**Thesis**

# Mosaic trisomy 15 in Prader-Willi Syndrome with maternal uniparental disomy

A case report and retrospective study on the occurrence of mosaic trisomy 15 in additional cases


submitted by

**Sabine Stefanie Pitzl**


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Assoz. Prof. Priv.-Doz. Dr.med. Jochen Bernd Geigl
Dr.scient.med. Lukas Kaufmann, MSc

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# Declaration of Academic Integrity

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of "Good Scientific Practice".

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Graz, 20th February 2026 Sabine Stefanie Pitzl m.p.

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## Zusammenfassung

**Einleitung:** Die maternale uniparentale Disomie (matUPD) wurde als molekulare Ursache für 36 % der Fälle mit Prader-Willi-Syndrom (PWS) identifiziert. Als primärer Mechanismus der Entstehung einer matUPD wird die Trisomie-Rettung diskutiert. Hierbei wird ein Non-Disjunction-Fehler in der Meiose I oder II korrigiert, indem aus einer trisomischen Zygote ein Chromosom entfernt wird. Non-disjunction-Fehler wurden mit fortgeschrittenem mütterlichem Alter in Verbindung gebracht. Hinweisend für diesen Mechanismus sind Fälle, in denen eine Mosaik-Trisomie zusätzlich zu einer uniparentalen Disomie im Gewebe oder in der Plazenta nachgewiesen wurde. In Bezug auf das PWS wurden bisher acht Fälle veröffentlicht, in denen eine Mosaik-Trisomie 15 (MT15) in fetalem Gewebe oder Blut von Betroffenen mit matUPD festgestellt wurde. Nur in zwei Fällen wurde eine MT15 aus peripheren Blutproben nachgewiesen. Laut Literatur wird bei Betroffenen mit gleichzeitiger MT15 und matUPD 15 ein schwererer Phänotyp diskutiert. Diese Arbeit beschreibt den zweiten dokumentierten Fall eines lebend geborenen männlichen Kindes mit matUPD bei PWS, bei dem zusätzlich eine MT15 im peripheren Blut nachgewiesen werden konnte. In Anlehnung an den beschriebenen Fallbericht wurden weitere Betroffene mit mütterlicher UPD und väterlicher UPD auf das Vorhandensein einer MT15 untersucht, unter der Annahme, dass das Vorkommen einer zusätzlichen MT15 höher sein könnte als bisher berichtet.

**Methoden:** Für die genetischen Analysen wurden Lymphozyten aus dem peripheren Blut des acht Tage alten Kindes entnommen. Zunächst wurden eine Whole-Exome-Sequenzierung und eine methylation-specific multiplex ligation-dependent probe amplification (MS MLPA) durchgeführt. Anschließend wurde eine Trio-Genotyp-Analyse (vom Säugling und beider Elternteile), unter Verwendung eines single nucleotide polymorphism (SNP)-Arrays, durchgeführt. Die zytogenetische Analyse erfolgte anhand einer Blutprobe, als das Kind 16 Monate alt war. Im Rahmen der retrospektiven Studie wurden mit PWS und Angelman-Syndrom diagnostizierte Probandinnen und Probanden mit einem auffälligen Methylierungsmuster am Chromosom 15 kontaktiert, wodurch 4 Probandinnen und Probanden in die Studie eingeschlossen werden konnten (3x maternale UPD und 1x paternale UPD). Bei diesen wurden periphere Blutproben mithilfe von SNP-array-Analysen und klassischer Zytogenetik auf das Vorhandensein einer MT15 untersucht.

**Ergebnisse:** In der genetischen Diagnostik des Kindes zeigte die MS MLPA eine Hypermethylierung in der kritischen Region von Prader-Willi auf Chromosom 15 sowie eine normale Kopienzahl, wodurch eine Deletion ausgeschlossen werden konnte. Trio-Genotyp und SNP-Array ergaben eine vollständige heterodisomische matUPD 15 sowie eine geringgradige MT15 (ca. 10 %). Die zytogenetische Untersuchung ergab einen normalen männlichen Karyotyp ohne Anzeichen einer Trisomie. Die Mutter des Kindes ist eine 38-jährige Erstgebärende. Klinisch weist das Kind einen relativ milden Phänotyp auf und zeigt bisher eine für das PWS altersgerechte Entwicklung ohne schwere kongenitale Fehlbildungen. In der retrospektiven Studie konnte bei den vier Probandinnen und Probanden keine MT15 festgestellt werden, weder durch die zytogenetische Analyse noch durch den SNP-Array.

**Diskussion:** Der Fallbericht liefert weitere Belege dafür, dass die Trisomie-Rettung nach Meiosefehlern der primäre Mechanismus bei der Entwicklung einer matUPD 15 ist. Außerdem zeigt sich auch in diesem Fall ein Zusammenhang einer matUPD mit erhöhtem mütterlichem Alter. Die klinischen Erkenntnisse im aktuellen Fall deuten zudem darauf hin, dass Betroffene mit geringgradiger MT15 und matUPD 15 im peripheren Blut nicht zwangsläufig einen schwereren PWS-Phänotyp aufweisen müssen. Zusätzlich untermauert der Fallbericht die Wirksamkeit der SNP-Array-Analyse zur Erkennung einer MT15. Diese Beobachtung aus der aktuellen Arbeit stützt die Empfehlung einer Array-Untersuchung bei zukünftigen Fällen mit UPD 15 zur Detektion einer möglichen MT15. Dies ist erforderlich, um in Zukunft die tatsächliche Häufigkeit einer MT15 bei UPD 15 sowie phänotypische Merkmale bei Betroffenen bestimmen zu können

## Abstract

**Introduction:** Maternal uniparental disomy (matUPD) has been identified as the molecular cause of 36% of the cases with Prader-Willi Syndrome (PWS). Trisomic rescue is discussed as the primary mechanism in the development of matUPD, which corrects a non-disjunction error in meiosis I or II by discarding one chromosome of a trisomic zygote. These non-disjunction errors have been linked to advanced maternal age (AMA). Evidence for this mechanism was provided by the detection of mosaicism for trisomy in affected individuals with uniparental disomy (UPD) or placental tissue. For PWS, eight cases have been published so far, detecting mosaic trisomy 15 (MT15) in fetal tissue or blood of individuals with matUPD. Only in two of the cases MT15 has been detected in peripheral blood samples. According to the literature, a more severe phenotype has been discussed for individuals with concurrent MT15 and matUPD 15. This thesis presents the case report of the second documented case of a live-born infant with matUPD in PWS, in which MT15 was found in peripheral blood. Consistent with this finding, further matUPD and paternal uniparental disomy (patUPD) cases were examined for the presence of MT15, under the assumption that its occurrence might be higher than previously reported.

**Methods:** Genetic testing was performed on the infant's lymphocytes extracted from peripheral blood at eight days of age. Methylation-specific multiplex ligation-dependent probe amplification (MS MLPA) was performed initially. Subsequently, a trio genotype analysis (infant and both parents) was performed using single nucleotide polymorphism (SNP) array. Cytogenetic analysis was performed at 16 months of age. The performed retrospective study involved surveying individuals with a previous diagnosis of PWS and Angelman Syndrome (AS) with an abnormal methylation on chromosome 15, resulting in the inclusion of four probands (three with matUPD 15 and one with patUPD 15). Peripheral blood samples were analysed for the presence of the MT15 utilising SNP array and classical cytogenetics.

**Results:** MS MLPA indicated hypermethylation in the Prader-Willi critical region on chromosome 15 and a normal copy number, ruling out a deletion. Trio-genotype and SNP array yielded a complete heterodisomic matUPD 15 as well as a low-grade

(approximately 10%) MT15. Cytogenetic analysis demonstrated a normal male karyotype, with no indication of trisomy. The boy was born to a 38-year-old primiparous woman and presented with a relatively mild phenotype. He presented age-appropriate development for PWS, with no severe congenital anomalies. In the retrospective study, MT15 was not identified in any of the four probands, either by cytogenetic analysis or by SNP array.

**Discussion:** The case report provides further evidence for a trisomic rescue after meiotic errors being the primary mechanism in the development of matUPD 15. A link between AMA and matUPD was also present. It also demonstrates that infants with MT15 and matUPD 15 found in peripheral blood may not present with a more severe phenotype or congenital anomalies, as previously suggested in the literature. This finding further supports the efficacy of SNP array analysis in detecting MT15. The current case report supports the recommendation for additional array analysis in future UPD 15 cases to detect a possible MT15. This is required to determine the actual frequency of MT15 in UPD 15 and further phenotypic characteristics in affected individuals in the future.

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# Abbreviations

**A**

**AB** abortus. 25
**aCGH** array based comparative genomic hybridization. 19, 23, 25, 43
**AF** amniotic fluid. 25
**AMA** advanced maternal age. E, F, 15, 23
**AS** Angelman Syndrome. E, 5, 6, 7, 11, 12, 15, 27, 28, 29, 39, 41, 44, 47, 48, 49
**ASD** atrial septal defect. 26, 32, 45

**B**

**BP** breaking point. 8
**BPI** biparental inheritance. 15, 37

**C**

**CMA** chromosomal microarray analysis. 18, 19
**CNV** copy number variations. 11, 18, 19, 33, 34, 35
**CPAP** continuous positive airway pressure. 31, 32
**CPG island** cytosine-phosphate-guanine island. 6, 36
**CPM** confined placental mosaicisim. 16, 17, 21, 24, 47
**CVS** chorionic villus sampling. 17, 18, 21, 22, 23, 24, 25, 43, 48

**D**

**DNA** Deoxyribonucleic acid. 6, 8, 12, 13, 19, 22, 25, 28, 36, 37, 48
**DR Institute** Diagnostic and Research Institute. 28, 29

**F**

**FB** fibroblasts. 25

**FISH** fluorescence in situ hybridization. 18, 21, 42

**G**

**GH** growth hormone. 1, 3, 33, 40

**H**

**HPO** human phenotype ontology. 28

**I**

**IC** imprinting center. 7
**ICD** imprinting center deletion. 9, 12
**ICR** imprinting control region. 5, 6, 7, 9
**ICSI** intracytoplasmic sperm injection. 31
**IQ** intelligence quotient. 3
**IUGR** intrauterine growth restriction. 21, 24, 26, 39, 45
**IVF** in vitro fertilization. 17

