

First time the girl was referred to genetic counseling at the age of one year and five months because of IUGR, small gain in weight and delayed motor development. She was tested by CMA and BWS/SRS MS-MLPA analyses using DNA extracted from peripheral blood. Both analyses were negative for genetic alterations.

The patient was repeatedly investigated by a medical geneticist at four and five years of age because of DD, behaviour problems, growth failure, and dysmorphic features. She had learning difficulties and was also diagnosed with attention deficit hyperactivity disorder. Roentgenogram of the patient´s hands revealed unusual changes of small hand bones (Figure 8). The patient was additionally tested by molecular analysis of Floating-Harbor syndrome, serum and urine analysis for inborn errors of metabolism, UPD(6, 7, 14) MS-MLPA and MS-SNuPE. As the results of all these analyses were normal, it was decided to perform trio ES analysis.

The initial result of trio ES was negative. However, two years later, the data of the ES was reanalyzed at the Broad Institute of MIT and Harvard. This analysis revealed a *de novo* heterozygous variant c.2229A>C, p.(Lys743Asn) in the *GNAS* gene (RefSeq NM_080425.3). Clinical presentation of the patient was therefore thoroughly revised and it was found that the girl has typical somatic features of the *GNAS*-gene-related ImpDis. As there were no clinically important changes in serum levels of PTH, calcium or phosphorus, PPHP was diagnosed.

This case demonstrates that clinical features of some ImpDis are unspecific and the process of determining the correct diagnosis of these ImpDis can be very difficult and long-lasting.

Figure 8: Roentgenogram of the patient´s hands at the age of four years and eight months. Note typical osseous manifestations of PPHP: brachydactyly, short and thick metacarpal bones (especially metacarpals IV and V), cone-shaped epiphyses, short phalanges and thick thumbs.

6. CONCLUSIONS

1. The results of the study of the frequency of genetic and methylation abnormalities among Estonian patients referred with clinical suspicion of SRS or BWS and selected according to previously published clinical diagnostic scoring systems for these syndromes were reported (Paper I).
   1.1. Molecular diagnostic tests confirmed the SRS diagnosis in 38% (5/13) of patients with clinical diagnosis of SRS according to the Bartholdi scoring system. In most of them (80%, 4/5) the most common molecular alteration causing SRS, hypomethylation at IC1, was detected, and one patient had a rare maternal duplication in 11p15.5–p15.4.
   1.2. Molecular abnormality, hypomethylation at IC2, was found in only one patient of 12 (8%) with clinical diagnosis of BWS according to the Weksberg clinical diagnostic system. Unexpectedly, MS-SNuPE and UPD (6, 7, 14) MS-MLPA analyses revealed an isolated TNDM (hypomethylation of *PLAGL1*) in the patient with the highest BWS scoring and without history of neonatal diabetes.
   1.3. The detection rate of the Bartholdi scoring system amongst Estonian patients with clinical suspicion of SRS (38%) is similar to those obtained by Bartholdi herself (39%). The detection rate of a new scoring system, the NH-CSS, in our SRS group was significantly higher (57%), but there were false negative results.
   1.4. The detection rate of the Weksberg scoring system in our BWS group (8%) was inexplicably significantly lower compared to the results of other similar studies (28–72%). The detection rate of consensus scoring system for BWS spectrum disorders was somewhat higher in our BWS group (20%), but still significantly lower than the published detection rate of 80%.
2. The nationwide prevalence of the most common ImpDis and time-trend changes in the live birth prevalence of these disorders in Estonia were reported (Paper II).
   2.1. From 1998 to the end of the study period, a total of 87 individuals with ImpDis were identified in Estonia. The most frequent ImpDis in Estonia were PWS (31% of patients with ImpDis), AS (17%), SRS (17%), BWS (14%) and PHP/PPHP (11%).
   2.2. The live birth prevalence of the most frequent ImpDis in Estonia in 2004–2016 was 1/13,599 for PWS, 1/27,198 for AS, 1/21,154 for BWS, 1/15,866 for SRS, and 1/27,198 for PHP/PPHP.
   2.3. The live birth prevalence of all ImpDis in Estonia in 2004–2016 was 1/3,462, and the overall prevalence of all ImpDis in 2018 was 1/17,132 (5.8/100,000). It can be concluded that, while each ImpDis, taken separately, is very rare, all ImpDis together are relatively common.
   2.4. The birth prevalence of PWS, AS and BWS found in Estonia is comparable with the prevalence previously reported in the literature. Unexpectedly, the live birth prevalence of SRS in Estonia (1/15,866)

was several times higher compared with the estimated SRS prevalence of 1/75,000–1/100,000. The live birth prevalence of the *GNAS*-gene-related ImpDis, PHP and PPHP, is also significantly higher than previously estimated, and can be compared with the prevalence of AS.

2.5. There was a statistically significant increase in the live birth prevalence of BWS and all ImpDis in Estonia during the years 1998–2016, that can be explained by both improved diagnostic methods as well as increased awareness of physicians of ImpDis. There was no statistically significant increase in the live birth prevalence of PWS, AS, SRS and PHP/PPHP during this period.

3. New molecular diagnostic tests for ImpDis were established in laboratory practice in Estonia (Paper I and II).
   3.1. During this study, an UPD (6, 7, 14) MS-MLPA and *GNAS* locus MS-MLPA analyses were implemented in the molecular laboratory of the Department of Clinical Genetics of Tartu University Hospital.
   3.2. The diagnostic effectiveness of PWS/AS MS-PCR and PWS/AS MS-MLPA analyses together was 6.4%, and the effectiveness of BWS/SRS MS-MLPA and UPD(7) MS-MLPA analyses together was 7.7%. These results are significantly lower than those published in previous similar studies (≥20%). However, this low result can be explained by the absence of preanalytical selection of patients in our routine clinical practice according to clinical scoring systems for these disorders.
4. Based on our study results and personal experience, we can conclude that the awareness of ImpDis among Estonian doctors and other medical specialists increased as a result of this study (Paper II and IV). Clinical and molecular investigations of Estonian patients referred to genetic counselling with clinical suspicion of ImpDis or other genetic disorder revealed several individuals with new rare ImpDis.
   4.1. During the years 1998–2011, we detected on average 1–3 new molecularly confirmed cases of ImpDis in Estonia per year. But, during the period 2012–2018, this number increased to 5–8 new cases of ImpDis per year.
   4.2. Altogether, eight patients with such rare ImpDis as *MKRN3* gene-related CPP, TS14, TNDM and MDS were detected in Estonia during this study.
   4.3. We detected and described a patient with atypical TNDM (Paper I), a patient with a rare combination of TS14 and mosaic trisomy 14 (Paper III), and a family with diagnoses of both *MKRN3* gene-related CPP and *CHD8* gene-related autism spectrum disorder.

This study demonstrated that ImpDis are relatively common genetic disorders which have very diverse molecular etiology and often unspecific or atypical clinical presentation. Moreover, the implementation of new diagnostic tests and methods has significantly improved the diagnostics of ImpDis in Estonia in the past few years, therefore, the prevalence of many ImpDis may further increase. All these facts play an important role in the diagnostics and the management of ImpDis in clinical practice.

REFERENCES

Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, *et al*.: Central precocious puberty caused by mutations in the imprinted gene MKRN3. N Engl J Med 2013;368:2467–2475.

Abreu AP, Macedo DB, Brito VN, Kaiser UB, Latronico AC: A new pathway in the control of the initiation of puberty: the MKRN3 gene. J Mol Endocrinol 2015;54:R131–139.

Acs OD, Peterfia B, Hollosi P, Haltrich I, Sallai A, Luczay A, *et al*.: [Rapid first-tier genetic diagnosis in patients with Prader-Willi syndrome]. Orv Hetil 2018;159:64–69.

Acuna-Hidalgo R, Veltman JA, Hoischen A: New insights into the generation and role of de novo mutations in health and disease. Genome Biol 2016;17:241.

Åkefeldt A, Gillberg C, Larsson C: Prader-Willi syndrome in a Swedish rural county: epidemiological aspects. Dev Med Child Neurol 1991;33:715–721.

Albright F, Burnett C, Smith P, Parson W: Pseudo-hypoparathyroidism, an example of Albright Bantam Syndrome. Endocrinology 1942;30:322–330.

Albright F, Forbes AP, Henneman PH: Pseudo-pseudohypoparathyroidism. Trans Assoc Am Physicians 1952;65:337–350.

Alders M, Maas SM, Kadouch DJM, van der Lip K, Bliek J, van der Horst CMAM, *et al*.: Methylation analysis in tongue tissue of BWS patients identifies the (EPI)genetic cause in 3 patients with normal methylation levels in blood. Eur J Med Genet 2014;57:293–297.

AlSalloum A, El Mouzan MI, AlHerbish A, AlOmer A, Qurashi M: Prevalence of selected congenital anomalies in Saudi children: a community-based study. Ann Saudi Med 2015;35:107–110.

Ammerpohl O, Martin-Subero JI, Richter J, Vater I, Siebert R: Hunting for the 5th base: Techniques for analyzing DNA methylation. Biochim Biophys Acta 2009; 1790:847–862.

Andersen WH, Rasmussen RK, Stromme P: Levels of cognitive and linguistic development in Angelman syndrome: a study of 20 children. Logoped Phoniatr Vocol 2001;26:2–9.

Angelman H: 'Puppet' Children A Report on Three Cases. Dev Med Child Neurol 1965;7:681–688.

Angulo MA, Butler MG, Cataletto ME: Prader-Willi syndrome: a review of clinical, genetic, and endocrine findings. J Endocrinol Invest 2015;38:1249–1263.

Arroyo Carrera I, Martínez-Frías ML, Egüés Jimeno J, García Martínez MJ, Eloína Cimadevilla Sánchez C, Bermejo Sánchez E: [Wiedemann-Beckwith syndrome: clinical and epidemiological analysis of a consecutive series of cases in Spain]. An Esp Pediatr 1999;50:161–165.

Asmus F, Hjermind LE, Dupont E, Wagenstaller J, Haberlandt E, Munz M, *et al*.: Genomic deletion size at the epsilon-sarcoglycan locus determines the clinical phenotype. Brain J Neurol 2007;130:2736–2745.

Azcona C, Stanhope R: Hypoglycaemia and Russell-Silver syndrome. J Pediatr Endocrinol Metab JPEM 2005;18:663–670.

Azzi S, Abi Habib W, Netchine I: Beckwith-Wiedemann and Russell-Silver Syndromes: from new molecular insights to the comprehension of imprinting regulation. Curr Opin Endocrinol Diabetes Obes 2014;21:30–38.

Azzi S, Rossignol S, Steunou V, Sas T, Thibaud N, Danton F, *et al*.: Multilocus methylation analysis in a large cohort of 11p15-related foetal growth disorders (Russell Silver and Beckwith Wiedemann syndromes) reveals simultaneous loss of methylation at paternal and maternal imprinted loci. Hum Mol Genet 2009;18:4724–4733.

Azzi S, Salem J, Thibaud N, Chantot-Bastaraud S, Lieber E, Netchine I, *et al*.: A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome. J Med Genet 2015; 52:446–453.

B Beckwith J: Extreme Cytomegaly of the Adrenal Fetal Cortex, Omphalocele, Hyperplasia of Kidneys and Pancreas, and Leydig-Cell Hyperplasia: Another Syndrome? Annu Meet West Soc Pediatr Res Los Angel CA Novemb 1963;

Bak M, Boonen SE, Dahl C, Hahnemann JMD, Mackay DJDG, Tumer Z, *et al*.: Genome-wide DNA methylation analysis of transient neonatal diabetes type 1 patients with mutations in ZFP57. BMC Med Genet 2016;17:29.

Bakker B, Sonneveld LJH, Woltering MC, Bikker H, Kant SG: A Girl With Beckwith-Wiedemann Syndrome and Pseudohypoparathyroidism Type 1B Due to Multiple Imprinting Defects. J Clin Endocrinol Metab 2015;100:3963–3966.

Bannister AJ, Kouzarides T: Regulation of chromatin by histone modifications. Cell Res 2011;21:381–395.

Baple EL, Poole RL, Mansour S, Willoughby C, Temple IK, Docherty LE, *et al*.: An atypical case of hypomethylation at multiple imprinted loci. Eur J Hum Genet EJHG 2011;19:360–362.

Bar C, Diene G, Molinas C, Bieth E, Casper C, Tauber M: Early diagnosis and care is achieved but should be improved in infants with Prader-Willi syndrome. Orphanet J Rare Dis 2017;12:118.

Barel O, Shalev SA, Ofir R, Cohen A, Zlotogora J, Shorer Z, *et al*.: Maternally inherited Birk Barel mental retardation dysmorphism syndrome caused by a mutation in the genomically imprinted potassium channel KCNK9. Am J Hum Genet 2008;83:193–199.

Barisic I, Boban L, Akhmedzhanova D, Bergman JEH, Cavero-Carbonell C, Grinfelde I, *et al*.: Beckwith Wiedemann syndrome: A population-based study on prevalence, prenatal diagnosis, associated anomalies and survival in Europe. Eur J Med Genet 2018;61:499–507.

Barlow DP, Bartolomei MS: Genomic imprinting in mammals. Cold Spring Harb Perspect Biol 2014;6.

Barlow DP, Stöger R, Herrmann BG, Saito K, Schweifer N: The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. Nature 1991;349:84–87.