**K**

**kb** kilobases. 5

**L**

**LB** liveborn. 25

**M**

**MA** maternal age. 25
**matUPD** maternal uniparental disomy. F, G, E, x, 1, 5, 8, 9, 10, 12, 13, 14, 15, 20, 21, 22, 23, 24, 25, 26, 27, 34, 37, 39, 40, 42, 43, 44, 46, 47, 49
**mb** megabase. 7, 8, 18
**MLPA** multiplex ligation-dependent probe amplification. 11, 12, 28, 43
**MP** metaphases. 39
**MS MLPA** methylation-specific multiplex ligation-dependent probe amplification. F, G, E, 11, 12, 23, 25, 28, 33, 35, 36, 44, 47
**MS PCR** methylation-specific polymerase chain reaction. 11
**MSA** microsatellite analysis. 22, 23, 25, 27

**MT15** mosaic trisomy 15. F, G, E, x, 20, 21, 22, 23, 24, 25, 27, 30, 37, 39, 42, 43, 44, 45, 46, 47, 48, 49

**N**

**N/A** not available. 26
**NAHR** non-allelic homologous recombination. 8
**NFS** not further specified. 25
**NGS** next-generation sequencing. 28

**O**

**OSA** oligo-small nucleotide polymorphism combination array. 11

**P**

**patUPD** paternal uniparental disomy. E, 8, 27, 39, 41
**PCR** polymerase chain reaction. 12
**PDA** patent ductus arteriosus. 26
**PFO** patent foramen ovale. 26, 32, 45
**PWCR** Prader-Willi critical region. E, 1, 5, 7, 8, 9, 10, 33, 43
**PWS** Prader-Willi Syndrome. F, G, E, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 20, 21, 22, 23, 27, 28, 29, 33, 34, 39, 40, 42, 43, 44, 45, 47, 48, 49
**PWS SRO** PWS shortest region of deletion overlap. 9

**R**

**ROH** region of homozygositiy. 19

**S**

**SGA** small for gestational age. 31, 45
**snoRNA** small nucleolar RNA. 6, 7
**SNP** single nucleotide polymorphism. F, G, E, ix, 11, 15, 19, 20, 23, 24, 25, 27, 29, 30, 34, 37, 39, 40, 42, 43, 44, 47, 48, 49

**T**

**T15** trisomy 15. 25

**U**

**UPD** uniparental disomy. G, E, F, 8, 9, 11, 12, 13, 15, 19, 24, 29, 30, 39, 40, 41, 42, 45, 47, 48, 49

**V**

**VSD** ventricular septal defect. 26, 45

**W**

**WES** whole-exome sequencing. 28, 33

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# Chapter 1

# Introduction

## 1.1 Prader-Willi Syndrome

The Prader-Willi Syndrome (PWS), also known as Prader-Labhart-Willi Syndrome, first described by Prader, Labhart and Willi in 1956 [1], is a rare contiguous gene syndrome associated with a parent-of-origin effect [2] due to genetic imprinting in the chromosomal region 15q11.2-q13. Some genes in this region on chromosome 15 are subject to maternal genetic imprinting and epigenetically inactivated through methylation on the maternal allele [3]. Therefore, these genes are only expressed on the active paternal allele. A loss of the paternal Prader-Willi critical region (PWCR) and subsequent loss of gene expression leads to the PWS specific phenotype [4]. Loss of gene expression may happen through different genetic mechanisms, mainly through 15q11.2-q13 paternal deletions, maternal uniparental disomy (matUPD) and imprinting centre defects [3].

The syndrome appears with an estimated prevalence of 1 in 10000 to 1 in 30000 and is a complex disorder affecting multiple systems of the body. Severe hypotonia, poor suck, and feeding difficulties are distinctive symptoms appearing in early infancy. Other characteristic features include hypogonadism with subsequent genital hypoplasia, hyperphagia and obesity in early childhood, phenotype-specific behavioural problems and distinct facial features as well as a short stature due to growth hormone (GH) deficiency. There may also be a developmental delay or mild intellectual disability associated with PWS [3].

### 1.1.1 Clinical presentation

Clinical presentations in PWS are complex and vary at different ages of the individuals [3].

- **Obesity and nutritional phases:** A hallmark of this syndrome is severe obesity and disordered eating with phases from feeding difficulties in infancy to hyperphagia in adolescence to adulthood. This can be categorised into 5 phases with 2 sub-phases in phases 1 and 2, as shown in Table 1. According to Miller et al. [5], most individuals with PWS go through phases 1 to 3, but only a small number of those transition to phase 4 in adulthood and experience improvement in their appetite control.

  Obesity is also one of the major complications of this syndrome and predisposes individuals to develop serious metabolic diseases often at a young age. This includes type 2 diabetes, dyslipidemia, and cardiovascular events [6]. The mechanisms involved in the development of obesity are complex and believed to be caused by hyperphagia, alterations in metabolic hormones that regulate food intake and decreased energy expenditure [6]. The proposed cause for the hyperphagia that appears in phase 3, starting at approximately 8 years, is a hypothalamic abnormality that results in the lack of feeling full and the expression of food-seeking behaviours, which may manifest in food hoarding and stealing. Individuals with PWS also have less muscle mass in comparison to unaffected individuals and show less overall activity, leading to decreased resting energy expenditure. Consequently, the body needs fewer calories, which, in combination with hyperphagia, further contributes to obesity [3].

Table 1: Clinical characteristics across nutritional phases

| **Phase** | **Median Age** | **Clinical Characteristics of Nutritional Phases** |
|---|---|---|
| 0 | Prenatal to birth | Decreased fetal movements and lower birth weight than siblings. |
| 1a/b | 0 – 25 months | Hypotonia at birth, weak cry and difficulty feeding.<br>Improvement and growing appetite by the 9th month. |
| 2a/b | 2.1 – 8 years | Turning point in weight chart with disproportionate weight gain, increasing appetite and interest in food by the age of 4.5 years. |
| 3 | 8 years – adulthood | Hyperphagic phase with no feeling of fullness and high weight gain. |
| 4 | Adulthood | Appetite is no longer insatiable and may vary. |

- **Hypotonia:** In addition to obesity, one of the most frequent symptoms across all PWS cases is hypotonia at birth, with a prevalence of 95-100% [3]. In comparison, the prevalence of PWS in all infants with hypotonia is about 10% and the syndrome should therefore be considered as a differential diagnosis in infantile hypotonia [7]. The origin of hypotonia in PWS is in the central nervous system and is associated with dysphagia, lethargy, poor suck, and poor appetite, which result in low weight and decreased weight gain in affected individuals during infancy. Although this hypotonia improves over time, adults often remain mildly affected by this symptom, characterised by decreased muscular tone and reduced muscle mass [3, 4].

- **Endocrine manifestations:** Hypothalamic dysfunction is a key feature in the cause of many endocrine manifestations that are part of the PWS specific phenotype. The main endocrine dysfunctions affect the GH, gonadal hormones, thyroid hormones, as well as glucose metabolism and the corticotropin system. In the context of gonadal hormone deficiency, both sexes are frequently affected by hypogonadism, which in most cases manifests as both peripheral and central hypogonadism or peripheral hypogonadism only. In boys, this is characterised by cryptorchidism in 92.7 - 100% of cases and in girls by hypoblastic labia minora. Hypogonadism also impacts pubertal development with a late start and incomplete puberty. Premature growing of pubic hair may also appear in some individuals [8].
  Deficiency of GH leads to the characteristic short height of individuals affected by PWS [4]. Without GH replacement, the average height of females is 148 cm and 155 cm in males [9].

- **Developmental delay and cognition:** In general, the development of children with PWS is delayed, but this delay may vary from case to case. These children take approximately twice as long to develop motor skills and reach milestones compared to their peers. Sitting is typically described as happening at 12 months and walking at 14 months. Development slows down over time, and weight gain and obesity also affect further motor development in older children [10]. Loss of expression of genes in the region 15q11-q14 has been linked to an affection of social and intellectual cognition. Even though the scores obtained in intelligence quotient (IQ) tests have a wide range, some individuals affected by PWS can reach normal IQ scores. The genetic impact on cognition seems to vary in relation to

the specific genetic subtype [11]. Delay in language development has also been described in PWS, as well as overall impaired language ability [10].

- **Behavioural symptoms:** Aside from the above-mentioned hyperphagia and food-seeking behaviours, other key behaviours in individuals with PWS are temper outbursts, anxiety, obsessive-compulsive behaviours, rigidity in routines and thinking, as well as social cognition deficits. Since many individuals with PWS show more than one key behaviour, and the behaviours may also overlap, it is not easy to categorise them. This is further complicated by the symptoms of mental health disorders that may also overlap with the behavioural features in PWS[12]. These compulsive behaviours and anxiety have been shown to even arise in early childhood at median ages from 3 years (compulsive behaviours) to 8 years (anxiety). Anxiety in general and in relation to food is a pervasive issue for individuals with PWS, and a higher frequency of other psychiatric conditions has been observed in association with it[13].

- **Psychiatric disorders:** Mental health disorders in PWS are very common, with one study stating that 89% of their testing cohort, consisting of 53 individuals with PWS, had at least one psychiatric diagnosis. Common psychiatric diagnoses are disruptive behaviour disorders, obsessive compulsive disorder and skin picking. Some individuals also showed general anxiety disorder, attention deficit hyperactivity disorder and psychotic disorder. Though the study did observe an effect of gender in relation to psychiatric diagnosis, there seems to be no association between genetic subtype and psychiatric diagnosis [14].

- **Characteristic facial features:** Distinct facial features in PWS have been described. These include a small bifrontal diameter, palpebral fissures shaped like an almond, a thin nasal bridge and thin upper vermilion, as well as a mouth with down-turned corners [4].

- **Differences in phenotypes:** Even though differences in the symptoms of PWS depending on the underlying molecular cause have been described, no clear and consistent genotype-phenotype correlations have been established. A large study from 2024 found that individuals with a deletion had higher rates of hypopigmentation, obesity, hyperphagia and delay in language development in comparison to the non-deletion group [15]. A higher risk of epilepsy in PWS with deletions has

also been described in multiple studies [13, 15, 16]. Individuals with matUPD are described as having a higher expressive language ability [17].

### 1.1.2 Genetic background

In this section, the Prader-Willi critical region (PWCR) on chromosome 15 and the effect of imprinting are described, as well as the various genetic mechanisms that lead to PWS, which is of great interest to this thesis.

PWS belongs to the imprinting disorders and is caused by loss of function of the paternally expressed genes in 15q11.2-q13. Other imprinting disorders, for example, are Beckwith-Wiedemann syndrome and AS, with AS also being of particular interest for this thesis since the same genetic region as in PWS is affected. In contrast to PWS, AS arises through loss of function of the maternally expressed gene *UBE3A* in neuronal cells [18].

#### 1.1.2.1 Imprinting

Genomic imprinting is a part of epigenetic regulation and refers to the mechanism by which one copy of a gene is silenced on either the maternal or paternal allele, thereby preventing it from being actively transcribed. The imprinted regions, mostly found on autosomes, are already specifically determined in the germline during gametogenesis [19]. By 2018, over 200 imprinted human genes had been identified, accounting for less than 1% of all genes, with some playing a crucial role in multiple physiological functions and growth [20]. Most of the imprinted genes are located in just a few specific gene clusters. These imprinted gene clusters contain approximately 1-2 kilobases (kb) long imprinting control regions (ICRs), which control the expression of neighbouring genes within the cluster. A balanced expression of both parental chromosomes, and consequently of imprinted genes, is imperative for normal physiological function, since the loss of one allele may result in an imprinting disorder [19], such as those described above.

A fact that makes genomic imprinting even more complex is that certain genes are expressed differently in specific tissues. While one gene may be expressed biallelically, meaning on both the paternal and maternal allele, in one tissue, it may only be expressed maternally in another tissue. This expression in a specific tissue may also

change during development, with genes being biallelically expressed during embryogenesis but then only monoallelically expressed during adulthood [21].

The molecular mechanism behind genomic imprinting is quite complex, with the two main mechanisms being Deoxyribonucleic acid (DNA) methylation and histone modification. Both DNA methylation and histone modification are key mechanisms in epigenetics and affect transcription, replication and recombination of chromosomes without changing the nucleotide sequence. In humans, methylation happens after DNA replication and occurs almost exclusively on cytosine in the sequence 5‘CpG-3‘, which is a dinucleotide sequence consisting of cytosine-phosphate-guanine. These sequences are often found in multiple copies near promoter regions of genes and are called cytosine-phosphate-guanine islands (CPG islands). The CPG islands are usually unmethylated. Methylation of the CPG islands leads to silencing of neighbouring promoter regions and therefore to loss of expression of genes [19].

The ICR of imprinted regions contains CPG islands, which have different methylation patterns on the maternal and paternal alleles. These differences in methylation play an important role in the regulation of gene expression. Histone modification follows exactly the methylation pattern of the ICR, resulting in the formation of transcriptionally inactive heterochromatin on the methylated allele and active euchromatin in the unmethylated allele. Consequently, the methylated allele is silenced, whilst the unmethylated allele is transcriptionally active in an imprinted region [19].

In case of PWS, the imprinted area causal to the phenotypic changes when the paternally expressed genes are lost, is located on the proximal arm of chromosome 15 in the region 15q11.2 - q13. This region contains differently imprinted areas with both maternally and paternally imprinted genes [3].

Figure 1 shows a map of this 15q11.2 - q13 region, including the specific critical regions for PWS and AS. As seen on this map, several genes (*MKRN3, MAGEL2, NDN, PWRN1, NPAP1, SNURF-SNRPN, IPW* and *small nucleolar RNA (snoRNA)* genes are localised here and only paternally expressed in humans [3].
Since PWS is a contiguous gene disorder, the loss of multiple genes is thought to be responsible for the phenotypic spectrum in PWS [3]. Even though the complete PWS phenotype cannot be pinpointed to loss of expression of one specific gene, the limited deletion of the *SNORD116* region has been shown to present typical features of PWS and consequently seems to be a main aetiological factor in the expression of the phenotype in PWS [22]. The *SNORD116* region is part of the snoRNA cluster in

the PWCR and consists of approximately 24 copies. These snoRNAs play a part in the alternative splicing in the modification of mRNA [3].

In contrast, AS results from the loss of expression of the maternally inherited *UBE3A*. This gene encodes for an ubiquitin-protein ligase, which is biallelically expressed in most tissues. One exception is the human brain, where it is only maternally expressed. Despite the identification of the *UBE3A* gene being causal for the AS phenotype, the regulation of this gene and the underlying mechanisms leading to neurological symptoms are still unknown [19].

The regulation of the imprinting in both syndromes (through methylation of ICRs, as described above [19]) is controlled from the same site. This site in region 15q11-q13 consists of two components, one for AS and one for PWS and is often referred to as imprinting center (IC) [3]. The IC is marked on Figure 1.

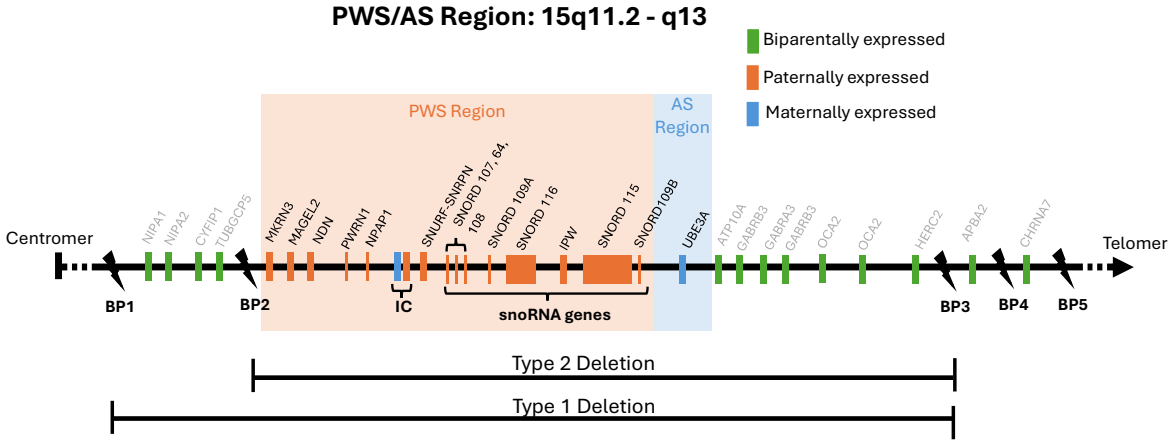



Figure 1: Simplified overview of the area 15q11.2 - q13.3 containing the 2.5 megabases (mb) long PWS/AS region. The map is adapted from Driscoll et al. [3]. In orange, the figure shows the genes located in the PWCR that are maternally imprinted and therefore only expressed paternally. These are *MKRN3, MAGEL2, NDN, PWRN1, NPAP1, SNURF-SNRPN*, as well as a cluster of snoRNA and *IPW*. There are multiple copies of *SNORD116* and *SNORD115*, which are not shown in the map. The gene *UBE3A*, which is associated with AS, is only expressed on the maternal allele in certain tissues, and is shown in blue. The genes that are not imprinted and therefore expressed biparentally are shown in green. The IC contains an "AS-critical" (maternal) and a "PWS-critical" (paternal) component and is located in the 5' region of SNURF-SNRPN. The map also shows the most common deletion breakpoints (BP) in PWS and AS, with type 1 deletions spanning from BP1 to BP3 and type 2 deletions spanning from BP2 to BP3 [3].

#### 1.1.2.2 Paternal deletion (15q11.2-q13 deletion)

Loss of function of the paternally expressed genes in 15q11.2-q13 may happen through different molecular causes, as demonstrated in Figure 2. The most common one is a microdeletion of the paternal PWCR allele. Microdeletions account for approximately 60% of all PWS cases [23] and most commonly happen between the proximal breaking point (BP) BP1 or BP2 and the distal BP3 [3]. As seen in Figure 1, the approximately 6 Mb type 1 deletion stretches from BP1 to BP3, while the shorter 5 Mb type 2 deletion stretches from BP2 to BP3 [3]. Breakage at these specific points is thought to result from unequal alignment of repeated DNA sequences, known as low copy repeats near the breakpoints during homologous recombination in meiosis [24], called non-allelic homologous recombination (NAHR) [25].

There are 4 genes (*NIPA1, NIPA2, CYFIP1, GCP5*) located between BP1 and BP2, which are missing in type 1 deletions, in contrast to type 2 deletions. It is suggested that these additional deleted genes are involved in the differences between individuals with type 1 deletion and those with type 2 deletion. These differences include a lower intellectual ability and compulsive behaviour [26].
In 2018, the largest cohort study of molecular classes in PWS revealed that among individuals with deletions, 38.9% had a type 1 deletion and 54.6% had a type 2 deletion. Only 6.6% had an atypical deletion, which is either smaller or larger than the two common deletions mentioned above [23].

#### 1.1.2.3 Maternal uniparental disomy)

Following 15q11.2-q13 deletion, maternal uniparental disomy (matUPD) is the second most common genetic mechanism causal to PWS and the main mechanism of interest for this thesis. According to the largest cohort study from 2018, matUPD was identified as the genetic cause in 36% of the individuals [23]. UPD is characterised as both copies of a chromosome originating from one parent, with either both being inherited maternally (matUPD) or paternally (patUPD) [27]. It was first described as a genetic concept by Engel in 1980 [27] and first associated with a genetic disease in PWS in 1989 [28]. As demonstrated in Figure 2, both chromosomes in matUPD in PWS are inherited from the mother. The absence of gene expression of the maternally imprinted genes in the PWCR thus results in the clinical manifestations of PWS [28].
UPD may be further categorised into complete heterodisomy, complete isodisomy and

heterodisomy/isodisomy (segmental UPD). In complete heterodisomy, both homologous chromosomes of one parent are inherited, while in isodisomy, there are two identical copies inherited from one homologue of a parent, therefore showing the same polymorphic loci. Segmental UPD results from prior crossing-over [29]. The types of UPD are illustrated in part II of Figure 3.

According to the largest cohort study mentioned above in Section 1.1.2.2, in 185 individuals diagnosed with matUPD, approximately 60% showed maternal segmental UPD, 30% showed heterodisomy and 13% showed isodisomy, with the remaining not being able to be determined because of a lack of genetic material to be analyzed [23]. This concludes that segmental UPD is the most common category found in matUPD 15.

Several mechanisms are possible for the development of UPD, with them often being some sort of rescue mechanism to avert lethal abnormalities in developing fetuses [30]. In discussion are monosomic rescue, trisomic rescue, gamete complementation and post-fertilisation error. While monosomic rescue and post-fertilisation error may only produce isodisomy, both isodisomy and heterodisomy may arise from trisomic rescue and gamete complementation [31]. The exact mechanism in discussion for the arising of matUPD due to meiotic errors will be described further in Section 1.2.