Bartholdi D, Krajewska-Walasek M, Õunap K, Gaspar H, Chrzanowska KH, Ilyana H, *et al*.: Epigenetic mutations of the imprinted IGF2-H19 domain in Silver-Russell syndrome (SRS): results from a large cohort of patients with SRS and SRS-like phenotypes. J Med Genet 2009;46:192–197.

Bartolomei MS, Zemel S, Tilghman SM: Parental imprinting of the mouse H19 gene. Nature 1991;351:153–155.

Barton SC, Surani MA, Norris ML: Role of paternal and maternal genomes in mouse development. Nature 1984;311:374–376.

Baskin B, Choufani S, Chen Y-A, Shuman C, Parkinson N, Lemyre E, *et al*.: High frequency of copy number variations (CNVs) in the chromosome 11p15 region in patients with Beckwith-Wiedemann syndrome. Hum Genet 2014;133:321–330.

Bastepe M, Frohlich LF, Hendy GN, Indridason OS, Josse RG, Koshiyama H, *et al*.: Autosomal dominant pseudohypoparathyroidism type Ib is associated with a heterozygous microdeletion that likely disrupts a putative imprinting control element of GNAS. J Clin Invest 2003;112:1255–1263.

Bastepe M, Pincus JE, Sugimoto T, Tojo K, Kanatani M, Azuma Y, *et al*.: Positional dissociation between the genetic mutation responsible for pseudohypoparathyroidism type Ib and the associated methylation defect at exon A/B: evidence for a long-range regulatory element within the imprinted GNAS1 locus. Hum Mol Genet 2001;10:1231–1241.

Bauer U-M, Daujat S, Nielsen SJ, Nightingale K, Kouzarides T: Methylation at arginine 17 of histone H3 is linked to gene activation. EMBO Rep 2002;3:39–44.

Begemann M, Leisten I, Soellner L, Zerres K, Eggermann T, Spengler S: Use of multilocus methylation-specific single nucleotide primer extension (MS-SNuPE) technology in diagnostic testing for human imprinted loci. Epigenetics 2012a;7:473–481.

Begemann M, Spengler S, Gogiel M, Grasshoff U, Bonin M, Betz RC, *et al*.: Clinical significance of copy number variations in the 11p15.5 imprinting control regions: new cases and review of the literature. J Med Genet 2012b;49:547–553.

Begemann M, Spengler S, Kanber D, Haake A, Baudis M, Leisten I, *et al*.: Silver-Russell patients showing a broad range of ICR1 and ICR2 hypomethylation in different tissues. Clin Genet 2011;80:83–88.

Begemann M, Zirn B, Santen G, Wirthgen E, Soellner L, Buttel H-M, *et al*.: Paternally Inherited IGF2 Mutation and Growth Restriction. N Engl J Med 2015;373:349–356.

Bens S, Kolarova J, Beygo J, Buiting K, Caliebe A, Eggermann T, *et al*.: Phenotypic spectrum and extent of DNA methylation defects associated with multilocus imprinting disturbances. Epigenomics 2016;8:801–816.

Berger SL: Histone modifications in transcriptional regulation. Curr Opin Genet Dev 2002;12:142–148.

Bessa DS, Macedo DB, Brito VN, Franca MM, Montenegro LR, Cunha-Silva M, *et al*.: High Frequency of MKRN3 Mutations in Male Central Precocious Puberty Previously Classified as Idiopathic. Neuroendocrinology 2017;105:17–25.

Beygo J, Kuchler A, Gillessen-Kaesbach G, Albrecht B, Eckle J, Eggermann T, *et al*.: New insights into the imprinted MEG8-DMR in 14q32 and clinical and molecular description of novel patients with Temple syndrome. Eur J Hum Genet EJHG 2017;25:935–945.

Bianchi F: Registro nazionale malattie rare. Epidemiologia di 44 malformazioni congenite rare in Italia. [Internet] 2002;Available from: http://www.iss.it/ binary/publ/publi/0236.1109328902.pdf

Bird LM: Angelman syndrome: review of clinical and molecular aspects. Appl Clin Genet 2014;7:93–104.

Bliek J, Verde G, Callaway J, Maas SM, De Crescenzo A, Sparago A, *et al*.: Hypomethylation at multiple maternally methylated imprinted regions including PLAGL1 and GNAS loci in Beckwith-Wiedemann syndrome. Eur J Hum Genet EJHG 2009;17:611–619.

Boonen SE, Mackay DJG, Hahnemann JMD, Docherty L, Gronskov K, Lehmann A, *et al*.: Transient neonatal diabetes, ZFP57, and hypomethylation of multiple imprinted loci: a detailed follow-up. Diabetes Care 2013;36:505–512.

Brioude F, Kalish JM, Mussa A, Foster AC, Bliek J, Ferrero GB, *et al*.: Expert consensus document: Clinical and molecular diagnosis, screening and management of

Beckwith-Wiedemann syndrome: an international consensus statement. Nat Rev Endocrinol 2018;14:229–249.
Brioude F, Oliver-Petit I, Blaise A, Praz F, Rossignol S, Le Jule M, *et al.*: CDKN1C mutation affecting the PCNA-binding domain as a cause of familial Russell Silver syndrome. J Med Genet 2013;50:823–830.
Brito VN, Latronico AC: Underdiagnosis of central precocious puberty in boys with loss-of-function mutations of MKRN3. J Pediatr 2017;183:202–203.
Brix B, Werner R, Staedt P, Struve D, Hiort O, Thiele S: Different pattern of epigenetic changes of the GNAS gene locus in patients with pseudohypoparathyroidism type Ic confirm the heterogeneity of underlying pathomechanisms in this subgroup of pseudohypoparathyroidism and the demand for a new classification of GNAS-related disorders. J Clin Endocrinol Metab 2014;99:E1564–1570.
Buchholz T, Jackson J, Robson L, Smith A: Evaluation of methylation analysis for diagnostic testing in 258 referrals suspected of Prader-Willi or Angelman syndromes. Hum Genet 1998;103:535–539.
Buckley RH, Dinno N, Weber P: Angelman syndrome: are the estimates too low? Am J Med Genet 1998;80:385–390.
Buiting K, Di Donato N, Beygo J, Bens S, von der Hagen M, Hackmann K, *et al.*: Clinical phenotypes of MAGEL2 mutations and deletions. Orphanet J Rare Dis 2014;9:40.
Buiting K, Gross S, Lich C, Gillessen-Kaesbach G, el-Maarri O, Horsthemke B: Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. Am J Hum Genet 2003;72:571–577.
Buiting K, Williams C, Horsthemke B: Angelman syndrome – insights into a rare neurogenetic disorder. Nat Rev Neurol 2016;12:584–593.
Bullman H, Lever M, Robinson DO, Mackay DJG, Holder SE, Wakeling EL: Mosaic maternal uniparental disomy of chromosome 11 in a patient with Silver-Russell syndrome. J Med Genet 2008;45:396–399.
Burd L, Vesely B, Martsolf J, Kerbeshian J: Prevalence study of Prader-Willi syndrome in North Dakota. Am J Med Genet 1990;37:97–99.
Butler MG: Prader-Willi Syndrome: Obesity due to Genomic Imprinting. Curr Genomics 2011;12:204–215.
Butler MG, Bittel DC, Kibiryeva N, Talebizadeh Z, Thompson T: Behavioral differences among subjects with Prader-Willi syndrome and type I or type II deletion and maternal disomy. Pediatrics 2004;113:565–573.
Butler MG, Fischer W, Kibiryeva N, Bittel DC: Array comparative genomic hybridization (aCGH) analysis in Prader-Willi syndrome. Am J Med Genet A 2008;146A:854–860.
Calvello M, Tabano S, Colapietro P, Maitz S, Pansa A, Augello C, *et al.*: Quantitative DNA methylation analysis improves epigenotype-phenotype correlations in Beckwith-Wiedemann syndrome. Epigenetics 2013;8:1053–1060.
Cammarata-Scalisi F, Avendano A, Stock F, Callea M, Sparago A, Riccio A: Beckwith-Wiedemann syndrome: clinical and etiopathogenic aspects of a model genomic imprinting entity. Arch Argent Pediatr 2018;116:368–373.
Carecchio M, Magliozzi M, Copetti M, Ferraris A, Bernardini L, Bonetti M, *et al.*: Defining the epsilon-sarcoglycan (SGCE) gene phenotypic signature in myoclonus-dystonia: a reappraisal of genetic testing criteria. Mov Disord Off J Mov Disord Soc 2013;28:787–794.

Carrera IA, de Zaldivar MS, Martin R, Begemann M, Soellner L, Eggermann T: Microdeletions of the 7q32.2 imprinted region are associated with Silver-Russell syndrome features. Am J Med Genet A 2016;170:743–749.
Cassidy FC, Charalambous M: Genomic imprinting, growth and maternal-fetal interactions. J Exp Biol 2018;221.
Cedar H, Bergman Y: Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 2009;10:295–304.
Chaudhry M, Wang X, Bamne MN, Hasnain S, Demirci FY, Lopez OL, *et al.*: Genetic variation in imprinted genes is associated with risk of late-onset alzheimer's disease. J Alzheimers Dis JAD 2015;44:989–994.
Cheon CK: Genetics of Prader-Willi syndrome and Prader-Will-Like syndrome. Ann Pediatr Endocrinol Metab 2016;21:126–135.
Cheung P, Lau P: Epigenetic regulation by histone methylation and histone variants. Mol Endocrinol Baltim Md 2005;19:563–573.
Choufani S, Shuman C, Weksberg R: Beckwith-Wiedemann syndrome. Am J Med Genet C Semin Med Genet 2010;154C:343–354.
Choufani S, Shuman C, Weksberg R: Molecular findings in Beckwith-Wiedemann syndrome. Am J Med Genet C Semin Med Genet 2013;163C:131–140.
Christianson AL, Viljoen DL, Winship WS, de la Rey M, van Rensburg EJ: Prader-Willi syndrome in South African patients – clinical and molecular diagnosis. South Afr Med J Suid-Afr Tydskr Vir Geneeskd 1998;88:711–714.
Cianferotti L, Brandi ML: Pseudohypoparathyroidism. Minerva Endocrinol 2018; 43:156–167.
Clarke DJ, Marston G: Problem behaviors associated with 15q- Angelman syndrome. Am J Ment Retard AJMR 2000;105:25–31.
Clayton-Smith J: A clinical and genetic study of Angelman syndrome. 1993;
Clayton-Smith J, Pembrey ME: Angelman syndrome. J Med Genet 1992;29:412–415.
Conlin LK, Thiel BD, Bonnemann CG, Medne L, Ernst LM, Zackai EH, *et al.*: Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. Hum Mol Genet 2010;19:1263–1275.
Conroy JM, Grebe TA, Becker LA, Tsuchiya K, Nicholls RD, Buiting K, *et al.*: Balanced translocation 46,XY,t(2;15)(q37.2;q11.2) associated with atypical Prader-Willi syndrome. Am J Hum Genet 1997;61:388–394.
Cooper WN, Luharia A, Evans GA, Raza H, Haire AC, Grundy R, *et al.*: Molecular subtypes and phenotypic expression of Beckwith-Wiedemann syndrome. Eur J Hum Genet EJHG 2005;13:1025–1032.
Cortessis VK, Azadian M, Buxbaum J, Sanogo F, Song AY, Sriprasert I, *et al.*: Comprehensive meta-analysis reveals association between multiple imprinting disorders and conception by assisted reproductive technology. J Assist Reprod Genet 2018;35:943–952.
Court F, Martin-Trujillo A, Romanelli V, Garin I, Iglesias-Platas I, Salafsky I, *et al.*: Genome-wide allelic methylation analysis reveals disease-specific susceptibility to multiple methylation defects in imprinting syndromes. Hum Mutat 2013;34:595–602.
Czyz W, Morahan JM, Ebers GC, Ramagopalan SV: Genetic, environmental and stochastic factors in monozygotic twin discordance with a focus on epigenetic differences. BMC Med 2012;10:93.
Dagli A, Buiting K, Williams CA: Molecular and Clinical Aspects of Angelman Syndrome. Mol Syndromol 2011;2:100–112.

Dagli AI, Mueller J, Williams CA, Dagli AI, Mueller J, Williams CA: Angelman Syndrome [Internet]. GeneReviews(R) 2017;Available from: http://www.ncbi.nlm.nih.gov/books/NBK1144/

Davies AF, Ogilvie CM: Prevalence of Angelman syndrome amongst referrals with epilepsy and developmental delay. Am J Med Genet A 2007;143A:2189–2191.

DeBaun MR, Tucker MA: Risk of cancer during the first four years of life in children from The Beckwith-Wiedemann Syndrome Registry. J Pediatr 1998;132:398–400.

DeChiara TM, Robertson EJ, Efstratiadis A: Parental imprinting of the mouse insulin-like growth factor II gene. Cell 1991;64:849–859.

Dias RP, Nightingale P, Hardy C, Kirby G, Tee L, Price S, *et al.*: Comparison of the clinical scoring systems in Silver-Russell syndrome and development of modified diagnostic criteria to guide molecular genetic testing. J Med Genet 2013;50:635–639.

Dimitrova-Mladenova MS, Stefanova EM, Glushkova M, Todorova AP, Todorov T, Konstantinova MM, *et al.*: Males with Paternally Inherited MKRN3 Mutations May Be Asymptomatic. J Pediatr 2016;179:263–265.

Docherty LE, Kabwama S, Lehmann A, Hawke E, Harrison L, Flanagan SE, *et al.*: Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype-phenotype correlation in an international cohort of patients. Diabetologia 2013;56:758–762.