#### 1.1.2.4 Imprinting defects

Imprinting defects are the rarest cause of PWS and are composed of microdeletions in the imprinting centre and imprinting defects by epimutation. These imprinting defects account for about 4% of all PWS cases [23]. In diagnostics, individuals with this genetic cause show a maternal methylation pattern of the PWCR but no 15q11.2-q13 deletion or maternal UPD [3]. In the case of imprinting center deletions (ICD), the deletions affect the PWS shortest region of deletion overlap (PWS SRO), which includes the promoter for the *SNRPN* gene and is the critical region responsible for controlling imprinting [33]. Individuals with imprinting defects by epimutation show the same maternal methylation pattern but without any detectable deletion in the ICR [3].

The differentiation of these two mechanisms is critical since an ICD has a risk of recurrence. The children of a man with an ICD on the maternal allele, which is silently inherited by his mother, have a 50% chance of inheriting the faulty allele and developing PWS [34]. This stands in contrast to most PWS individuals with 15q11.2-q13 deletion or maternal UPD being de novo events [3].

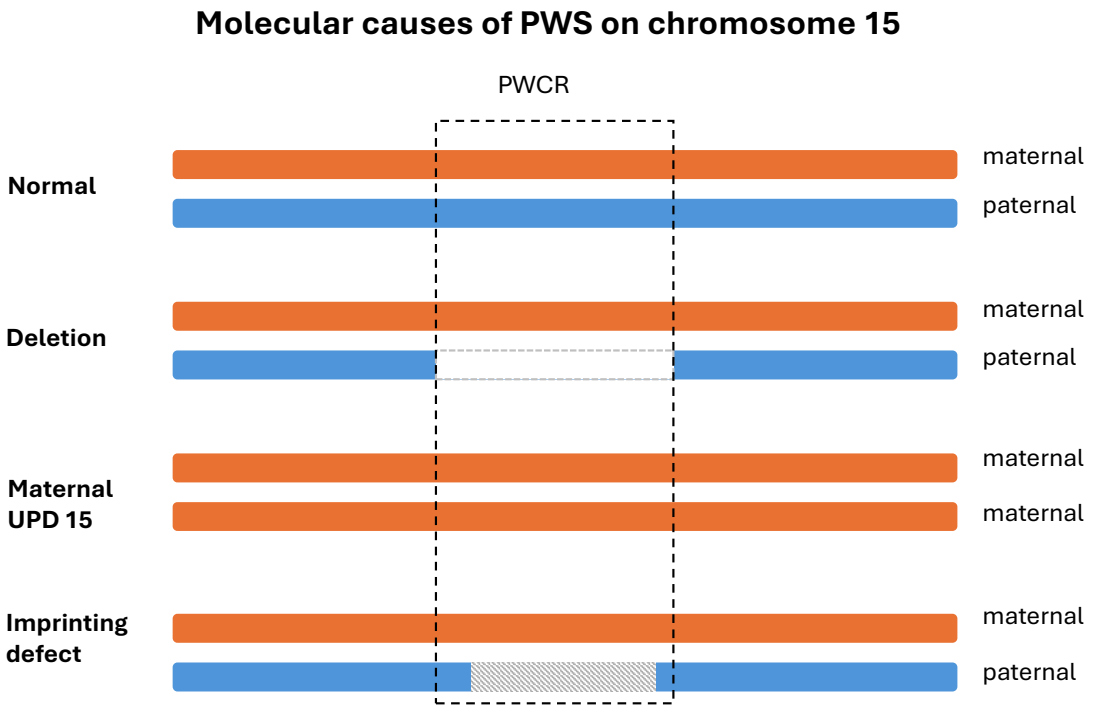



Figure 2: Molecular causes of PWS (illustration adapted from Hassan et al. [32]). This figure provides a simplified representation of the molecular causes on chromosome 15 that lead to the phenotypic changes in PWS. Deletion of the PWCR on the paternal chromosome is the most common cause of PWS (60%), followed by matUPD (36%) and imprinting defects (4%). Each of the molecular causes leads to a loss of expression of the maternally imprinted genes [23] as marked in the illustration.

### 1.1.3 Diagnostics

The clinical diagnosis of PWS is challenging due to the absence of distinct clinical features and symptoms, which may also manifest differently depending on the individual's age. To reduce misdiagnosis, clinical consensus criteria for PWS were established in 1996 [35]. Today, with definitive molecular testing available, it is suggested that these criteria should only be used to raise suspicion that PWS may be present, as the criteria are too strict to ensure the identification of all individuals relevant for testing. Irrespective of the criteria, all infants with hypotonia and poor suck are recommended for testing, as are children with additional developmental delay and excessive eating. In adolescents and adults, testing is recommended in individuals with excessive eating/central obesity, cognitive impairment and hypogonadism or behavioural problems typical for the syndrome [36].

Molecular genetic testing and analysis of the methylation status of the critical region for PWS and consequently AS is the most sensitive approach to diagnosing both syndromes and is recommended as the initial step in diagnostics. For analysis of the methylation status, methylation-specific polymerase chain reaction (MS PCR) and MS MLPA are possible tools. While MS PCR only detects the methylation status at one specific genetic locus, MS MLPA detects the methylation status at multiple loci across the critical region and also assesses genomic dosage and copy number variations (CNV). This complementary assessment of genomic dosage provides additional knowledge about the underlying molecular causes [37].

According to Driscoll et al. [3], oligo-small nucleotide polymorphism combination array (OSA) should also be used as a diagnostic tool alongside methylation analysis, as this array will provide additional information about deletions and chromosomal abnormalities. In addition, isodisomy (segmental and complete) in case of UPD may also be detected through the use of SNP microarray.

Since MS MLPA is often the preferred tool in testing for the methylation status [37], this method will be further described.

#### 1.1.3.1 Methylation-specific MLPA

The multiplex ligation-dependent probe amplification (MLPA) was first described in 2002 as a fast, easy-to-use, and sensitive method to detect copy number variants not

only in chromosomal sequences but also in single exons. The method uses ligation-dependent polymerase chain reaction (PCR) as a basis and can analyse multiple target sequences at once, while requiring only small DNA samples [38].

The methylation-specific multiplex ligation-dependent probe amplification (MS MLPA) is a variation of the classic multiplex ligation-dependent probe amplification (MLPA). This method enables the identification of copy numbers and the evaluation of methylation status. This is achieved by dividing the reaction into two parts after the probes are ligated to the sample DNA. One reaction is used to detect the copy numbers, while the other reaction is used to detect the methylation status. For the detection of the methylation status, the MS MLPA contains probes that have a recognition site for a methylation-sensitive endonuclease (HhaI). This endonuclease will not cut the probe-sample DNA if the recognition site is methylated, leading to the amplification of the fragment during PCR. Unmethylated recognition sites will be cut and, therefore, not amplified. Through the analysis of these amplified fragments and a comparison of the test sample with the reference sample, the methylation status of the test sample can be determined [39].

Complete methylation or hypermethylation in the PWS region indicates PWS, while hypomethylation or no methylation at all is suspicious for AS. The kit, mostly used in diagnostics for PWS/AS contains probes specific to the critical region of both syndromes on chromosome 15, as well as methylation-sensitive probes and control probes. As for the genetic causes, 15q11-13 deletions and even ICD can be confirmed with this method. In contrast, it is not possible to differentiate between UPD or an imprinting centre defect. Therefore, additional testing is needed if an abnormal methylation pattern is detected in MS MLPA [37]. This additional testing may be performed as DNA polymorphism testing of both the individual and the parents [3]. One option for this is a CytoSNP-Array, which will be explained further in Subsection 1.3.3.

## 1.2 Trisomic rescue as the basis of matUPD and mosaic trisomy 15

Trisomic rescue has been discussed as the primary mechanism in the development of matUPD in PWS since a correlation between a meiotic origin of UPD and trisomic cells in placental tissues was found [40]. This has been further connected by multiple

cases published, in which trisomic cell lines in placental tissue were present in children with PWS and confirmed matUPD [41, 42], which will be further elaborated in Section 1.5.
The prerequisite for the trisomic rescue mechanism is an error in meiosis that leads to the formation of a trisomic zygote. The subsequent mitotic loss of one of the three chromosomes of the zygote is then considered a trisomic rescue. This trisomic rescue may result in UPD if both chromosomes from only one parent are retained [30]. Furthermore, trisomic rescue may lead to mosaic trisomy in the placenta or certain tissues in the fetus [43], which will be further discussed in Section 1.3. The possible outcomes of trisomic rescue after meiotic errors (trisomic cell lines, matUPD, and "normal" disomy) are shown as an overview in part I of Figure 3.

The basis for the development of matUPD in case of PWS are segregation errors happening in female meiosis. Depending on the timing of these errors happening, during meiosis I or II, a heterodisomic or isodisomic UPD arises [29].

Meiosis in humans consists of two cell division cycles, meiosis I and meiosis II, with the ultimate goal of producing haploid gametes for reproduction. After initial DNA replication, meiosis I begins with two homologous chromosomes, each consisting of two sister chromatids (2n, 2c). These homologues then segregate and are each transferred to a cell (1n, 2c). Meiosis I is a complex process, as genetic recombination occurs before segregation, where parts of the chromatids of homologous chromosomes exchange genetic material through crossing over. Division of the cells in meiosis II then proceeds similarly to a normal mitotic division and produces haploid gametes (1n, 1c) by segregation of the sister chromatids [45].

Errors in the segregation of the homologous chromosomes or chromatids may occur through non-disjunction. This results in an unequal distribution of the genetic material in the gametes [44], which is also illustrated in part II of Figure 3. Here, it can be seen that in non-disjunction happening during meiosis I, both homologues are transferred to one cell, while in non-disjunction during meiosis II, both sister chromatids are transferred to one cell.