Docherty LE, Rezwan FI, Poole RL, Jagoe H, Lake H, Lockett GA, *et al.*: Genome-wide DNA methylation analysis of patients with imprinting disorders identifies differentially methylated regions associated with novel candidate imprinted genes. J Med Genet 2014;51:229–238.

Docherty LE, Rezwan FI, Poole RL, Turner CLS, Kivuva E, Maher ER, *et al.*: Mutations in NLRP5 are associated with reproductive wastage and multilocus imprinting disorders in humans. Nat Commun 2015;6:8086.

Driscoll DJ, Miller JL, Schwartz S, Cassidy SB, Driscoll DJ, Miller JL, *et al.*: Prader-Willi Syndrome [Internet]. GeneReviews(R) 2017;Available from: http://www.ncbi.nlm.nih.gov/books/NBK1330/

Eggermann K, Bliek J, Brioude F, Algar E, Buiting K, Russo S, *et al.*: EMQN best practice guidelines for the molecular genetic testing and reporting of chromosome 11p15 imprinting disorders: Silver-Russell and Beckwith-Wiedemann syndrome. Eur J Hum Genet EJHG 2016;24:1377–1387.

Eggermann T, Gonzalez D, Spengler S, Arslan-Kirchner M, Binder G, Schonherr N: Broad clinical spectrum in Silver-Russell syndrome and consequences for genetic testing in growth retardation. Pediatrics 2009;123:e929–931.

Eggermann T, Heilsberg A-K, Begemann M, Soellner L, Bens S, Siebert R, *et al.*: Additional molecular findings in 11p15-associated imprinting disorders: An urgent need for multi-locus testing. J Mol Med 2014a;92:769–777.

Eggermann T, Leisten I, Binder G, Begemann M, Spengler S: Disturbed methylation at multiple imprinted loci: an increasing observation in imprinting disorders. Epigenomics 2011;3:625–637.

Eggermann T, Mergenthaler S, Eggermann K, Albers A, Linnemann K, Fusch C, *et al.*: Identification of interstitial maternal uniparental disomy (UPD) (14) and complete maternal UPD(20) in a cohort of growth retarded patients. J Med Genet 2001;38:86–89.

Eggermann T, Netchine I, Temple IK, Tümer Z, Monk D, Mackay D, *et al*.: Congenital imprinting disorders: EUCID.net – a network to decipher their aetiology and to improve the diagnostic and clinical care. Clin Epigenetics 2015a;7:23.

Eggermann T, Perez de Nanclares G, Maher ER, Temple IK, Tumer Z, Monk D, *et al*.: Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. Clin Epigenetics 2015b;7:123.

Eggermann T, Schneider-Ratzke B, Begemann M, Spengler S: Isolated hypermethylation of GRB10 (7p12.2) in a Silver-Russell syndrome patient carrying a 20p13 microdeletion. Clin Genet 2014b;85:399–400.

Eggermann T, Spengler S, Begemann M, Binder G, Buiting K, Albrecht B, *et al*.: Deletion of the paternal allele of the imprinted MEST/PEG1 region in a patient with Silver-Russell syndrome features. Clin Genet 2012;81:298–300.

Ehara H, Ohno K, Takeshita K: Frequency of the Prader-Willi syndrome in the San-in district, Japan. Brain Dev 1995;17:324–326.

Elli FM, Linglart A, Garin I, de Sanctis L, Bordogna P, Grybek V, *et al*.: The Prevalence of GNAS Deficiency-Related Diseases in a Large Cohort of Patients Characterized by the EuroPHP Network. J Clin Endocrinol Metab 2016;101:3657–3668.

Elliott M, Maher ER: Beckwith-Wiedemann syndrome. J Med Genet 1994;31:560–564.

Fenton TR: A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. BMC Pediatr 2003;3:13.

Ferguson-Smith AC, Bourc'his D: The discovery and importance of genomic imprinting. eLife 2018;7.

Ferguson-Smith AC, Cattanach BM, Barton SC, Beechey CV, Surani MA: Embryological and molecular investigations of parental imprinting on mouse chromosome 7. Nature 1991;351:667–670.

Finer S, Holland ML, Nanty L, Rakyan VK: The hunt for the epiallele. Environ Mol Mutagen 2011;52:1–11.

Fontana L, Bedeschi MF, Maitz S, Cereda A, Fare C, Motta S, *et al*.: Characterization of multi-locus imprinting disturbances and underlying genetic defects in patients with chromosome 11p15.5 related imprinting disorders. Epigenetics 2018;13:897–909.

Fountain MD, Aten E, Cho MT, Juusola J, Walkiewicz MA, Ray JW, *et al*.: The phenotypic spectrum of Schaaf-Yang syndrome: 18 new affected individuals from 14 families. Genet Med Off J Am Coll Med Genet 2017;19:45–52.

Fountain MD, Schaaf CP: Prader-Willi Syndrome and Schaaf-Yang Syndrome: Neurodevelopmental Diseases Intersecting at the MAGEL2 Gene. Dis Basel Switz 2016;4.

Franco MM, Prickett AR, Oakey RJ: The role of CCCTC-binding factor (CTCF) in genomic imprinting, development, and reproduction. Biol Reprod 2014;91:125.

Gardner RJ, Mungall AJ, Dunham I, Barber JC, Shield JP, Temple IK, *et al*.: Localisation of a gene for transient neonatal diabetes mellitus to an 18.72 cR3000 (approximately 5.4 Mb) interval on chromosome 6q. J Med Genet 1999;36:192–196.

Gaston V, Le Bouc Y, Soupre V, Burglen L, Donadieu J, Oro H, *et al*.: Analysis of the methylation status of the KCNQ1OT and H19 genes in leukocyte DNA for the diagnosis and prognosis of Beckwith-Wiedemann syndrome. Eur J Hum Genet EJHG 2001;9:409–418.

Gayatri S, Bedford MT: Readers of histone methylarginine marks. Biochim Biophys Acta 2014;1839:702–710.

Geoffron S, Abi Habib W, Chantot-Bastaraud S, Dubern B, Steunou V, Azzi S, *et al*.: Chromosome 14q32.2 Imprinted Region Disruption as an Alternative Molecular Diagnosis of Silver-Russell Syndrome. J Clin Endocrinol Metab 2018;103:2436–2446.

Gicquel C, Rossignol S, Cabrol S, Houang M, Steunou V, Barbu V, *et al*.: Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. Nat Genet 2005;37:1003–1007.

Golbabapour S, Majid NA, Hassandarvish P, Hajrezaie M, Abdulla MA, Hadi AHA: Gene silencing and Polycomb group proteins: an overview of their structure, mechanisms and phylogenetics. Omics J Integr Biol 2013;17:283–296.

Gonzalgo ML, Liang G: Methylation-sensitive single-nucleotide primer extension (Ms-SNuPE) for quantitative measurement of DNA methylation. Nat Protoc 2007; 2:1931–1936.

Goto M, Kagami M, Nishimura G, Yamagata T: A patient with Temple syndrome satisfying the clinical diagnostic criteria of Silver-Russell syndrome. Am J Med Genet A 2016;170:2483–2485.

Grafodatskaya D, Choufani S, Basran R, Weksberg R: An Update on Molecular Diagnostic Testing of Human Imprinting Disorders. J Pediatr Genet 2017;6:3–17.

Graham JMJ, Zadeh N, Kelley M, Tan ES, Liew W, Tan V, *et al*.: KCNK9 imprinting syndrome-further delineation of a possible treatable disorder. Am J Med Genet A 2016;170:2632–2637.

Greer EL, Shi Y: Histone methylation: a dynamic mark in health, disease and inheritance. Nat Rev Genet 2012;13:343–357.

Gregg C: Known unknowns for allele-specific expression and genomic imprinting effects. F1000prime Rep 2014;6:75.

Grewal SIS, Elgin SCR: Heterochromatin: new possibilities for the inheritance of structure. Curr Opin Genet Dev 2002;12:178–187.

Gronskov K, Poole RL, Hahnemann JMD, Thomson J, Tumer Z, Brondum-Nielsen K, *et al*.: Deletions and rearrangements of the H19/IGF2 enhancer region in patients with Silver-Russell syndrome and growth retardation. J Med Genet 2011;48:308–311.

Grunewald A, Djarmati A, Lohmann-Hedrich K, Farrell K, Zeller JA, Allert N, *et al*.: Myoclonus-dystonia: significance of large SGCE deletions. Hum Mutat 2008; 29:331–332.

Guettard E, Portnoi M-F, Lohmann-Hedrich K, Keren B, Rossignol S, Winkler S, *et al*.: Myoclonus-dystonia due to maternal uniparental disomy. Arch Neurol 2008; 65:1380–1385.

Haggarty P, Hoad G, Campbell DM, Horgan GW, Piyathilake C, McNeill G: Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. Am J Clin Nutr 2013;97:94–99.

Halliday J, Oke K, Breheny S, Algar E, J Amor D: Beckwith-Wiedemann syndrome and IVF: a case-control study. Am J Hum Genet 2004;75:526–528.

Hanna CW, Peñaherrera MS, Saadeh H, Andrews S, McFadden DE, Kelsey G, *et al*.: Pervasive polymorphic imprinted methylation in the human placenta. Genome Res 2016;26:756–767.

Hatada I, Ohashi H, Fukushima Y, Kaneko Y, Inoue M, Komoto Y, *et al*.: An imprinted gene p57KIP2 is mutated in Beckwith-Wiedemann syndrome. Nat Genet 1996; 14:171–173.

Haug MG, Brendehaug A, Houge G, Kagami M, Ogata T: Mosaic upd(14)pat in a patient with mild features of Kagami-Ogata syndrome. Clin Case Rep 2018;6:91–95.
Heksch R, Kamboj M, Anglin K, Obrynba K: Review of Prader-Willi syndrome: the endocrine approach. Transl Pediatr 2017;6:274–285.
Henry I, Bonaiti-Pellie C, Chehensse V, Beldjord C, Schwartz C, Utermann G, *et al*.: Uniparental paternal disomy in a genetic cancer-predisposing syndrome. Nature 1991;351:665–667.
Higurashi M, Iijima K, Sugimoto Y, Ishikawa N, Hoshina H, Watanabe N, *et al*.: The birth prevalence of malformation syndromes in Tokyo infants: a survey of 14,430 newborn infants. Am J Med Genet 1980;6:189–194.
Higurashi M, Iijima S, Takeshita T, Oda M, Takadaya K, Watanabe N: Incidence of malformation syndromes and chromosomal abnormalities in 22,063 newborn infants in Tokyo. Jinrui Idengaku Zasshi Jpn J Hum Genet 1985;30:1–8.
Higurashi M, Oda M, Iijima K, Iijima S, Takeshita T, Watanabe N, *et al*.: Livebirth prevalence and follow-up of malformation syndromes in 27,472 newborns. Brain Dev 1990;12:770–773.
Hoffmann K, Heller R: Uniparental disomies 7 and 14. Best Pract Res Clin Endocrinol Metab 2011;25:77–100.
Horsthemke B: Epimutations in human disease. Curr Top Microbiol Immunol 2006; 310:45–59.
Hosoki K, Kagami M, Tanaka T, Kubota M, Kurosawa K, Kato M, *et al*.: Maternal uniparental disomy 14 syndrome demonstrates prader-willi syndrome-like phenotype. J Pediatr 2009;155:900–903.e1.
Huang C-L, Lan M-Y, Chang Y-Y, Hsu C-Y, Lai S-C, Chen R-S, *et al*.: Large SGCE deletion contributes to Taiwanese myoclonus-dystonia syndrome. Parkinsonism Relat Disord 2010;16:585–589.
Hyllus D, Stein C, Schnabel K, Schiltz E, Imhof A, Dou Y, *et al*.: PRMT6-mediated methylation of R2 in histone H3 antagonizes H3 K4 trimethylation. Genes Dev 2007;21:3369–3380.
Ibrahim A, Kirby G, Hardy C, Dias RP, Tee L, Lim D, *et al*.: Methylation analysis and diagnostics of Beckwith-Wiedemann syndrome in 1,000 subjects. Clin Epigenetics 2014;6:11.
Inbar-Feigenberg M, Choufani S, Cytrynbaum C, Chen Y-A, Steele L, Shuman C, *et al*.: Mosaicism for genome-wide paternal uniparental disomy with features of multiple imprinting disorders: diagnostic and management issues. Am J Med Genet A 2013;161A:13–20.
Inoue T, Yagasaki H, Nishioka J, Nakamura A, Matsubara K, Narumi S, *et al*.: Molecular and clinical analyses of two patients with UPD(16)mat detected by screening 94 patients with Silver-Russell syndrome phenotype of unknown aetiology. J Med Genet 2019;56:413–418.
Ioannides Y, Lokulo-Sodipe K, Mackay DJG, Davies JH, Temple IK: Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet 2014;51:495–501.
Jirtle RL: Genomic Imprinting Website [Internet]. Geneimprint 2018;Available from: http://www.geneimprint.com
Jobling R, Stavropoulos DJ, Marshall CR, Cytrynbaum C, Axford MM, Londero V, *et al*.: Chitayat-Hall and Schaaf-Yang syndromes:a common aetiology: expanding the phenotype of MAGEL2-related disorders. J Med Genet 2018;55:316–321.