As seen in part II of Figure 3, complete heterodisomy may be the result of a non-disjunction error in Meiosis I with no recombination of the chromatids, leading to two distinct sets of alleles spanning the entire length of the chromosome. Meaning the child receives both alleles of the mother. Meiosis II segregation then proceeds normally and subsequent fertilisation results in trisomy, where two chromosomes are inherited from

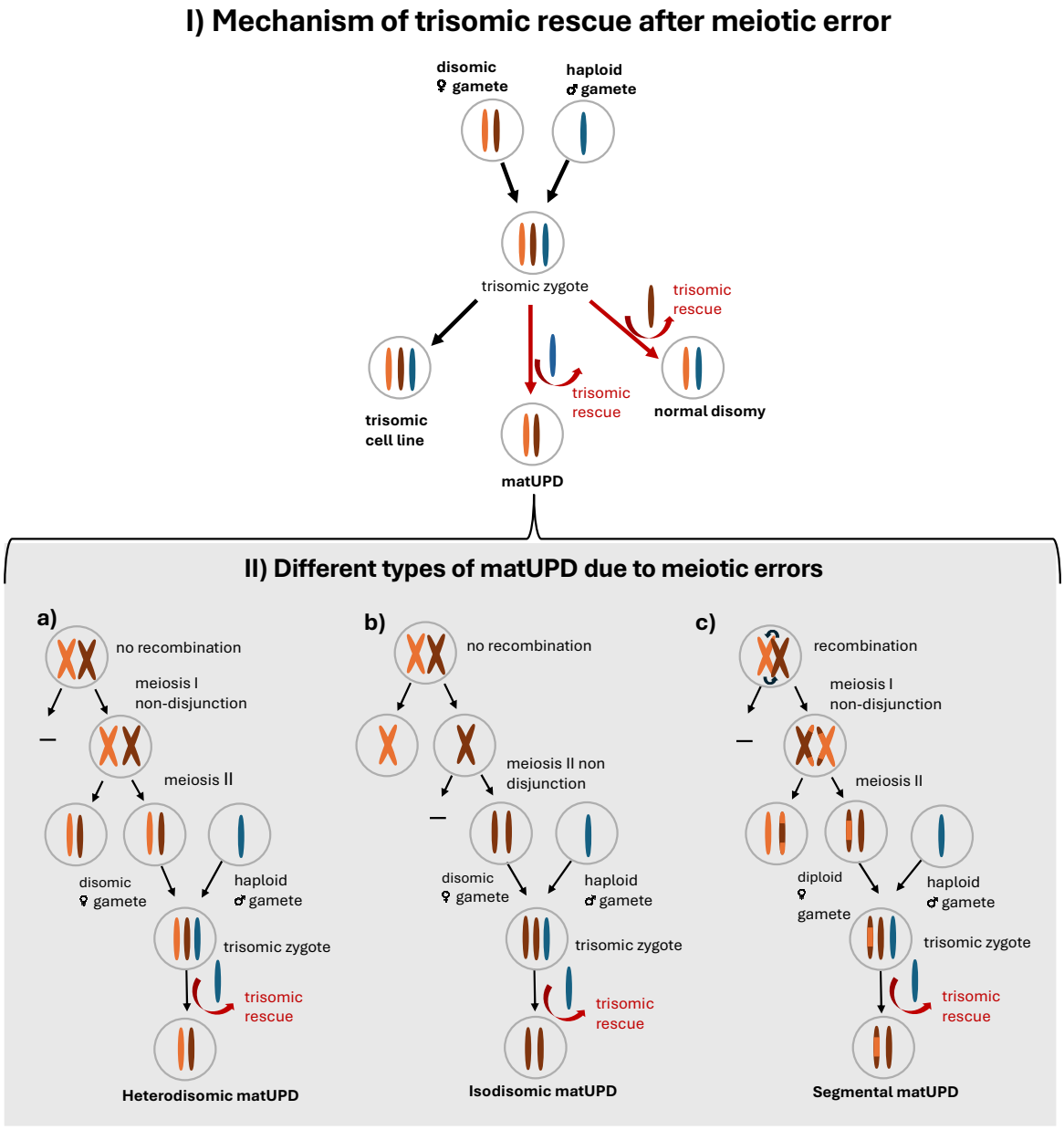



Figure 3: (I) Overview of the outcome of trisomic rescue happening after a meiotic error in a female gamete. If a disomic female gamete is fertilised by a normal haploid male sperm, a trisomic zygote may result. The trisomic rescue would result in the mitotic loss of one chromosome during the early mitotic divisions of the zygote and, if successful, result in a normal disomic zygote. If the paternal chromosome is lost, matUPD arises. A trisomic cell line may also persist [30].
(II) Illustration of the meiotic errors leading to matUPD before trisomic rescue [adapted from [44]]: a) complete heterodisomic matUPD arises from meiosis I non-disjunction, following normal meiosis II segregation and loss of the paternal chromosome. b) In complete isodisomic matUPD meiosis I segregation of the homologous chromosomes happens normally, but non-disjunction of the sister chromatids in meiosis II leads to a trisomic zygote. c) illustrates segmental matUPD which arises due to recombination (crossing over) happening before non-disjunction errors in either meiosis I or II, according to the mechanisms described before. For demonstration, only meiosis I non-disjunction is illustrated.

the mother and one from the father. During the process of trisomic rescue, one of the chromosomes is degraded to obtain a disomic cell line. If both chromosomes from the mother are retained matUPD results [29].
Complete isodisomy, on the other hand, may result from a non-disjunction error in meiosis II, with both chromosomes being identical copies of one maternal chromosome. As with heterodisomy, in complete isodisomy, there is also no recombination happening before segregation and therefore both chromosomes show the same polymorphic loci along the entire length [29].
In case of recombination taking place before the non-disjunction event in either meiosis I or II, both heterodisomy and isodisomy may appear as segmental UPD. In this case, some segments of the individuals' chromosomes are identical, showing the same polymorphisms, while other parts do not [29].

Advanced maternal age (AMA) and a higher likelihood of matUPD in PWS have been linked multiple times. It is assumed that the occurrence of non-disjunction events in meiosis increases with maternal age [9, 23, 46]. Additionally, a systematic review of the literature on mosaicism for autosomal trisomies revealed that the percentage of AMA is higher in individuals with UPD 15 compared to those with biparental inheritance (BPI). In individuals with BPI of chromosome 15, 78% of cases were associated with AMA, compared with 87% in UPD. Overall, they found a significant (p=0.0026) difference with 78% of UPD cases to be associated with AMA versus 48% of the cases with BPI for all chromosomes analysed (7, 14, 15, 16). Therefore, it also raises the question of a supposed preferential disposal mechanism for one inherited chromosome [47].

#### 1.2.0.1 Other possible mechanisms in the formation of matUPD

MatUPD in PWS may also arise in a small percentage due to a post-zygotic error, in which the initial error is paternal and therefore not associated with higher maternal age. In this case, the female gamete is normal and fertilised by a nullisomic sperm, followed by mitotic duplication of the maternal chromosome. The arising matUPD would then be isodisomic, as the second chromosome is a copy of the inherited maternal chromosome. In contrast to PWS, in AS the post-zygotic error would be associated with advanced maternal age [48].
This mechanism of formation of matUPD in PWS was hypothesised in cases in which mosaicism of isodisomic matUPD cells and euploid cells was able to be detected via SNP array [49, 50].

## 1.3 Mosaicism

Mosaicism in genetics refers to the simultaneous presence of more than one cell line in humans or a certain tissue. Not all cells in the human body are genetically alike due to post-zygotic mutations, which are then multiplied by mitosis, generating cellular lines with variations in their genetic material. Depending upon the timing of the mutation event during development, mosaicism can affect the whole body or just certain tissues [45].
In chromosomal mosaicism, some cell lines exhibit a loss or gain of a chromosome, rendering them aneuploid [45]. This can be further categorised into general mosaicism, originating from an early error in mitosis, where mosaicism is present in the whole organism, and confined mosaicism. In confined mosaicism, the aneuploidy only affects a certain tissue in the body [43].

A topic relevant for this thesis is confined placental mosaicisim (CPM), in which aneuploidies and mosaicism might be found in placental tissue but not in human tissue [51]. Trisomies of multiple chromosomes in CPM, including chromosome 15, are associated with structural anomalies of the fetus as well as fetal growth restriction, low birth weight and preterm birth of babies. Although these findings have been associated with CPM, structural fetal anomalies may also be due to undetected chromosomal mosaicism in the fetus, which may not be found if the percentage of mosaicism is low in the examined tissue [52].

### 1.3.1 Development of mosaicism

Mosaicism may develop from mitotic or meiotic errors leading to aneuploidy (trisomy or monosomy) and, as mentioned above, may express itself differently depending on the timing of the error occurring [43].

A meiotic error in a zygote being corrected during the cleavage stage, a stage of rapid mitotic division of the zygote after fertilisation, may produce abnormal aneuploid cells and result in mosaicism. In case of trisomic cells, correction via trisomic rescue may happen, which was already described in Section 1.2.

Following trisomy and trisomic rescue, there are two scenarios possible: (I) The fetus may develop from the rescued diploid cells and the abnormal cells will be isolated in the trophoblast, which further contributes to the development of the placenta and

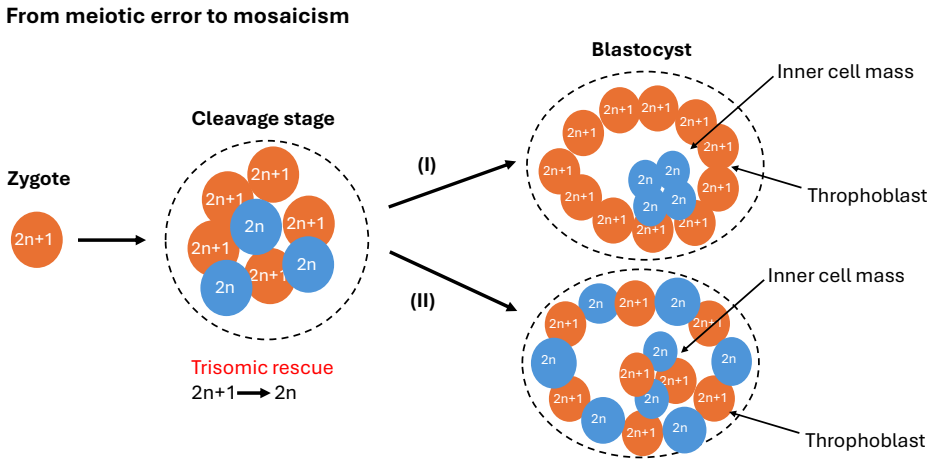



Figure 4: Development of mosaicism from meiotic errors, adapted from Taylor et al. [43]. Non-disjunction during meiosis leads to the formation of an aneuploid zygote (2n+1). Correction via trisomic rescue during the cleavage stage may result in (I) mosaicism in only the trophoblast or (II) mosaicism in both the trophoblast and inner cell mass.

produces CPM. Or (II) the trisomic cells are not isolated and the trophoblast and inner cell mass, further forming the embryo, will both show mosaicism [43]. Both scenarios are demonstrated in Figure 4.