Johnson JP, Beischel L, Schwanke C, Styren K, Crunk A, Schoof J, *et al*.: Over-representation of pregnancies conceived by artificial reproductive technology in prenatally identified fetuses with Beckwith-Wiedemann syndrome. J Assist Reprod Genet 2018;

Kagami M, Kurosawa K, Miyazaki O, Ishino F, Matsuoka K, Ogata T: Comprehensive clinical studies in 34 patients with molecularly defined UPD(14)pat and related conditions (Kagami-Ogata syndrome). Eur J Hum Genet EJHG 2015;23:1488–1498.

Kagami M, Matsubara K, Nakabayashi K, Nakamura A, Sano S, Okamura K, *et al*.: Genome-wide multilocus imprinting disturbance analysis in Temple syndrome and Kagami-Ogata syndrome. Genet Med Off J Am Coll Med Genet 2017a;19:476–482.

Kagami M, Nagai T, Fukami M, Yamazawa K, Ogata T: Silver-Russell syndrome in a girl born after in vitro fertilization: partial hypermethylation at the differentially methylated region of PEG1/MEST. J Assist Reprod Genet 2007;24:131–136.

Kagami M, Nagasaki K, Kosaki R, Horikawa R, Naiki Y, Saitoh S, *et al*.: Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients. Genet Med Off J Am Coll Med Genet 2017b;19:1356–1366.

Kalish JM, Conlin LK, Bhatti TR, Dubbs HA, Harris MC, Izumi K, *et al*.: Clinical features of three girls with mosaic genome-wide paternal uniparental isodisomy. Am J Med Genet A 2013a;161A:1929–1939.

Kalish JM, Conlin LK, Mostoufi-Moab S, Wilkens AB, Mulchandani S, Zelley K, *et al*.: Bilateral pheochromocytomas, hemihyperplasia, and subtle somatic mosaicism: the importance of detecting low-level uniparental disomy. Am J Med Genet A 2013b;161A:993–1001.

Kamiya M, Judson H, Okazaki Y, Kusakabe M, Muramatsu M, Takada S, *et al*.: The cell cycle control gene ZAC/PLAGL1 is imprinted – a strong candidate gene for transient neonatal diabetes. Hum Mol Genet 2000;9:453–460.

Kanber D, Giltay J, Wieczorek D, Zogel C, Hochstenbach R, Caliebe A, *et al*.: A paternal deletion of MKRN3, MAGEL2 and NDN does not result in Prader-Willi syndrome. Eur J Hum Genet EJHG 2009;17:582–590.

Kanduri C: Long noncoding RNAs: Lessons from genomic imprinting. Biochim Biophys Acta 2016;1859:102–111.

Kappil M, Lambertini L, Chen J: Environmental Influences on Genomic Imprinting. Curr Environ Health Rep 2015;2:155–162.

Kawashima S, Nakamura A, Inoue T, Matsubara K, Horikawa R, Wakui K, *et al*.: Maternal Uniparental Disomy for Chromosome 20: Physical and Endocrinological Characteristics of Five Patients. J Clin Endocrinol Metab 2018a;103:2083–2088.

Kawashima S, Nakamura A, Inoue T, Matsubara K, Horikawa R, Wakui K, *et al*.: Maternal Uniparental Disomy for Chromosome 20: Physical and Endocrinological Characteristics of Six Patients [Internet]; in : ESPE Abstracts (2018). 2018b. Available from: http://abstracts.eurospe.org/hrp/0089/eposters/hrp0089p1-p186_eposter.pdf

Kellum R: Is HP1 an RNA detector that functions both in repression and activation? J Cell Biol 2003;161:671–672.

Kim J, Bretz CL, Lee S: Epigenetic instability of imprinted genes in human cancers. Nucleic Acids Res 2015;43:10689–10699.

King DA, Fitzgerald TW, Miller R, Canham N, Clayton-Smith J, Johnson D, *et al*.: A novel method for detecting uniparental disomy from trio genotypes identifies a significant excess in children with developmental disorders. Genome Res 2014; 24:673–687.

Kyllerman M: On the prevalence of Angelman syndrome. Am J Med Genet 1995; 59:405; author reply 403–404.
Lai KY, Skuse D, Stanhope R, Hindmarsh P: Cognitive abilities associated with the Silver-Russell syndrome. Arch Dis Child 1994;71:490–496.
Lakshman R, Forouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw K-T, *et al.*: Early age at menarche associated with cardiovascular disease and mortality. J Clin Endocrinol Metab 2009;94:4953–4960.
Lande A, Kroken M, Rabben K, Retterstol L: Temple syndrome as a differential diagnosis to Prader-Willi syndrome: Identifying three new patients. Am J Med Genet A 2018;176:175–180.
de Lange IM, Verrijn Stuart AA, van der Luijt RB, Ploos van Amstel HK, van Haelst MM: Macrosomia, obesity, and macrocephaly as first clinical presentation of PHP1b caused by STX16 deletion. Am J Med Genet A 2016;170:2431–2435.
Lawson-Yuen A, Wu B-L, Lip V, Sahoo T, Kimonis V: Atypical cases of Angelman syndrome. Am J Med Genet A 2006;140:2361–2364.
Ledbetter DH, Riccardi VM, Airhart SD, Strobel RJ, Keenan BS, Crawford JD: Deletions of chromosome 15 as a cause of the Prader-Willi syndrome. N Engl J Med 1981;304:325–329.
Lee HS, Jin H-S, Shim YS, Jeong HR, Kwon E, Choi V, *et al.*: Low Frequency of MKRN3 Mutations in Central Precocious Puberty Among Korean Girls. Horm Metab Res Horm Stoffwechselforschung Horm Metab 2016;48:118–122.
Li E, Zhang Y: DNA methylation in mammals. Cold Spring Harb Perspect Biol 2014;6:a019133.
Liehr T, Brude E, Gillessen-Kaesbach G, Konig R, Mrasek K, von Eggeling F, *et al.*: Prader-Willi syndrome with a karyotype 47,XY,+min(15)(pter–>q11.1:) and maternal UPD 15 – case report plus review of similar cases. Eur J Med Genet 2005;48:175–181.
Liehr T, Ewers E, Hamid AB, Kosyakova N, Voigt M, Weise A, *et al.*: Small supernumerary marker chromosomes and uniparental disomy have a story to tell. J Histochem Cytochem Off J Histochem Soc 2011;59:842–848.
Lionti T, Reid SM, White SM, Rowell MM: A population-based profile of 160 Australians with Prader-Willi syndrome: trends in diagnosis, birth prevalence and birth characteristics. Am J Med Genet A 2015;167A:371–378.
Liu J, Erlichman B, Weinstein LS: The stimulatory G protein alpha-subunit Gs alpha is imprinted in human thyroid glands: implications for thyroid function in pseudohypoparathyroidism types 1A and 1B. J Clin Endocrinol Metab 2003;88:4336–4341.
Liu L, Bai J, Qu Y, Jin Y, Wang H, Song F: [A study on genetic diagnosis for Angelman syndrome]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi Zhonghua Yixue Yichuanxue Zazhi Chin J Med Genet 2009;26:495–498.
Loke YJ, Hannan AJ, Craig JM: The Role of Epigenetic Change in Autism Spectrum Disorders. Front Neurol 2015;6:107.
Lopez SJ, Dunaway K, Islam MS, Mordaunt C, Vogel Ciernia A, Meguro-Horike M, *et al.*: UBE3A-mediated regulation of imprinted genes and epigenome-wide marks in human neurons. Epigenetics 2017;12:982–990.
Lossie AC, Whitney MM, Amidon D, Dong HJ, Chen P, Theriaque D, *et al.*: Distinct phenotypes distinguish the molecular classes of Angelman syndrome. J Med Genet 2001;38:834–845.
Luk H-M: Temple syndrome misdiagnosed as Silver-Russell syndrome. Clin Dysmorphol 2016;25:82–83.

Luk HM, Lo IFM: Angelman syndrome in Hong Kong Chinese: A 20 years' experience. Eur J Med Genet 2016;59:315–319.
Macdonald WA: Epigenetic mechanisms of genomic imprinting: common themes in the regulation of imprinted regions in mammals, plants, and insects. Genet Res Int 2012;2012:585024.
Macedo DB, Abreu AP, Reis ACS, Montenegro LR, Dauber A, Beneduzzi D, *et al*.: Central precocious puberty that appears to be sporadic caused by paternally inherited mutations in the imprinted gene makorin ring finger 3. J Clin Endocrinol Metab 2014;99:E1097–1103.
Macedo DB, Franca MM, Montenegro LR, Cunha-Silva M, Best DS, Abreu AP, *et al*.: Central Precocious Puberty Caused by a Heterozygous Deletion in the MKRN3 Promoter Region. Neuroendocrinology 2018;107:127–132.
Mackay DJG, Boonen SE, Clayton-Smith J, Goodship J, Hahnemann JMD, Kant SG, *et al*.: A maternal hypomethylation syndrome presenting as transient neonatal diabetes mellitus. Hum Genet 2006;120:262–269.
Mackay DJG, Callaway JLA, Marks SM, White HE, Acerini CL, Boonen SE, *et al*.: Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. Nat Genet 2008;40:949–951.
Mackay DJG, Eggermann T, Buiting K, Garin I, Netchine I, Linglart A, *et al*.: Multi-locus methylation defects in imprinting disorders. Biomol Concepts 2015;6:47–57.
Maeda T, Higashimoto K, Jozaki K, Yatsuki H, Nakabayashi K, Makita Y, *et al*.: Comprehensive and quantitative multilocus methylation analysis reveals the susceptibility of specific imprinted differentially methylated regions to aberrant methylation in Beckwith-Wiedemann syndrome with epimutations. Genet Med Off J Am Coll Med Genet 2014;16:903–912.
Mantovani G, Ballare E, Giammona E, Beck-Peccoz P, Spada A: The gsalpha gene: predominant maternal origin of transcription in human thyroid gland and gonads. J Clin Endocrinol Metab 2002;87:4736–4740.
Mantovani G, Bastepe M, Monk D, de Sanctis L, Thiele S, Usardi A, *et al*.: Diagnosis and management of pseudohypoparathyroidism and related disorders: first international Consensus Statement. Nat Rev Endocrinol 2018;14:476–500.
Mantovani G, de Sanctis L, Barbieri AM, Elli FM, Bollati V, Vaira V, *et al*.: Pseudo-hypoparathyroidism and GNAS epigenetic defects: clinical evaluation of albright hereditary osteodystrophy and molecular analysis in 40 patients. J Clin Endocrinol Metab 2010;95:651–658.
Marques AC, Ponting CP: Intergenic lncRNAs and the evolution of gene expression. Curr Opin Genet Dev 2014;27:48–53.
Matarazzo V, Muscatelli F: Natural breaking of the maternal silence at the mouse and human imprinted Prader-Willi locus: A whisper with functional consequences. Rare Dis Austin Tex 2013;1:e27228.
Maupetit-Mehouas S, Azzi S, Steunou V, Sakakini N, Silve C, Reynes C, *et al*.: Simultaneous hyper- and hypomethylation at imprinted loci in a subset of patients with GNAS epimutations underlies a complex and different mechanism of multilocus methylation defect in pseudohypoparathyroidism type 1b. Hum Mutat 2013;34:1172–1180.
McCarthy J, Lupo PJ, Kovar E, Rech M, Bostwick B, Scott D, *et al*.: Schaaf-Yang syndrome overview: Report of 78 individuals. Am J Med Genet A 2018;176:2564–2574.

McGrath J, Solter D: Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell 1984;37:179–183.
Merner N, Forgeot d'Arc B, Bell SC, Maussion G, Peng H, Gauthier J, *et al.*: A de novo frameshift mutation in chromodomain helicase DNA-binding domain 8 (CHD8): A case report and literature review. Am J Med Genet A 2016;170A:1225–1235.
Mertz LGB, Christensen R, Vogel I, Hertz JM, Nielsen KB, Grønskov K, *et al.*: Angelman syndrome in Denmark. birth incidence, genetic findings, and age at diagnosis. Am J Med Genet A 2013;161A:2197–2203.
Metz C, Cave H, Bertrand AM, Deffert C, Gueguen-Giroux B, Czernichow P, *et al.*: Neonatal diabetes mellitus: chromosomal analysis in transient and permanent cases. J Pediatr 2002;141:483–489.
Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold J-A, Kimonis V, *et al.*: Nutritional phases in Prader-Willi syndrome. Am J Med Genet A 2011;155A:1040–1049.
Mitchell BD, Pollin TI: Genomic imprinting in diabetes. Genome Med 2010;2:55.
Moelans CB, Atanesyan L, Savola SP, van Diest PJ: Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA). Methods Mol Biol Clifton NJ 2018;1708:537–549.
Molinaro A, Tiosano D, Takatani R, Chrysis D, Russell W, Koscielniak N, *et al.*: TSH elevations as the first laboratory evidence for pseudohypoparathyroidism type Ib (PHP-Ib). J Bone Miner Res Off J Am Soc Bone Miner Res 2015;30:906–912.
Monk D: Genomic imprinting in the human placenta. Am J Obstet Gynecol 2015; 213:S152–162.
Monk D, Morales J, den Dunnen JT, Russo S, Court F, Prawitt D, *et al.*: Recommendations for a nomenclature system for reporting methylation aberrations in imprinted domains. Epigenetics 2018;13:117–121.
Monk M: Genomic imprinting. Memories of mother and father. Nature 1987;328:203–204.
Monk M: Genomic imprinting. Genes Dev 1988;2:921–925.
Mouallem M, Shaharabany M, Weintrob N, Shalitin S, Nagelberg N, Shapira H, *et al.*: Cognitive impairment is prevalent in pseudohypoparathyroidism type Ia, but not in pseudopseudohypoparathyroidism: possible cerebral imprinting of Gsalpha. Clin Endocrinol (Oxf) 2008;68:233–239.
Mulchandani S, Bhoj EJ, Luo M, Powell-Hamilton N, Jenny K, Gripp KW, *et al.*: Maternal uniparental disomy of chromosome 20: a novel imprinting disorder of growth failure. Genet Med Off J Am Coll Med Genet 2016;18:309–315.
Mussa A, Molinatto C, Baldassarre G, Riberi E, Russo S, Larizza L, *et al.*: Cancer Risk in Beckwith-Wiedemann Syndrome: A Systematic Review and Meta-Analysis Outlining a Novel (Epi)Genotype Specific Histotype Targeted Screening Protocol. J Pediatr 2016a;176:142–149.e1.
Mussa A, Russo S, de Crescenzo A, Freschi A, Calzari L, Maitz S, *et al.*: Fetal growth patterns in Beckwith-Wiedemann syndrome. Clin Genet 2016b;90:21–27.
Mussa A, Russo S, De Crescenzo A, Chiesa N, Molinatto C, Selicorni A, *et al.*: Prevalence of Beckwith-Wiedemann syndrome in North West of Italy. Am J Med Genet A 2013;161A:2481–2486.
Mussa A, Russo S, Larizza L, Riccio A, Ferrero GB: (Epi)genotype-phenotype correlations in Beckwith-Wiedemann syndrome: a paradigm for genomic medicine. Clin Genet 2016c;89:403–415.