For mitotic errors, the timing of the error plays a major role. An error in the first to second division during cleavage may lead to mosaicism throughout all embryonic tissues, whereas it might be confined, if it occurs later in development [43].

Chromosomal mosaicism in the preimplantation stage of a zygote appears to be quite common, especially in in vitro fertilization (IVF), with a systematic review finding that up to 73% of preimplantation embryos after IVF were mosaic. In addition, the results showed that these mosaic embryos, consisting of diploid and aneuploid cell lines, are viable, supporting the theory that there appears to be a selection bias against aneuploid cells during development [53].

### 1.3.2 Prenatal testing for trisomies and mosaicism

For the detection of chromosomal abnormalities, including trisomies and mosaicism, there are two invasive procedures in prenatal testing: chorionic villus sampling (CVS) and amniocentesis. CVS may be performed earlier (10 - 11 weeks) in pregnancy than amniocentesis (from 14th weeks). In CVS, cytotrophoblasts and mesodermal fibrob-

lasts are obtained and used for a short-term culture and a long-term culture. In amniocentesis, trophoblasts, fibroblasts and epithelium can be extracted from amniotic fluid and cultured [54].
Since there is a high likelihood of detection of mosaicism in short-term culture in CVS, both cell types should be analysed [54]. Mosaicism in cytotrophoblasts resembles placental mosaicism and may only be secondarily developed and confined to the placenta and not necessarily found in the fetal tissue [55].
In reference to CVS, approximately 1-2% of the samples show mosaicism, mostly consisting of disomic and trisomic cell lines, with only 13% of the abnormal cell lines being true fetal mosaicism. True fetal mosaicism hereby refers to the confirmation of the mosaicism through amniocentesis and detection of abnormal cell lines in amniotic fluid, since amniotic fluid contains fetal cells and not only placental tissue [56].
Karyotyping via culture is still the recommended screening tool in patients when chromosomal aneuploidy is suspected during pregnancy, to provide assessment of fetal risk. For diagnostic purposes chromosomal microarray analysis (CMA) is commonly preferred [55].

For karyotyping, G-banding with Giemsa staining has been the gold standard for many years and referred to as “classical“ or “conventional“ karyotyping. For this method, the chromosomes are treated with a proteolytic enzyme and then stained, resulting in light and dark bands on the chromosomes. One band consists of approximately 7.5 Mb. In chromosomes obtained from metaphase, a band resolution of up to 400 bands is possible, whereas in high-resolution banding, a resolution of 550 - 850 bands may be achieved. For this, the chromosomes are obtained in prometaphase. The band resolution plays an important role in detecting possible structural aberrations [57].

### 1.3.3 CytoSNP-Array in the detection of mosaicism

Historically, the detection of aneuploidies and mosaicism was conducted cytogenetically by classical karyotyping, as described above. However, even with high-resolution karyotyping using fluorescence in situ hybridization (FISH), these techniques only offer a limited resolution for detecting duplications and deletions. This has been improved by the introduction of CMA, which encompasses array-based techniques that analyse CNV in chromosomal material, offering higher resolution and an easier approach than classical karyotyping [58]. CNVs hereby refer to copy number gains or losses of certain

DNA sections, which are already formed in the germline [59].
CMA as a general term refers to array based comparative genomic hybridization (aCGH) and SNP arrays. While aCGH compares the individuals DNA with a control sample, in SNP arrays, polymorphic markers for specific sites on the DNA are used [58].

These SNPs play a part in the variability of the human genome and are spread all over our DNA. As the name already suggests, the polymorphism only affects one nucleotide, which may differ in individuals. Some SNPs are more common than others, with common SNPs occurring in more than 5% of all humans and rare SNPs occurring in less than 5%. SNPs are found on average approximately every thousand base pairs and are an ideal DNA marker for genetic analysis [19].

With the use of these polymorphisms as markers, SNP arrays can detect regions of homozygosity (ROHs) in addition to aneuploidies and CNVs, by simultaneously providing information about the genotype and the number of alleles being present at each SNP locus analysed [59]. Information about the genotype may be presented as homozygous, heterozygous, or hemizygous. In homozygosity, both alleles are the same; in heterozygosity, the alleles are different, and hemizygosity refers to only one allele being present at the locus [60].
ROHs are contiguous regions in the genome of homozygous genotypes that are present in the germline of an individual [59]. For instance, a SNP array may be utilised to diagnose an isodisomic UPD, as this area appears as a ROH, since both copies of the chromosome are identical. Heterodisomy can also be detected using SNP array analysis, but only after comparison with the DNA of the parents [61].

Information about the number of alleles at the SNP loci enables the method to also detect the gain and loss of chromosomal material, such as aneuploidies and mosaicism. Conlin et al. [61] demonstrated that the detection of mosaicism through SNP array is more sensitive than traditional karyotyping, with the detection limit of mosaicism being approximately 5% in their study, which is even lower than estimated in previous studies (10-20%). It also eliminates a possible bias from traditional karyotyping, as many cells in interphase and metaphase can be studied [61]. In traditional karyotyping, a limited number of cells are studied only in metaphase. Izumi et al. [49] confirmed the use of SNP array and suggested it to be the ideal tool for the detection of imprinting disorders, as it will sufficiently detect low-level mosaicism. They subsequently published the first

case of mosaic trisomy 15 cells found in peripheral blood in a live-born individuals with matUPD in PWS.

In cases of mosaic trisomy, the SNP array technology allows the number of different haplotypes to be determined. This also helps to clarify the chromosome constellation [61].

## 1.4 Trisomy 15 versus mosaic trisomy 15 phenotype

### 1.4.1 Trisomy 15

The most common and clinically relevant aneuploidies are trisomies and monosomies, with trisomies having three copies of one chromosome and monosomies missing one copy [62]. Approximately 35% of spontaneous abortuses show trisomy or monosomy, making aneuploidies the leading cause of miscarriage, with most aneuploid pregnancies not making it to term [63]. There is no single cause to be found for the occurrence of aneuploidy, but a multi-step process is under discussion, with advanced maternal age being involved in many of the errors happening [63]. Except for complete trisomy of chromosomes 13, 18 and 21, all other autosomal trisomies are only compatible with life if genetic mosaicism is present. On the other hand, complete monosomies of autosomes are not viable in fetal or embryonic development [62].

### 1.4.2 Mosaic trisomy 15 phenotype

Trisomies have been shown to be the most common chromosomal aberration in spontaneous miscarriages [64], of which 7.6% account for trisomy 15. Even though complete trisomy of chromosome 15 is described as non-viable in most literature, two cases of live-born infants with trisomy 15 have been reported, with both babies showing multiple congenital abnormalities and both dying on day 4 after birth [65, 66].

In contrast, several cases of live-born infants with MT15 have been published so far [67–69]. A clear characteristic phenotype has not yet been described for these individuals. However, many exhibit similar features and congenital anomalies. The most common are congenital heart defects and distinct facial features. As for facial features, a broad nasal bridge, upturned nasal tip, large nose, small mouth or micrognathia/ret-

rognathia and abnormalities of the ears have been described. Other findings include intrauterine growth restriction (IUGR), genital anomalies like an anteriorly placed anus and hypoplastic labia majora or scrotum, digit anomalies and brain anomalies [67, 68].

A correlation between the level of MT15 found in cultured amniocytes from amniocentesis and abnormal fetal outcome has been indicated as well, with a lower level of MT15 being in favour of a better fetal outcome and fewer congenital anomalies. In addition, in prenatal diagnosis, abnormal ultrasound associated with MT15 may be IUGR, congenital heart defects and oligohydramnios [70].

## 1.5 Mosaic trisomy 15 and matUPD in literature

Mosaic trisomy 15 and matUPD in PWS have been linked in a few publications, often also in regards to prenatal diagnostics [71, 72].
One of the earliest documented cases was published by Cassidy et al. in 1992 [41]. In their case report, trisomy 15 cells were found in CVS during prenatal testing of a fetus due to advanced maternal age. Subsequent molecular analysis of the child after birth, performed due to clinical features aligning with PWS, determined matUPD 15 as the cause of the syndrome. This finding led to them proposing trisomic rescue as the underlying mechanism, with the trisomic cells found in CVS preceding the matUPD 15.

As in the case report of Cassidy et al.[41], the presence of trisomy 15 in CVS in association with matUPD has been detected in other cases. Since the trisomic cell lines often only appeared in CVS but not in follow-up amniocentesis, CPM for the MT15 was suggested [42, 73, 74]. Another interesting case was published by Roberts et al. in 1997 [74], who found trisomy 15 cells in CVS and then retrospectively also managed to find MT15 in placental tissue of the aborted fetus who was diagnosed with matUPD 15. In this case MT15 was found in 46% of the placental cells studied with FISH. They did not find any signs of MT15 in amniocytes, fetal tissue, or fetal blood which further indicated CPM.
Adding to this, Walczak et al. [75] retrospectively also found trisomy 15 cells confined in the placental tissue of an infant previously diagnosed with maternal heterodisomy and PWS.

### 1.5.1 Mosaic trisomy 15 found in tissue or blood alongside matUPD 15

The simultaneous presence of PWS due to matUPD and MT15 in fetal tissue or blood is rare, and to date, only eight cases, including five live-born, have been described in the literature [49, 76–82]. These publications are summarised in Table 2 and Table 3. Table 2 presents genetic testing and results from cytogenetics and molecular analysis, while Table 3 provides an overview of the phenotypic and clinical features of the published cases.

In seven of the cases [76–82] MT15 alongside matUPD 15 was detected in fetal skin and in only two cases [49, 78] in lymphocytes from blood samples.

Milunsky et al. [76] reported on a liveborn female infant with heterodisomic matUPD 15, who died at the age of 6 weeks. They found MT15 in amniocytes, fibroblasts and lung tissue of the infant. The MT15 was detected in fibroblasts in cytogenetics as well as in microsatellite analysis (MSA). No trisomy was detected in lymphocytes. The infant exhibited multiple congenital anomalies and distinct facial features, as described in Table 3.

In 1997, Slater et al. [77] published a case report of a fetus that was aborted, after MSA of DNA from amniocytes showed heterodisomic matUPD 15. The MSA was performed after MT15 was detected in the initial CVS performed due to advanced maternal age. Amniocentesis following CVS showed a normal, unremarkable karyotype. Notably, after determination of the pregnancy, cultured skin samples from the autopsy detected 4% MT15. The autopsy of the fetus was otherwise described as unremarkable.

Devriendt et al. [79] described a 3.3-year-old girl with MT15 found in fibroblasts, as well as an additional cell line with trisomy X in fibroblasts and lymphocytes. The presence of MT15 was also detected by MSA in DNA of fibroblasts. MatUPD 15 was detected in lymphocytes and described as heterodisomic. The girl was described as having distinct facial features and congenital anomalies. They concluded that the origins of both aneuploidies are unrelated and occurred in different stages. Further suggesting that the trisomy 15 arose as the result of a meiotic error, while the trisomy X arose as a postzygotic error. However, it is possible that the trisomic rescue occurred simultaneously with the post-zygotic gain of the additional X chromosome.

In their study of karyotype/phenotype correlations in rare mosaic trisomies, Hsu et al. [80] included the case of an aborted fetus (XIII-8) with matUPD and MT15 found in CVS, amniotic fluid, a skin and cord sample, as well as fetal membrane in cytogenetics. No further information about a specific phenotype was described, only that the autopsy revealed a narrowing of the proximal segment of the cord. Type of matUPD 15 was also not further specified.

In 2000, Olander et al. [81] reported on a boy with matUPD 15 with a severe phenotype in PWS, including distinct facial features and multiple congenital anomalies, which they linked to the presence of MT15 found in cultured fibroblasts of the boy. The matUPD 15 was detected using MSA and described as isodisomic. Their case is the only one to have isodisomic matUPD 15, raising the question whether matUPD 15 may have developed due to a postzygotic mitotic error, in contrast to trisomic rescue.

Aparicio et al. [82] published the latest report in 2019, in which they described a liveborn infant with matUPD 15 in PWS, who also showed MT15 in amniotic fluid and cultured skin. PWS was diagnosed using MS MLPA. MT15 was also verified by aCGH alongside cytogenetics in amniocytes. Multiple congenital anomalies had already been detected on fetal ultrasound and the infant later showed distinct facial features, including upslanted palpebral fissures, a thin upper lip with downturned corners of the mouth and dysplastic, rotated ears.

The initial documentation of the presence of MT15 in the blood of a fetus with matUPD 15 was reported by Christian et al. [78] in 1996, who included a case in their study which had been previously published in an abstract by Rocklin et al. [83]. In amniocentesis performed due to AMA, MT15 was detected in three separate cultures in cytogenetics, thus leading to the termination of the pregnancy. MT15 was further found using cytogenetic analysis in skin fibroblasts, kidney tissue, lung tissue, fetal membrane and a blood sample obtained from the fetal cord in autopsy. MSA of amniocytes and fibroblasts verified MT15 and heterodisomic matUPD 15 in the fetus. Other than a two-vessel umbilical cord and malrotation of bowel found in the autopsy, no further anomalies or phenotypic features were described.

Izumi et al. [49] then published the first case of MT15 being detected in the peripheral blood of a live-born female infant with matUPD 15. In patient 2 presented in their study, they found trisomic cells in cytogenetics and via SNP array. Cytogenetics yielded a mosaicism of 2.5% (1/40 cells) and the SNP array a mosaicism of approximately 5-10%. MatUPD was categorized as mixed iso-/heterodisomic (segmental).

The girl presented with mild upslanted palpebral fissures and a mild adduction of the thumbs. IUGR of the girl, breech presentation and mild hypotonia were described, but no congenital anomalies.

Another case of interest not depicted in Tables 3 and 2, as matUPD was only suspected but not verified, was described by Silva et al. [72]. They found MT15 cytogenetically in CVS, amniocytes and cultured fetal skin of an aborted fetus. In SNP array, the mosaic level was found to be between 26-29% in CVS and amniocytes, making this the case with the highest levels of trisomy 15 found in CVS, which was not CPM. They suspected a meiosis I non-disjunction error and matUPD, but had difficulties in the assessment of UPD via SNP array, linking this to the high level of mosaicism in their case.

Table 2: Part I of the overview of cases with MT15 and matUPD in literature. Depicting maternal age, family history, matUPD type, outcome of pregnancy (LB/AB) and results of genetic analysis as described by the authors.

| Case | MA | Family history | LB/ AB | matUPD Type | Cytogenetics (T15 cells detected) | Molecular analysis |
|---|---|---|---|---|---|---|
| **(I) Milunsky et al. 1996 [76]** | 32 | | LB; died at 6 weeks | hetero-disomy | AF 44% (4/9 colonies)<br>FB 80% (40/50 cells)<br>Lung tissue 85% (17/20 cells)<br>Lymphocytes 0% (0/100 cells)<br>- | MSA:<br>FB: MT15 (2 mat, 1 pat)<br>lymphocytes: matUPD |
| **(II) Slater et al. 1997 [77]** | 38 | | AB | hetero-disomy | CVS cultured 43.3% (13/30 cells; 2 cultures)<br>amniocytes 0% (0/3 cultures)<br>Cultured skin (autopsy) 4% (4/100 cells)<br>- | MSA:<br>amniocytes: matUPD |
| **(III) Christian et al. 1996 [78] Case 1/ Rocklin et al. 1994 [83]** | 37 | prior child with trisomy 21 | AB | hetero-disomy | AF 44% (12/27 cells; 3 cultures)<br>Skin FB 100% (10/10 cells)<br>Kidney 100% (10/10 cells)<br>Lung 100% (10/10 cells<br>Fetal membrane 30% (3/10 cells)<br>blood sample (cord) 18.5% (35/189 cells)<br>- | MSA:<br>acrshortaf culture & FB: MT15 & matUPD |
| **(IV) Devriendt et al. 1997 [79]** | 42 | | LB | hetero-disomy | Lymphocytes 0%<br>47, XX+15 0/35 cells<br>(47, XXX 35/35 cells)<br>Cultured FB<br>47, XX+15 in 16 cells<br>47, XXX in 15 cells<br>- | MSA<br>Lymphocytes: matUPD<br>FB: MT15 (mat. & faint pat.) |
| **(V) Hsu et al. 1997 [80] Case XIII-8** | 43 | | AB | NFS | AF 11/28 cells<br>Skin 2/10 cells<br>Cord 5/10 cells<br>CVS 4/9 cells<br>Membrane 6/10 cells<br>- | DNA study NFS of aborted tissue: matUPD |
| **(VI) Olander et al. 2000 [81]** | 33 | | LB | Iso-disomy | Cultured lymphocytes 0%<br>0/21 cells (age 2 days)<br>0/100 cells (age 7 months)<br>Cultured FB 13.4%<br>5/67 cells (age 5 months)<br>- | MSA:<br>matUPD |
| **(VII) Izumi et al. 2013 [49] Patient 2** | 41 | | LB | Mixed iso-/hetero-disomy | Peripheral blood 2,5% (1/40 cells) | SNP array:<br>peripheral blood: 5-10%<br>T15 & matUPD in most cells |
| **(VIII) Aparicio et al. 2019 [82]** | 35 | prior mis-carriage | LB | NFS | acrshortaf 8% (4/50 cells)<br>Cultured skin 2-3% (of 400 cells)<br>Blood and urine 0% | aCGH:<br>amniocytes: T15<br>MS MLPA:<br>abnormal methylation pattern & normal copy number |

*Abbreviations: maternal age (MA), liveborn (LB), abortus (AB), amniotic fluid (AF), not further specified (NFS), fibroblasts (FB), trisomy 15 (T15), microsatellite analysis (MSA), array based comparative genomic hybridization (aCGH)*

Table 3: Part II of the overview of cases with MT15 and matUPD described in the literature. Depicting prenatal findings, phenotype and congenital anomalies described by the authors.

| Publication | Prenatal findings | Facial features | Other features | Congenital anomalies |
|---|---|---|---|---|
| **(I) Milunsky et al. 1996 [76]** | IUGR | triangular face, relative telecanthus, laterally prominent eyebrows, large anterior fontanelle, high forehead, bitemporal receding hairline, epicanthal folds, short bulbous nose, broad and flat nasal bridge, upturned nasal tip with anteverted nostrils, long philtrum, small mouth and tongue, micrognathia, low set posteriorly angulated ears, bilateral helical ear pits, ptosis | high arched palate, nuchal fold, short sternum, widely set nipples, anteriorly placed anus, overlapping fingers with hypoplastic nails, excess creases on all extremities, overlapping toes and broad halluces, hypermobile joints, 13 ribs bilaterally | two-vessel umbilical cord, VSD, PDA, dilated coronary sinus, long and redundant tricuspid valve, choroid plexus cyst, small grade I intra-ventricular haemorrhage bilaterally, some calcification of the lenticular striate vessels |
| **(II) Slater et al. 1997 [77]** | N/A | N/A | N/A | autopsy was unremarkable |
| **(III) Christian et al. 1996 [78] Case 1/ Rocklin et al. 1994 [83]** | N/A | N/A | N/A | two-vessel umbilical cord malrotation of bowel |
| **(IV) Devriendt et al. 1997 [79]** | N/A | bilateral epicanthic folds, downturned mouth, poor facial expression | clinodactyly of the fifth fingers, internal strabismus of the left eye, sticky saliva, small external genitalia, anteriorly placed anus, small hands | VSD, bicuspid aortic valve, mild peripheral pulmonary stenosis, horseshoe kidneys with vesico-ureteral reflux |
| **(V) Hsu et al. 1997 [80] Case XIII-8** | IUGR | N/A | N/A | narrowing of proximal segment of cord |
| **(VI) Olander et al. 2000 [81]** | breech presentation, premature contractions, oligo-hydramnios, unilateral pyelactasis | low-set posteriorly angulated ears with slight under folding of the superior helices, prominent nasal bridge, short horizontal palpebral fissures, ptosis, micrognathia, long philtrum, small mouth at 8 months mild upslant of the palpebral fissures & a more prominent chin | high-arched palate, undescended testes, hypoplastic scrotum, small penis with chordee, inguinal hernias, single palmar creases, generalized brachydactyly with slightly tapered fingers | VSD, PDA, PFO, prominent subarachnoid spaces over the fronto-temporal and parieto-occipital regions |
| **(VII) Izumi et al. 2013 [49] Patient 2** | IUGR breech presentation | mild upslanted palpebral fissures | bilateral mild adduction of thumbs, mild hypotonia | - |
| **(VIII) Aparicio et al. 2019 [82]** | multiple congenital anomalies on fetal scan | brachycephaly, upslanted palpebral fissures, thin upper lip with downturned corners of the mouth, dysplastic rotated ears | hypotonia, right cryptorchidism, three deep horizontal palmar creases on hands | ASD, VSD, PDA, vesico-ureteral reflux grade II |

*Abbreviations: not available (N/A), ventricular septal defect (VSD), atrial septal defect (ASD), patent foramen ovale (PFO), patent ductus arteriosus (PDA)*

## 1.6 Aim of this thesis

In reference to the existing literature described above, it is of great interest to publish similar cases, to delineate a possible phenotype for individuals that present with matUPD in PWS and MT15. This includes how individuals differ in clinical findings and outcomes compared to those presenting only with PWS and no MT15. It may also yield important information for prenatal testing, as a common severe phenotype in individuals with this condition may alter how the prenatal finding of MT15 cells is interpreted in the context of fetal outcome.