Nakamura Y, Matsumoto T, Tamakoshi A, Kawamura T, Seino Y, Kasuga M, *et al*.: Prevalence of idiopathic hypoparathyroidism and pseudohypoparathyroidism in Japan. J Epidemiol 2000;10:29–33.

Nakka P, Smith SP, O'Donnell-Luria AH, McManus KF, Mountain JL, Ramachandran S, *et al*.: Characterization of prevalence and health consequences of uniparental disomy in four million individuals from the general population. bioRxiv 2019; 540955.

de Nanclares GP, Fernandez-Rebollo E, Santin I, Garcia-Cuartero B, Gaztambide S, Menendez E, *et al*.: Epigenetic defects of GNAS in patients with pseudohypoparathyroidism and mild features of Albright's hereditary osteodystrophy. J Clin Endocrinol Metab 2007;92:2370–2373.

Netchine I, Rossignol S, Dufourg M-N, Azzi S, Rousseau A, Perin L, *et al*.: 11p15 imprinting center region 1 loss of methylation is a common and specific cause of typical Russell-Silver syndrome: clinical scoring system and epigenetic-phenotypic correlations. J Clin Endocrinol Metab 2007;92:3148–3154.

Neuheuser L, Meyer R, Begemann M, Elbracht M, Eggermann T: Next generation sequencing and imprinting disorders: Current applications and future perspectives: Lessons from Silver-Russell syndrome. Mol Cell Probes 2019;

Nishioka J, Shima H, Fukami M, Yatsuga S, Matsumoto T, Ushijima K, *et al*.: The first Japanese case of central precocious puberty with a novel MKRN3 mutation. Hum Genome Var 2017;4:17017.

Noeker M, Wollmann HA: Cognitive development in Silver-Russell syndrome: a sibling-controlled study. Dev Med Child Neurol 2004;46:340–346.

Ogata T, Kagami M: Kagami-Ogata syndrome: a clinically recognizable upd(14)pat and related disorder affecting the chromosome 14q32.2 imprinted region. J Hum Genet 2016;61:87–94.

Õiglane E, Õunap K, Bartsch O, Rein R, Talvik T: Sudden death of a girl with Prader-Willi syndrome. Genet Couns Geneva Switz 2002;13:459–464.

Õiglane-Shlik E: Angelman and Prader-Willi syndromes in Estonia 2007;

Õiglane-Shlik E, Rein R, Tillmann V, Talvik T, Õunap K: A female with Angelman syndrome and unusual limb deformities. Pediatr Neurol 2005;33:66–69.

Õiglane-Shlik E, Talvik T, Zordania R, Põder H, Kahre T, Raukas E, *et al*.: Prevalence of Angelman syndrome and Prader-Willi syndrome in Estonian children: sister syndromes not equally represented. Am J Med Genet A 2006a;140:1936–1943.

Õiglane-Shlik E, Zordania R, Varendi H, Antson A, Magi M-L, Tasa G, *et al*.: The neonatal phenotype of Prader-Willi syndrome. Am J Med Genet A 2006b;140:1241–1244.

Okun N, Sierra S, GENETICS COMMITTEE, SPECIAL CONTRIBUTORS: Pregnancy outcomes after assisted human reproduction. J Obstet Gynaecol Can JOGC J Obstet Gynecol Can JOGC 2014;36:64–83.

Õunap K, Reimand T, Magi M-L, Bartsch O: Two sisters with Silver-Russell phenotype. Am J Med Genet A 2004;131:301–306.

Pajusalu S, Pfundt R, Vissers LELM, Kwint MP, Reimand T, Õunap K, *et al*.: Identifying long indels in exome sequencing data of patients with intellectual disability [Internet]. . bioRxiv 2018 [cited 2019 Jan 28];Available from: http://biorxiv.org/lookup/doi/10.1101/244756

Parikh S, Goldstein A, Karaa A, Koenig MK, Anselm I, Brunel-Guitton C, *et al*.: Patient care standards for primary mitochondrial disease: a consensus statement from the

Mitochondrial Medicine Society. Genet Med Off J Am Coll Med Genet 2017; 19:1380.
Pauwels S, Ghosh M, Duca RC, Bekaert B, Freson K, Huybrechts I, *et al*.: Dietary and supplemental maternal methyl-group donor intake and cord blood DNA methylation. Epigenetics 2017;12:1–10.
Peall KJ, Dijk JM, Saunders-Pullman R, Dreissen YEM, van Loon I, Cath D, *et al*.: Psychiatric disorders, myoclonus dystonia and SGCE: an international study. Ann Clin Transl Neurol 2016;3:4–11.
Peall KJ, Kurian MA, Wardle M, Waite AJ, Hedderly T, Lin J-P, *et al*.: SGCE and myoclonus dystonia: motor characteristics, diagnostic criteria and clinical predictors of genotype. J Neurol 2014;261:2296–2304.
Pelc K, Boyd SG, Cheron G, Dan B: Epilepsy in Angelman syndrome. Seizure 2008; 17:211–217.
Perez-Nanclares G, Romanelli V, Mayo S, Garin I, Zazo Seco C, Fernandez-Rebollo E, *et al*.: Detection of hypomethylation syndrome among patients with epigenetic alterations at the GNAS locus. J Clin Endocrinol Metab 2012;97:E1060-1067.
Peters SU, Goddard-Finegold J, Beaud*et al*, Madduri N, Turcich M, Bacino CA: Cognitive and adaptive behavior profiles of children with Angelman syndrome. Am J Med Genet A 2004;128A:110–113.
Petersen MB, Brøndum-Nielsen K, Hansen LK, Wulff K: Clinical, cytogenetic, and molecular diagnosis of Angelman syndrome: estimated prevalence rate in a Danish county. Am J Med Genet 1995;60:261–262.
Plagge A, Gordon E, Dean W, Boiani R, Cinti S, Peters J, *et al*.: The imprinted signaling protein XL alpha s is required for postnatal adaptation to feeding. Nat Genet 2004;36:818–826.
Polak M, Cave H: Neonatal diabetes mellitus: a disease linked to multiple mechanisms. Orphanet J Rare Dis 2007;2:12.
Poole RL, Docherty LE, Al Sayegh A, Caliebe A, Turner C, Baple E, *et al*.: Targeted methylation testing of a patient cohort broadens the epigenetic and clinical description of imprinting disorders. Am J Med Genet A 2013;161A:2174–2182.
Poyatos D, Camprubi C, Gabau E, Nosas R, Villatoro S, Coll MD, *et al*.: [Prader Willi syndrome patients: study of 77 patients]. Med Clin (Barc) 2009;133:649–656.
Pozharny Y, Lambertini L, Ma Y, Ferrara L, Litton CG, Diplas A, *et al*.: Genomic loss of imprinting in first-trimester human placenta. Am J Obstet Gynecol 2010; 202:391.e1–8.
Prader A, Labhart A, Willi H: Ein Syndrom von Adipositas, Kleinwuchs, Kryptorchismus und Oligophrenie nach myatonieartigem Zustand im Neugeborenenalter. Schweizerische Medizinische Wochenschrift 1956;86:1260–1261.
Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC: The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. J Med Genet 1999;36:837–842.
Rachad L, El Kadmiri N, Slassi I, El Otmani H, Nadifi S: Genetic Aspects of Myoclonus-Dystonia Syndrome (MDS). Mol Neurobiol 2017;54:939–942.
Rao DS, Parfitt AM, Kleerekoper M, Pumo BS, Frame B: Dissociation between the effects of endogenous parathyroid hormone on adenosine 3',5'-monophosphate generation and phosphate reabsorption in hypocalcemia due to vitamin D depletion: an acquired disorder resembling pseudohypoparathyroidism type II. J Clin Endocrinol Metab 1985;61:285–290.

Reik W, Brown KW, Schneid H, Le Bouc Y, Bickmore W, Maher ER: Imprinting mutations in the Beckwith-Wiedemann syndrome suggested by altered imprinting pattern in the IGF2-H19 domain. Hum Mol Genet 1995;4:2379–2385.

Richard-De Ceaurriz B, Leymarie C, Godefroy A, Collignon P, Sigaudy S, Truc P: [Neonatal presentation of Prader-Willi syndrome: A report of five cases]. Arch Pediatr Organe Off Soc Francaise Pediatr 2017;24:1115–1120.

Richer LP, Shevell MI, Miller SP: Diagnostic profile of neonatal hypotonia: an 11-year study. Pediatr Neurol 2001;25:32–37.

Rochtus A, Martin-Trujillo A, Izzi B, Elli F, Garin I, Linglart A, *et al*.: Genome-wide DNA methylation analysis of pseudohypoparathyroidism patients with GNAS imprinting defects. Clin Epigenetics 2016;8:10.

Roze E, Apartis E, Clot F, Dorison N, Thobois S, Guyant-Marechal L, *et al*.: Myoclonus-dystonia: clinical and electrophysiologic pattern related to SGCE mutations. Neurology 2008;70:1010–1016.

Roze E, Lang AE, Vidailhet M: Myoclonus-dystonia: classification, phenomenology, pathogenesis, and treatment. Curr Opin Neurol 2018;31:484–490.

Russell A: A syndrome of intra-uterine dwarfism recognizable at birth with cranio-facial dysostosis, disproportionately short arms, and other anomalies (5 examples). Proc R Soc Med 1954;47:1040–1044.

Saal HM, Adam MP, Ardinger HH: Russell-Silver Syndrome [Internet]. GeneReviews(R) 2011; Available from: http://www.ncbi.nlm.nih.gov/books/NBK1324/?report=classic

Sachwitz J, Strobl-Wildemann G, Fekete G, Ambrozaityte L, Kucinskas V, Soellner L, *et al*.: Examinations of maternal uniparental disomy and epimutations for chromosomes 6, 14, 16 and 20 in Silver-Russell syndrome-like phenotypes. BMC Med Genet 2016;17:20.

Sahoo T, Bacino CA, German JR, Shaw CA, Bird LM, Kimonis V, *et al*.: Identification of novel deletions of 15q11q13 in Angelman syndrome by array-CGH: molecular characterization and genotype-phenotype correlations. Eur J Hum Genet EJHG 2007;15:943–949.

Sahoo T, Peters SU, Madduri NS, Glaze DG, German JR, Bird LM, *et al*.: Microarray based comparative genomic hybridization testing in deletion bearing patients with Angelman syndrome: genotype-phenotype correlations. J Med Genet 2006;43:512–516.

Sanchez-Delgado M, Riccio A, Eggermann T, Maher ER, Lapunzina P, Mackay D, *et al*.: Causes and Consequences of Multi-Locus Imprinting Disturbances in Humans. Trends Genet TIG 2016;32:444–455.

de Sanctis L, Giachero F, Mantovani G, Weber G, Salerno M, Baroncelli GI, *et al*.: Genetic and epigenetic alterations in the GNAS locus and clinical consequences in Pseudohypoparathyroidism: Italian common healthcare pathways adoption. Ital J Pediatr 2016;42:101.

Sano S, Matsubara K, Nagasaki K, Kikuchi T, Nakabayashi K, Hata K, *et al*.: Beckwith-Wiedemann syndrome and pseudohypoparathyroidism type Ib in a patient with multilocus imprinting disturbance: a female-dominant phenomenon? J Hum Genet 2016;61:765–769.

Santa María L, Curotto B, Cortés F, Rojas C, Alliende MA: [Molecular diagnosis of Prader-Willi and Angelman syndromes: methylation, cytogenetics and FISH analysis]. Rev Med Chil 2001;129:367–374.

Sato K, Iwakoshi M, Shimokawa O, Sakai H, Ohta T, Saitoh S, *et al.*: Angelman syndrome caused by an identical familial 1,487-kb deletion. Am J Med Genet A 2007;143A:98–101.

Schaaf CP, Gonzalez-Garay ML, Xia F, Potocki L, Gripp KW, Zhang B, *et al.*: Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. Nat Genet 2013;45:1405–1408.

Schreiner F, Gohlke B, Hamm M, Korsch E, Woelfle J: MKRN3 mutations in familial central precocious puberty. Horm Res Paediatr 2014;82:122–126.

Schulze A, Mogensen H, Hamborg-Petersen B, Graem N, Ostergaard JR, Brøndum-Nielsen K: Fertility in Prader-Willi syndrome: a case report with Angelman syndrome in the offspring. Acta Paediatr Oslo Nor 1992 2001;90:455–459.

Settas N, Dacou-Voutetakis C, Karantza M, Kanaka-Gantenbein C, Chrousos GP, Voutetakis A: Central precocious puberty in a girl and early puberty in her brother caused by a novel mutation in the MKRN3 gene. J Clin Endocrinol Metab 2014;99:E647–651.

Severi G, Bernardini L, Briuglia S, Bigoni S, Buldrini B, Magini P, *et al.*: New patients with Temple syndrome caused by 14q32 deletion: Genotype-phenotype correlations and risk of thyroid cancer. Am J Med Genet A 2016;170A:162–169.

Sheridan MB, Bytyci Telegrafi A, Stinnett V, Umeh CC, Mari Z, Dawson TM, *et al.*: Myoclonus-dystonia and Silver-Russell syndrome resulting from maternal uniparental disomy of chromosome 7. Clin Genet 2013;84:368–372.

Shin Y-L: An update on the genetic causes of central precocious puberty. Ann Pediatr Endocrinol Metab 2016;21:66–69.

Shuman C, Beckwith JB, Weksberg R: Beckwith-Wiedemann Syndrome [Internet]. GeneReviews(R) 2016;Available from: https://www.ncbi.nlm.nih.gov/books/NBK1394/

Sibbons C, Morris JK, Crolla JA, Jacobs PA, Thomas NS: De novo deletions and duplications detected by array CGH: a study of parental origin in relation to mechanisms of formation and size of imbalance. Eur J Hum Genet EJHG 2012; 20:155–160.

Silver HK, Kiyasu W, George J, Deamer WC: Syndrome of congenital hemihypertrophy, shortness of stature, and elevated urinary gonadotropins. Pediatrics 1953;12:368–376.

Skaar DA, Li Y, Bernal AJ, Hoyo C, Murphy SK, Jirtle RL: The human imprintome: regulatory mechanisms, methods of ascertainment, and roles in disease susceptibility. ILAR J 2012;53:341–358.

Smith A, Egan J, Ridley G, Haan E, Montgomery P, Williams K, *et al.*: Birth prevalence of Prader-Willi syndrome in Australia. Arch Dis Child 2003;88:263–264.

Soellner L, Begemann M, Mackay DJG, Grønskov K, Tümer Z, Maher ER, *et al.*: Recent Advances in Imprinting Disorders. Clin Genet 2017;91:3–13.

Soellner L, Monk D, Rezwan FI, Begemann M, Mackay D, Eggermann T: Congenital imprinting disorders: Application of multilocus and high throughput methods to decipher new pathomechanisms and improve their management. Mol Cell Probes 2015;29:282–290.

Soubry A, Murphy SK, Wang F, Huang Z, Vidal AC, Fuemmeler BF, *et al.*: Newborns of obese parents have altered DNA methylation patterns at imprinted genes. Int J Obes 2005 2015;39:650–657.

Stanisławska J, Olszewski WL: RNA interference – significance and applications. Arch Immunol Ther Exp (Warsz) 2005;53:39–46.

Statistics Estonia. Stat Est 2018;Available from: www.stat.ee
Steffenburg S, Gillberg CL, Steffenburg U, Kyllerman M: Autism in Angelman syndrome: a population-based study. Pediatr Neurol 1996;14:131–136.
Strømme P: Aetiology in severe and mild mental retardation: a population-based study of Norwegian children. Dev Med Child Neurol 2000;42:76–86.
Summers JA, Allison DB, Lynch PS, Sandler L: Behaviour problems in Angelman syndrome. J Intellect Disabil Res JIDR 1995;39 (Pt 2):97–106.
Sun Y, Nicholls RD, Butler MG, Saitoh S, Hainline BE, Palmer CG: Breakage in the SNRPN locus in a balanced 46,XY,t(15;19) Prader-Willi syndrome patient. Hum Mol Genet 1996;5:517–524.
Surani MA, Barton SC, Norris ML: Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 1984;308:548–550.
Tafaj O, Juppner H: Pseudohypoparathyroidism: one gene, several syndromes. J Endocrinol Invest 2017;40:347–356.
Tee L, Lim DH, Dias RP, Baudement M-O, Slater AA, Kirby G, *et al.*: Epimutation profiling in Beckwith-Wiedemann syndrome: relationship with assisted reproductive technology. Clin Epigenetics 2013;5:23.
Temple I, Mackay D: Diabetes Mellitus, 6q24-Related Transient Neonatal [Internet]; in: GeneReviews((R)). Seattle (WA), University of Washington, Seattle, 2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1534/
Temple IK, Cockwell A, Hassold T, Pettay D, Jacobs P: Maternal uniparental disomy for chromosome 14. J Med Genet 1991;28:511–514.
Temple IK, Mackay DJ, Docherty L: Diabetes Mellitus, 6q24-Related Transient Neonatal [Internet]. GeneReviews(R) 2015;Available from: http://www.ncbi.nlm.nih.gov/books/NBK1534/
Thomson AK, Glasson EJ, Bittles AH: A long-term population-based clinical and morbidity profile of Angelman syndrome in Western Australia: 1953–2003. Disabil Rehabil 2006a;28:299–305.
Thomson AK, Glasson EJ, Bittles AH: A long-term population-based clinical and morbidity review of Prader-Willi syndrome in Western Australia. J Intellect Disabil Res JIDR 2006b;50:69–78.
Thorburn MJ, Wright ES, Miller CG, Smith-Read EH: Exomphalos-macroglossia-gigantism syndrome in Jamaican infants. Am J Dis Child 1970;119:316–321.
Turan S: Current Nomenclature of Pseudohypoparathyroidism: Inactivating Parathyroid Hormone/Parathyroid Hormone-Related Protein Signaling Disorder. J Clin Res Pediatr Endocrinol 2017;9:58–68.
Turan S, Thiele S, Tafaj O, Brix B, Atay Z, Abali S, *et al.*: Evidence of hormone resistance in a pseudo-pseudohypoparathyroidism patient with a novel paternal mutation in GNAS. Bone 2015;71:53–57.
Tuysuz B, Kartal N, Erener-Ercan T, Guclu-Geyik F, Vural M, Perk Y, *et al.*: Prevalence of Prader-Willi syndrome among infants with hypotonia. J Pediatr 2014; 164:1064–1067.
Uibo O, Grünberg H, Kallas E, Talvik T: Lastehaiguste propedeutika algtõed., Tartu Ülikooli Kirjastus, 2010.
Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L: Pseudohypoparathyroidism – epidemiology, mortality and risk of complications. Clin Endocrinol (Oxf) 2016;84:904–911.

Vals M-A, Kahre T, Mee P, Muru K, Kallas E, Zilina O, *et al*.: Familial 1.3-Mb 11p15.5p15.4 Duplication in Three Generations Causing Silver-Russell and Beckwith-Wiedemann Syndromes. Mol Syndromol 2015a;6:147–151.

Vals M-A, Oiglane-Shlik E, Noukas M, Shor R, Peet A, Kals M, *et al*.: Coffin-Siris Syndrome with obesity, macrocephaly, hepatomegaly and hyperinsulinism caused by a mutation in the ARID1B gene. Eur J Hum Genet EJHG 2014;22:1327–1329.

Vals M-A, Yakoreva M, Kahre T, Mee P, Muru K, Joost K, *et al*.: The Frequency of Methylation Abnormalities Among Estonian Patients Selected by Clinical Diagnostic Scoring Systems for Silver-Russell Syndrome and Beckwith-Wiedemann Syndrome. Genet Test Mol Biomark 2015b;19:684–691.

Varela MC, Fridman C, Koiffmann CP: Diagnosis of patients with Prader-Willi and Angelman Syndromes: the importance of an overall investigation. Genet Mol Biol 2002;25:07–12.

Vendrame M, Loddenkemper T, Zarowski M, Gregas M, Shuhaiber H, Sarco DP, *et al*.: Analysis of EEG patterns and genotypes in patients with Angelman syndrome. Epilepsy Behav EB 2012;23:261–265.

Veselovska L, Smallwood SA, Saadeh H, Stewart KR, Krueger F, Maupetit-Méhouas S, *et al*.: Deep sequencing and de novo assembly of the mouse oocyte transcriptome define the contribution of transcription to the DNA methylation landscape. Genome Biol 2015;16:209.

Vogels A, Van Den Ende J, Keymolen K, Mortier G, Devriendt K, Legius E, *et al*.: Minimum prevalence, birth incidence and cause of death for Prader-Willi syndrome in Flanders. Eur J Hum Genet EJHG 2004;12:238–240.

de Vries L, Gat-Yablonski G, Dror N, Singer A, Phillip M: A novel MKRN3 missense mutation causing familial precocious puberty. Hum Reprod Oxf Engl 2014;29:2838–2843.

de Vries L, Kauschansky A, Shohat M, Phillip M: Familial central precocious puberty suggests autosomal dominant inheritance. J Clin Endocrinol Metab 2004;89:1794–1800.

Wakeling EL, Amero SA, Alders M, Bliek J, Forsythe E, Kumar S, *et al*.: Epigenotype-phenotype correlations in Silver-Russell syndrome. J Med Genet 2010;47:760–768.

Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, *et al*.: Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol 2017;13:105–124.

Wang H, Nettleton D, Ying K: Copy number variation detection using next generation sequencing read counts. BMC Bioinformatics 2014;15:109.

Wang J, Liu J, Gao Y, Wang K, Jiang K: Autism spectrum disorder early in development associated with CHD8 mutations among two Chinese children. BMC Pediatr 2018;18:338.

Wang JC, Passage MB, Yen PH, Shapiro LJ, Mohandas TK: Uniparental heterodisomy for chromosome 14 in a phenotypically abnormal familial balanced 13/14 Robertsonian translocation carrier. Am J Hum Genet 1991;48:1069–1074.

van Wegberg AMJ, MacDonald A, Ahring K, Bélanger-Quintana A, Blau N, Bosch AM, *et al*.: The complete European guidelines on phenylketonuria: diagnosis and treatment. Orphanet J Rare Dis 2017;12:162.

Weksberg R, Shuman C, Beckwith JB: Beckwith-Wiedemann syndrome. Eur J Hum Genet EJHG 2010;18:8–14.

Whittington J, Holland A, Webb T, Butler J, Clarke D, Boer H: Academic under-achievement by people with Prader-Willi syndrome. J Intellect Disabil Res JIDR 2004;48:188–200.
Whittington JE, Holland AJ, Webb T, Butler J, Clarke D, Boer H: Population prevalence and estimated birth incidence and mortality rate for people with Prader-Willi syndrome in one UK Health Region. J Med Genet 2001;38:792–798.
Wiedemann H: Complexe malformatif familial avec hernie ombilicale et macroglossie – un "syndrome nouveau"? J Genet Hum 1964;13:223–32.
Wiedemann HR: Frequency of Wiedemann-Beckwith syndrome in Germany; rate of hemihyperplasia and of tumours in affected children. Eur J Pediatr 1997;156:251.
Williams CA, Driscoll DJ, Dagli AI: Clinical and genetic aspects of Angelman syndrome. Genet Med Off J Am Coll Med Genet 2010;12:385–395.
Wollmann HA, Kirchner T, Enders H, Preece MA, Ranke MB: Growth and symptoms in Silver-Russell syndrome: review on the basis of 386 patients. Eur J Pediatr 1995;154:958–968.
Xie T, Plagge A, Gavrilova O, Pack S, Jou W, Lai EW, *et al.*: The alternative stimulatory G protein alpha-subunit XLalphas is a critical regulator of energy and glucose metabolism and sympathetic nerve activity in adult mice. J Biol Chem 2006;281:18989–18999.
Yakoreva M, Kahre T, Pajusalu S, Ilisson P, Zilina O, Tillmann V, *et al.*: A New Case of a Rare Combination of Temple Syndrome and Mosaic Trisomy 14 and a Literature Review. Mol Syndromol 2018;9:182–189.
Yakoreva M, Kahre T, Zordania R, Reinson K, Teek R, Tillmann V, *et al.*: A retrospective analysis of the prevalence of imprinting disorders in Estonia from 1998 to 2016. Eur J Hum Genet 2019.
Yakoreva M, Vals M-A, Kahre T, Õunap K: Imprinting disorders: a literature review and presentation of cases. Eesti Arst 2017;96(1):22–35.
Yamazawa K, Ogata T, Ferguson-Smith AC: Uniparental disomy and human disease: an overview. Am J Med Genet C Semin Med Genet 2010;154C:329–334.
Yao R, Zhang C, Yu T, Li N, Hu X, Wang X, *et al.*: Evaluation of three read-depth based CNV detection tools using whole-exome sequencing data. Mol Cytogenet 2017;10:30.
Yasin H, Gibson WT, Langlois S, Stowe RM, Tsang ES, Lee L, *et al.*: A distinct neuro-developmental syndrome with intellectual disability, autism spectrum disorder, characteristic facies, and macrocephaly is caused by defects in CHD8. J Hum Genet 2019;64:271–280.
Yorifuji T, Matsubara K, Sakakibara A, Hashimoto Y, Kawakita R, Hosokawa Y, *et al.*: Abnormalities in chromosome 6q24 as a cause of early-onset, non-obese, non-autoimmune diabetes mellitus without history of neonatal diabetes. Diabet Med J Br Diabet Assoc 2015;32:963–967.
Yu S, Gavrilova O, Chen H, Lee R, Liu J, Pacak K, *et al.*: Paternal versus maternal transmission of a stimulatory G-protein alpha subunit knockout produces opposite effects on energy metabolism. J Clin Invest 2000;105:615–623.
Zadeh N, Graham JMJM: KCNK9 Imprinting Syndrome; in: GeneReviews((R)). Seattle (WA), University of Washington, Seattle, 2017.
Zarate YA, Mena R, Martin LJ, Steele P, Tinkle BT, Hopkin RJ: Experience with hemihyperplasia and Beckwith-Wiedemann syndrome surveillance protocol. Am J Med Genet A 2009;149A:1691–1697.

Zazo C, Thiele S, Martin C, Fernandez-Rebollo E, Martinez-Indart L, Werner R, *et al*.: Gsalpha activity is reduced in erythrocyte membranes of patients with pseudo-hypoparathyroidism due to epigenetic alterations at the GNAS locus. J Bone Miner Res Off J Am Soc Bone Miner Res 2011;26:1864–1870.

Zhang Y, Tycko B: Monoallelic expression of the human H19 gene. Nat Genet 1992;1:40–44.

Zhao Y, Sun H, Wang H: Long noncoding RNAs in DNA methylation: new players stepping into the old game. Cell Biosci 2016;6:45.

Zilina O, Kahre T, Talvik I, Õiglane-Shlik E, Tillmann V, Õunap K: Mosaicism for maternal uniparental disomy 15 in a boy with some clinical features of Prader-Willi syndrome. Eur J Med Genet 2014a;57:279–283.

Zilina O, Teek R, Tammur P, Kuuse K, Yakoreva M, Vaidla E, *et al*.: Chromosomal microarray analysis as a first-tier clinical diagnostic test: Estonian experience. Mol Genet Genomic Med 2014b;2:166–175.

Zimprich A, Grabowski M, Asmus F, Naumann M, Berg D, Bertram M, *et al*.: Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. Nat Genet 2001;29:66–69.

SUMMARY IN ESTONIAN

Vermimishäired Eestis

Vermimishäired on rühm harva esinevaid pärilikke haigusi, mis on tingitud vermitud ehk imprinditud geenide ekspressiooni muutustest tänu geneetilistele või epigeneetilistele muutustele genoomis. Kuigi neli klassikalist ning kõige sagedasemat vermimishäiret – Prader-Willi sündroom (PWS; OMIM #176270), Angelmani sündroom (AS; OMIM #105830), Beckwith-Wiedemanni sündroom (BWS; OMIM #130650) ja Silver-Russelli sündroom (SRS; OMIM #180860) – olid esimest korda kliiniliselt kirjeldatud juba 20. sajandi 50–60-ndatel aastatel [Prader *et al*., 1956; Angelman, 1965; B Beckwith, 1963; Russell, 1954; Silver *et al*., 1953; Wiedemann, 1964], jäi nende haiguste molekulaarne mehhanism teadmata veel järgmise kahe aastakümne jooksul, kuni 1980. aastate keskpaigani.

1984-ndal aastal avaldasid Davor Solter Wistar Instituudist (Philadelphia, USA) ning Azim Surani Loomade Füsioloogia AFRC Instituudist (Cambridge, UK) koos kolleegidega tulemused katsetest hiire embrüotega, mis sisaldasid kahte kas ainult emapoolsete või ainult isapoolsete kromosoomide komplekti [Barton *et al*., 1984; McGrath, and Solter, 1984; Surani *et al*., 1984]. Need katsed demonstreerisid, et emapoolsete kromosoomide komplekt ei ole funktsionaalselt võrdne isapoolsete kromosoomide komplektiga ja ka vastupidi ning embrüote normaalse arengu jaoks on tarvis ühte kromosoomide komplekti mõlemalt vanemalt. Eeldati, et mõnel geenil tekib peale viljastamist vanem-spetsiifiline ekspressioon ning seda nähtust nimetati genoomseks vermimiseks [Monk, 1987; Monk, 1988].

1991-ndal aastal määrati ja kaardistati hiire genoomis kolm esimest vermitud geeni, *Igf2r*, *Igf2* ja *H19* [Barlow *et al*., 1991; DeChiara *et al*., 1991; Ferguson-Smith *et al*., 1991; Bartolomei *et al*., 1991]. Aasta hiljem kinnitati monoalleelne vanem-spetsiifiline ekspressioon ka inimese *H19* geenil [Zhang, and Tycko, 1992]. Alates sellest ajast on inimese genoomis avastatud rohkem kui 100 vermitud geeni. Lisaks on leitud veel umbes 100 inimese geeni, millel on ennustatud, kuid mitte kinnitatud, vermimine [Jirtle, 2018].

Aastate jooksul on molekulaarsed ja kliinilised uuringud näidanud, et vermitud geenid on olulised mitte ainult prenataalse arengu jaoks, vaid ka paljude postnataalsete protsesside jaoks. Patoloogilised muutused vermitud geenide ekspressioonis võivad oluliselt mõjutada postnataalset kasvu, aju-funktsiooni, käitumist, hormonaalseid ja metaboolseid süsteeme ning põhjustada kompleksset sündroomi. Vaatamata suurele hulgale avastatud vermitud geenidele, on teadaolevate kaasasündinud vermimishäirete arv tagasihoidlik. Praegu on teada ainult 13 kliiniliselt tunnustatud kaasasündinud vermimishäiret: PWS, AS, BWS, SRS, *GNAS*-geeniga seotud ImpDis – pseudohüpoparatüreoidism ja pseudopseudohüpoparatüreoidism (PHP/PPHP; OMIM #103580, #603233, #612462, #612463), tsentraalne enneaegne puberteet (CPP; OMIM #615346), Temple sündroom (TS14; OMIM #616222), transitoorne neonataalne

diabeet (TNDM; OMIM #601410), müokloonus-düstoonia sündroom (MDS; OMIM #604149), Kagami-Ogata sündroom (KOS; OMIM #608149), 20. kromosoomi emapoolse uniparentaalse disoomia sündroom (UPD(20)mat; OMIM #617352), Schaaf-Yangi sündroom (SYS; OMIM #615547) ja Birk-Bareli sündroom (OMIM #612292). Peale selle on mõned uniparentaalsed disoomiad (UPD), nagu näiteks emapoolne 6. ja 16. kromosoomi UPD, mis võivad teoreetiliselt mõjutada vermitud geenide funktsiooni, kuid nendega seotud fenotüüp on kõige tõenäolisemalt põhjustatud kaasnevast mosaiiksest trisoomiast kas platsentas või organismi enda kudedes.

Vermimishäirete molekulaarne etioloogia ja kliiniline avaldumine on väga varieeruvad, mistõttu nende diagnostika on keeruline. Arvatakse, et märkimisväärne osa vermimishäirete juhtudest jääb diagnoosimata. Vermimishäirete kliiniline avaldumine ja molekulaarsed mehhanismid on varasemalt põhjalikult uuritud ning kirjanduses kirjeldatud, kuid ainult piiratud arv uuringuid on varem käsitlenud vermimishäirete esinemissagedust ja muid epidemioloogilisi andmeid. Peaaegu kõik eelnevad tööd on keskendunud PWS, AS ja BWS epidemioloogiale. Viimase kümne aasta jooksul on läbi viidud ainult mõned nendest uuringutest. Täpne vermimishäirete esinemissagedus on jäänud seega ebaselgeks.

Eesti lasteneuroloog Eve Õiglane-Šlik uuris aastatel 2000–2004 kahte kõige levinumat vermimishäiret – PWS ja AS. Oma doktoritöö käigus uuris ta Eestis nende sündroomide kliinilist avaldumist, geneetilist etioloogiat, varajase äratundmise ja diagnostika võimalusi ning esinemissagedust. Doktoritöö tulemusena õnnestus tal leida täpne PWS ja AS esinemissagedus Eestis aastatel 1984–2004 [Õiglane-Shlik, 2007; Õiglane-Shlik jt, 2006a]. Tema töö motiveeris meid jätkama vermimishäirete uurimist ja andis ka võimaluse teha järeldusi nende kahe vermimishäire esinemissageduse ning kliinilise ja molekulaarse diagnostika efektiivsuse muutumisest Eestis.

2014. aastal, kui me seda uuringuprojekti alustasime, oli Eestis vermimishäiretega patsientide arv peaaegu 1,7 korda väiksem kui 2018. aastal. 2014. aastal moodustasid kaks klassikalist ja kõige levinumat vermimishäiret – PWS ja AS ligikaudu kaks kolmandikku kõigist vermimishäirete juhtudest. Meie uuringu alguses ei olnud Eestis ühtegi patsienti, kellel oleks molekulaarselt kinnitatud TNDM, CPP või MDS. Samuti sai enamik teiste haruldaste vermimishäirete juhtusid, nagu PHP/PPHP, BWS ja SRS, diagnoositud selle uuringu käigus.

Käesoleva uuringu eesmärgid

Käesoleva uuringu eesmärkideks oli:

1. Uurida geneetiliste ja epigeneetiliste muutuste esinemissagedust Eesti patsientide seas, kes olid uuringusse valitud eelnevalt publitseeritud SRS ja BWS kliiniliste diagnostiliste skooringusüsteemide järgi (Artikkel I);

2. Leida Eestis sagedamini esinevate vermimishäirete üldine esinemissagedus ning esinemissagedus vastsündinute seas ning määrata selle ajalist muutust (Artikkel II);
3. Juurutada Eestis uusi molekulaarseid diagnostilisi teste vermimishäirete diagnoosimiseks ning hinnata nende efektiivsust (Artikkel I ja II);
4. Tõsta Eesti arstide teadlikkust vermimishäirete osas (Artikkel II ja IV) ning kirjeldada uusi haruldasi vermimishäireid Eestis (Artikkel III).

Patsientide ja meetodite lühikirjeldus

Vermimishäirete epidemioloogiliseks uurimiseks viidi läbi retrospektiivne uuring, mille käigus analüüsiti kõikide Eestis aastatel 1998–2014 diagnoositud vermimishäiretega patsientide kliinilisi ning laboratoorseid andmeid. Aastatel 2014–2018 teostati ka prospektiivne uuring, mille eesmärgiks oli leida Eestis võimalikult palju vermimishäiretega patsiente. Vajalik kliiniline ja molekulaarne informatsioon saadi SA Tartu Ülikooli Kliinikumi ühendlabori kliinilise geneetika keskuse molekulaardiagnostika labori andmebaasist ning elektroonsest haiguslugude süsteemist. Epidemioloogilise uuringu käigus koostati vermimishäiretega patsientide andmekogu.

Geneetiliste ja epigeneetiliste muutuste esinemissageduse uurimiseks Eesti patsientide seas, kes täidavad SRS või BWS kliinilisi diagnostilisi kriteeriume, kasutati Bartholdi *et al*. (2009) ning Weksberg *et al*. (2010) skooringusüsteeme. Patsientide kliiniliste andmete reanalüüs teostati kasutades uusi kliinilisi diagnostilisi skooringusüsteeme – Netchine-Harbisoni kliinilise skooringusüsteemi (ingl *Netchine-Harbison Clinical Score System* ehk NH-CSS) [Netchine *et al*., 2007; Azzi *et al*., 2015] SRS-grupis ning BWS spektrihäirete konsensuse skooringusüsteemi [Brioude *et al*., 2018] BWS-grupis. Kokku kaasati uuringusse 48 SRS/BWS kliinilise kahtlusega patsienti. Kõiki patsiente analüüsiti 11p15.5 regiooni muutuste suhtes kasutades metülatsiooni-spetsiifilist MLPA-analüüsi (ingl *Methylation-Specific Multiplex Ligation-dependent Probe Amplification* ehk MS-MLPA). Enamusele patsientidest teostati ka MS-SNuPE (ingl *Methylation-Specific Single Nucleotide Primer Extension*) ja/või 6., 7. ja 14. kromosoomide vermitud regioonide MS-MLPA analüüs.

Informatsioon vermimishäirete kliinilisest pildist, diagnostikast, jälgimise printsiipidest ning ravi võimalustest edastati korduvalt arstidele Eesti-sisestel meditsiinikonverentsidel ning erinevate raviosakondade (kaasaarvatud lasteneuroloogia, neonatoloogia, endokrinoloogia ja üldpediaatria osakonnad) sisestel loengutel.

Peamised tulemused ja järeldused

1. Esitasime tulemused uuringust, kus määrasime geneetiliste ja epigeneetiliste muutuste esinemissageduse Eesti patsientide seas, kes olid valitud eelnevalt publitseeritud SRS ja BWS kliiniliste diagnostiliste skooringusüsteemide järgi (Artikkel I).
   1.1. Molekulaarsed testid kinnitasid SRS diagnoosi 38%-l (5/13) SRS kliinilise diagnoosiga patsientidest vastavalt Bartholdi skooringusüsteemile. Enamikul neist (80%, 4/5) tuvastati sagedaseim SRS molekulaarne muutus – IC1 hüpometülatsioon. Lisaks leiti ühel patsiendil harva esinev emapoolne 11p15.5–p15.4 regiooni duplikatsioon.
   1.2. BWS-grupis leiti BWS molekulaarne muutus (IC2 hüpometülatsioon) ainult ühel patsiendil 12-st (8%). Kõigil 12-l patsiendil oli kliiniline BWS diagnoos pandud vastavalt Weksbergi kliinilisele skooringusüsteemile. Kõrgeima BWS skooringuga patsiendil leiti ootamatult isoleeritud TNDM (*PLAGL1* geeni hüpometülatsioon).
   1.3. Bartholdi skooringusüsteemi avastamise määr Eesti SRS kliinilise kahtlusega patsientide seas (38%) on sarnane Bartholdi enda omaga (39%). Uue skooringusüsteemi NH-CSS avastamise määr oli meie SRS-grupis kõrgem (57%), kuid esines ka vale-negatiivseid tulemusi.
   1.4. Weksbergi skooringusüsteemi avastamise määr oli meie BWS-grupis tunduvalt madalam (8%) võrreldes teiste sarnaste uuringute tulemustega (28–72%). BWS spektrihäirete konsensuse skooringusüsteemi avastamise määr oli meie BWS-grupis mõnevõrra kõrgem (20%), kuid siiski oluliselt madalam kui publitseeritud avastamise määr 80%.
2. Esitasime andmed sagedamini esinevate vermimishäirete esinemissagedusest ning nende haiguste esinemissageduse ajalisest muutusest Eestis (Artikkel II).
   2.1. Alates 1998. aastast ning kuni selle uurimistöö perioodi lõpuni tuvastati Eestis kokku 87 vermimishäirega isikut. Kõige sagedasemad vermimishäired Eestis on PWS (31%), AS (17%), SRS (17%), BWS (14%) ja PHP/PPHP (11%).
   2.2. Aastatel 2004–2016 oli sagedasemate vermimishäirete esinemissagedus Eestis 1/13599 elussünni kohta PWS puhul, 1/27198 AS puhul, 1/21154 BWS puhul, 1/15866 SRS ja 1/27198 PHP/PPHP puhul.
   2.3. Aastatel 2004–2016 oli kõikide vermimishäirete esinemissagedus Eestis 1/3462 elussünni kohta ning 2018. aastal üldine vermimishäirete esinemissagedus 1/17132 (5,8/100000). Seega võib järeldada, et iga vermimishäire eraldi võetuna on väga harva esinev, kuid kõik vermimishäired koos on suhteliselt levinud.
   2.4. Eestis leitud PWS, AS ja BWS esinemissagedus on võrreldav kirjanduses publitseeritud nende haiguste esinemissagedusega. SRS esinemissagedus Eestis (1/15866) on ootamatult mitu korda kõrgem kui eeldatav SRS esinemissagedus 1/75000–1/100000. *GNAS*-geeniga

seotud vermimishäirete (PHP ja PPHP) esinemissagedus on samuti eeldatavast oluliselt kõrgem, võrreldav AS esinemissagedusega.

2.5. Aastatel 1998–2016 esines statistiliselt oluline BWS ja kõigi vermimishäirete esinemissageduse tõus, mida võib seletada nii paranenud diagnostika võimaluste kui ka arstide tõusnud teadlikkusega vermimishäirete osas. PWS, AS, SRS ja PHP/PPHP esinemissageduse tõus ei olnud sel perioodil statistiliselt oluline.

3. Juurutasime Eestis rutiinsesse laboripraktikasse uusi molekulaardiagnostilisi teste vermimishäirete diagnostikaks (Artikkel I ja II).
   3.1. Selle uurimistöö käigus juurutati SA Tartu Ülikooli Kliinikumi Kliinilise geneetika keskuse molekulaardiagnostika laboris UPD(6, 7, 14) MS-MLPA ja *GNAS* lookuse MS-MLPA analüüsid.
   3.2. PWS/AS MS-PCR ja PWS/AS MS-MLPA analüüside totaalne diagnostiline efektiivsus oli 6,4% ning BWS/SRS MS-MLPA ja UPD(7) MS-MLPA analüüside totaalne efektiivsus 7,7%. Need tulemused on oluliselt madalama diagnostilise efektiivsusega võrreldes eelmiste sarnaste uuringutega (≥20%), mis võib olla seletatav patsientide eelvaliku puudumisega meie rutiinses kliinilises praktikas.
4. Meie uurimistöö tulemuste ja isikliku kogemuse põhjal võib öelda, et selle uurimistöö käigus tõusis oluliselt Eesti arstide teadlikkus vermimishäiretest (Artikkel II ja IV). Leidsime Eestis mitu isikut uute harva esinevate vermimishäiretega.
   4.1. Molekulaarselt kinnitatud vermimishäirete juhtude arv suurenes. Kui aastatel 1998–2011 tuvastasime Eestis keskmiselt 1–3 uut molekulaarselt kinnitatud vermimishäire juhtumit aastas, siis aastatel 2012–2018 tõusis see arv 5–8 haigusjuhtumini aastas.
   4.2. Selle uurimistöö käigus leidsime Eestis kokku kaheksa patsienti selliste harva esinevate vermimishäiretega nagu CPP, TS14, TNDM ja MDS.
   4.3. Leidsime ja kirjeldasime atüüpilise TNDM patsienti (Artikkel I), harva esineva TS14 ja mosaiikse 14. kromosoomi trisoomia kombinatsiooniga patsienti (Artikkel III) ning perekonda, kus leidsime nii *MKRN3* geeniga seotud CPP kui ka *CHD8* geeniga seotud autismispektri häire.

See uurimistöö näitas, et vermimishäired on suhteliselt levinud pärilikud haigused, millel on väga mitmekesine molekulaarne etioloogia ja sageli mittespetsiifiline või ebatüüpiline kliiniline avaldumine. Uute diagnostiliste testide ja meetodite kasutusele võtmine viimastel aastatel parandas oluliselt vermimishäirete diagnostikat Eestis ning seetõttu võib paljude vermimishäirete esinemissagedus tulevikus veelgi suureneda.

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PUBLICATIONS

CURRICULUM VITAE

Name: Maria Yakoreva
Date of birth: January 7, 1986, Tallinn, Estonia
Citizenship: Russian
Address: 2 L. Puusepa Street, 51014, Tartu, Estonia
Phone: +372 7319499
E-mail: maria.yakoreva@kliinikum.ee

Education and employment:

1992–2004	Maardu Gymnasium
2004–2010	University of Tartu, Faculty of Medicine, Degree in Medicine
2010–2014	University of Tartu, Faculty of Medicine, Residency training in medical genetics
2014–2016	Tartu University Hospital, United Laboratories, Department of Clinical genetics, laboratory specialist
2014–…	University of Tartu, Faculty of Medicine, PhD studies in medical genetics
2014–…	Tartu University Hospital, United Laboratories, Department of Clinical genetics, Medical Geneticist

Visits to research/clinical institutions during PhD studies

January 2015	Institute of Human Genetics, RWTH Aachen University, Aachen, Germany. Supervisor: Prof Thomas Eggermann, PhD.

Scientific work and professional organizations

Research fields:	Clinical genetics, imprinting disorders
Publications:	Five in international journals (the first or shared first author in three), two in Eesti Arst (Estonian Medical Journal), 12 published international or local conference abstracts.
Membership:	Member of the Estonian Society of Human Genetics.

List of Publications

Yakoreva M, Kahre T, Žordania R, Reinson K, Teek R, Tillmann V, Peet A, Õiglane-Shlik E, Pajusalu S, Murumets Ü, Vals M-A, Mee P, Wojcik MH, Õunap K. (2019). A retrospective analysis of the prevalence of imprinting disorders in Estonia: time trend from 1998–2016 and comparison with previously published data. Eur J Hum Genet, Jun.

Yakoreva M, Kahre T, Pajusalu S, Ilisson P, Žilina O, Tillmann V, Reimand T, Õunap K. (2018). A New Case of a Rare Combination of Temple Syndrome and Mosaic Trisomy 14 and a Literature Review. Mol Syndromol, Jul; 9(4):182–189.

Yakoreva M, Vals M-A, Kahre T, Õunap K. (2017). Imprinting disorders: a literature review and presentation of cases. Estonian Medical Journal (in Estonian), 96(1):22–35.

Vals MA, **Yakoreva M**, Kahre T, Mee P, Muru K, Joost K, Teek R, Soellner L, Eggermann T, Õunap K. (2015). The Frequency of Methylation Abnormalities Among Estonian Patients Selected by Clinical Diagnostic Scoring Systems for Silver-Russell Syndrome and Beckwith-Wiedemann Syndrome. Genet Test Mol Biomarkers, Dec;19(12):684–91.

Pajusalu S, Žilina O, **Yakoreva M**, Tammur P, Kuuse K, Mölter-Väär T, Nõukas M, Reimand T, Õunap K. (2015). The Diagnostic Utility of Single Long Contiguous Stretches of Homozygosity in Patients without Parental Consanguinity. Mol Syndromol. 6: 134–139.

Laidre P, Soplepmann J, Uibo O, Raime K, **Yakoreva M**, Mirka G, Roomere H, Õunap K. (2015). Familial adenomatous polyposis: review and case report. Estonian Medical Journal (in Estonian), 94(1):38–43.

Žilina O, Teek R, Tammur P, Kuuse K, **Yakoreva M**, Vaidla E, Mölter-Väär T, Reimand T, Kurg A, Õunap K. (2014). Chromosomal microarray analysis as a first-tier clinical diagnostic test: Estonian experience. Molecular Genetics and Genomic Medicine, 2(2):166–175.

ELULOOKIRJELDUS

Nimi: Maria Yakoreva
Sünniaeg: 7. jaanuar 1986, Tallinn
Kodakondsus: Vene
Aadress: L. Puusepa 2, 51014, Tartu
Telefon: +372 7319499
E-post: maria.yakoreva@kliinikum.ee

Haridus- ja ametikäik

1992–2004	Maardu Gümnaasium
2004–2010	Tartu Ülikool, arstiteaduskond, arstiõpe
2010–2014	Tartu Ülikool, arstiteaduskond, meditsiinigeneetika residentuur
2014–2016	Tartu Ülikooli Kliinikum, Ühendlabor, kliinilise geneetika keskus, laborispetsialist
2014–…	Tartu Ülikool, arstiteaduskond, doktoriõpe arstiteaduse (meditsiinigeneetika) erialal
2014–…	Tartu Ülikooli Kliinikum, Ühendlabor, kliinilise geneetika keskus, meditsiinigeneetik

Külastused teadus- või kliinilistesse asutustesse doktoriõppe jooksul

Jaanuar 2015	Inimesegeneetika instituut, RWTH Aacheni Ülikool, Aachen, Saksamaa. Juhendaja: Prof Thomas Eggermann, PhD.

Teadus- ja erialane tegevus

Uurimisvaldkonnad:	Kliiniline geneetika, vermimishäired
Publikatsioonid:	5 rahvusvahelistes ajakirjades (kolmes esimene või jagatud esimene autor), 2 Eesti Arstis, 12 publitseeritud konverentsiettekande teesi.
Liikmelisus:	Eesti Inimesegeneetika Ühingu liige.

Publikatsioonide nimekiri

Yakoreva M, Kahre T, Žordania R, Reinson K, Teek R, Tillmann V, Peet A, Õiglane-Shlik E, Pajusalu S, Murumets Ü, Vals M-A, Mee P, Wojcik MH, Õunap K. (2019). A retrospective analysis of the prevalence of imprinting disorders in Estonia: time trend from 1998–2016 and comparison with previously published data. Eur J Hum Genet, Jun.

Yakoreva M, Kahre T, Pajusalu S, Ilisson P, Žilina O, Tillmann V, Reimand T, Õunap K. (2018). A New Case of a Rare Combination of Temple Syndrome and Mosaic Trisomy 14 and a Literature Review. Mol Syndromol, Jul;9(4):182–189.

Yakoreva M, Vals M-A, Kahre T, Õunap K. (2017). Vermimishäired: kirjanduse ülevaade ja haigusjuhtude kirjeldus. Eesti Arst, 96(1):22–35.

Vals MA, **Yakoreva M**, Kahre T, Mee P, Muru K, Joost K, Teek R, Soellner L, Eggermann T, Õunap K. (2015). The Frequency of Methylation Abnormalities Among Estonian Patients Selected by Clinical Diagnostic Scoring Systems for Silver-Russell Syndrome and Beckwith-Wiedemann Syndrome. Genet Test Mol Biomarkers, Dec;19(12):684–91.

Pajusalu S, Žilina O, **Yakoreva M**, Tammur P, Kuuse K, Mölter-Väär T, Nõukas M, Reimand T, Õunap K. (2015). The Diagnostic Utility of Single Long Contiguous Stretches of Homozygosity in Patients without Parental Consanguinity. Mol Syndromol, 6: 134–139.

Laidre P, Soplepmann J, Uibo O, Raime K, **Yakoreva M**, Mirka G, Roomere H, Õunap K. (2015). Perekondlik adenomatoosne polüpoos: ülevaade ja ühe perekonna haigusjuht. Eesti Arst, 94(1):38–43.

Žilina O, Teek R, Tammur P, Kuuse K, **Yakoreva M**, Vaidla E, Mölter-Väär T, Reimand T, Kurg A, Õunap K. (2014). Chromosomal microarray analysis as a first-tier clinical diagnostic test: Estonian experience. Molecular Genetics and Genomic Medicine, 2(2):166–175.

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