At the same time, each new report provides possible new insights into the mechanisms that lead to the formation of the MT15 in these cases. Additionally, most of the cases [76–79, 81] were described before the year 2000, using classical cytogenetics, such as karyotyping, as tools to analyse the samples, and further using MSA for molecular analysis. In only the latest two cases, [49, 82], array-based technology was used in the genetic analysis, making the current case report in this thesis even more interesting as a SNP array was used.

This new possibility of genetic analysis also raises the question of whether many of the case reports did not find MT15 in blood lymphocytes, because the testing was not sensitive enough. If trisomic rescue is one of the primary mechanisms for the development of matUPD in PWS as well as patUPD in AS, then it would be expected that a greater number of individuals would carry low-level MT15 in tissue or blood. This finding contrasts with the relatively limited number of published cases to date.

For this reason, in addition to the case report, a retrospective study was conducted to investigate possible low-level MT15 in lymphocytes of individuals with known matUPD 15 or patUPD 15.

# Chapter 2

# Materials and methods

## 2.1 Case report

Information for the case report was obtained from the medical reports from the maternity clinic and the Diagnostic and Research Institute (DR Institute) of Human Genetics at the Medical University of Graz, Austria. Two follow-up interviews with the parents of the boy were also conducted via online call when the infant was approximately 7 months old and 27 months old. The family consented to the publication of this case.

Initial genetic testing was performed at the DR Institute of Human Genetics of the Medical University of Graz. DNA was isolated from peripheral blood lymphocytes of the boy at eight days of age. The blood was sampled in EDTA tubes.
Phenotypic screening via next-generation sequencing (NGS) was performed using whole-exome sequencing (WES) and trio-HPO analysis. Following human phenotype ontology (HPO) terms were used: Neonatal hypotonia HP:0001319, Neonatal respiratory distress HP:0002643, Hydronephrosis HP:0000126, Soft, doughy skin HP:0001027, Poor Suck HP:0002033.

For specific analysis of the PWS/AS locus, a MS MLPA was performed, using the MLPA SALSA Kit ME028D1 kit from MRC-Holland B.V. (Amsterdam, The Netherlands). The analysis of the DNA was conducted according to the established general protocol.

For further specification, a trio genotype analysis using the Infinium CytoSNP-850K BeadChip from Illumina, Inc. (San Diego, CA, USA) was performed on both the boy

and the parents' blood. The array was conducted according to the manufacturer's protocols and scanned using the NextSeq 550.

Karyotyping of the index patient's peripheral blood was performed at approximately 16 months of age to detect trisomic cells. 14 metaphases from cultured lymphocytes were analysed using a 550-band resolution, following accredited methods according to ISO 15189. Repeated analysis with SNP array could not be performed as only peripheral blood in heparin tubes was available.

## 2.2 Retrospective study

The study design was submitted to the ethics committee of the Medical University of Graz (EK No. 1050/2024) and approved. For the retrospective study, genetic data of individuals diagnosed with PWS and AS at the DR Institute of Human Genetics of the Medical University of Graz were surveyed. Individuals with proven UPD or an abnormal methylation pattern on chromosome 15 were included.

As the institute only covers genetic testing and counselling for three federal states in Austria and the syndromes are quite rare, only ten individuals fit these criteria. The individuals had an age range of 2 to 35 years. Of the ten individuals, nine were diagnosed with PWS and one with AS. Contact information of the individuals' families was obtained from the institute's database. As some individuals were initially diagnosed a few years ago and stored on an outdated IT system, there was often limited contact information available.

Six families were contacted by phone and two families by post, as there was no telephone number on file. Two families were unable to be contacted, as their contact information was no longer up to date.
In two cases, the individuals had already passed away and one of the families contacted postally did not respond. In the end, four families agreed to participate in the study, three probands with PWS and one with AS. The information and declaration of consent for the study were sent out by mail and an appointment at the institute was scheduled.

The families of probands 1 and 2 came to the institute in person for further counselling and to sign the consent form. Further information about the proband's health information and a phenotype evaluation was conducted. The families of probands 3 and 4 sent in the signed consent form.

For the study, additional blood sampling of the probands and their respective fathers or mothers was required in some cases, if there was no or insufficient material left from the initial testing at the institute.
Peripheral blood was obtained from proband no. 2 and their respective mother, as well as from the mother of proband no. 1.

A cytogenetic study of the individual's blood, collected in Na-heparin tubes, was performed. For karyotyping, blood was used from the initial diagnostics at the institute for patient 3. For patient 2, the blood was obtained specifically for the study at the age of 25. In the case of proband no. 1, no peripheral blood for cytogenetics was obtained, as the proband is quite young and the team decided against additional blood drawing. In proband 2, 30 metaphases were analysed from cultured lymphocytes, and in proband 3, 20 metaphases were analysed using a 550-band resolution, following accredited methods according to ISO 15189.

A SNP array was performed on peripheral blood from the probands and their respective parent using the Infinium CytoSNP-850K BeadChip from Illumina, Inc. (San Diego, CA, USA). The array was conducted according to the manufacturer's protocols and scanned using the NextSeq 550. Genetic material of the probands was used from the initial work-up at diagnosis. Results from the probands and parents were compared for the detection of UPD type. Results of the probands were analysed for the presence of MT15.

# Chapter 3

# Results

## 3.1 Case report

The centre of this thesis is the index patient, who is a male infant. The mother was 38 years old at birth. The medical history of the mother was unremarkable. The boy was conceived with intracytoplasmic sperm injection (ICSI) and was the first pregnancy and child of the mother. One younger sibling was born after the index patient to the parents. The child was conceived naturally and healthy.

A vanishing twin was ablated using radio-frequency in the second trimester. The remainder of the pregnancy and further testing with a non-invasive fetal trisomy test and fetal ultrasound were unremarkable.

The boy was born prematurely via cesarean section at gestational age 35+3, after hospitalisation of the mother due to vasa praevia and mild vaginal bleeding.
He was small for gestational age (SGA) with a birth weight of 2090g, body length of 46cm and head circumference of 33cm. Upon birth, he expressed a weak cry and hypotonic muscle tone as well as bradycardia (50-100 beats/min). Initially, bag-mask ventilation was required and the boy was adapted to nasal continuous positive airway pressure (CPAP) after spontaneous breathing became more regular. An APGAR Score of 6/8/9 was assigned, and he was transferred to the neonatal intensive care unit at minute 10 after birth.
Caffeine was administered parenterally to stimulate spontaneous breathing, as well as fluid substitution. Hypotonic muscle tone persisted in the intensive care unit and the

boy showed low suck and dysphagia. A gastric tube was placed to administer nutrition. Weight gain under enteral nutrition was satisfactory and the boy gained his initial birth weight back on day 7 after birth. Sufficient swallowing was achieved by day 9 and nasal CPAP could be stopped on day 10. He received phototherapy from day 3 to day 5 due to hyperbilirubinemia.
The boy was transferred from the intensive care unit to the general ward on day 9 and was discharged to home care on day 26 after birth. Due to the persistent muscular hypotonia and difficulties swallowing, further genetic and metabolic testing was recommended.

### 3.1.1 Clinical findings

The boy was described as having a fleeing chin and low-set ears. Contractures of the third phalanx on both sides were described. He also presented with ankyloglossia.

Ultrasound of the kidneys revealed a grade 2 hydronephrosis on the right kidney and a grade 1-2 hydronephrosis on the left kidney. Cranial ultrasound showed a slightly larger left lateral ventricle but was otherwise unremarkable. Initially, echocardiography detected an atrial septal defect (ASD) of the secondary type with left-to-right shunt. On check-up before discharge, it was described as a physiological patent foramen ovale (PFO) with left-to-right shunt. Upon birth, undescended testes were present with low inhibin B blood levels, but testes were confirmed to be located intra-abdominally.

At approximately 7 months, a follow-up with the family revealed no further health issues for the boy. The contracture of the 3rd phalanx on the right hand had improved. The parents described the boy as having hypermobile joints and having lower muscle tone.

At the second online call, when the boy was 27 months, the family again reported no further health issues. The previously diagnosed hydronephrosis grade 2 of the right kidney was downgraded to grade 1. Follow-up echocardiography of the heart revealed normal cardiac function. The contracture of the 3rd phalanx was barely noticeable anymore, according to the parents. The undescended testis were surgically lowered at the age of 16 months. The parents reported that the boy still expressed hypermobile joints at nearly two years, especially the ankles, and that muscular hypotonia was still present. A previously diagnosed scoliosis of the spine also seemed to have improved with the help of a torso brace.

### 3.1.2 Development

At the 7-month follow-up, the family reported that the boy no longer needed a gastric tube for feeding. GH treatment was started at 4 months of age. At this age, his parents described him as a calm and alert baby, and he was already searching for eye contact. During the video call, the boy appeared to be aware of the video picture.

The boy began to sit independently at 15 months of age, started to stand at around 18 months and started walking with an aid at approximately 22 months. He began crawling at around 20 months. Before crawling, he slid around on his belly. The parents have stated that the child's fine motor skills, such as grasping, have never been a cause for concern.

At 27 months, according to parental report, the boy demonstrated a relatively high level of verbal output. However, his speech is unintelligible, lacking semantic context. He could express "Mama" and "Papa" as words and used these semi-frequently. His weight was 10.4 kilograms (under the 3rd percentile), and his height was 86 centimetres (25th percentile). Over the last year, he had gained only 1 kilogram of body weight.

As a consequence of his underweight status, he continued to receive supplemental food at the time of the second follow-up. The parents reported that a slowly increasing appetite had been observed over the preceding weeks. He had always had an interest in food and wanted to eat everything he saw. But he usually did not finish the food given to him.

### 3.1.3 Genetic analysis

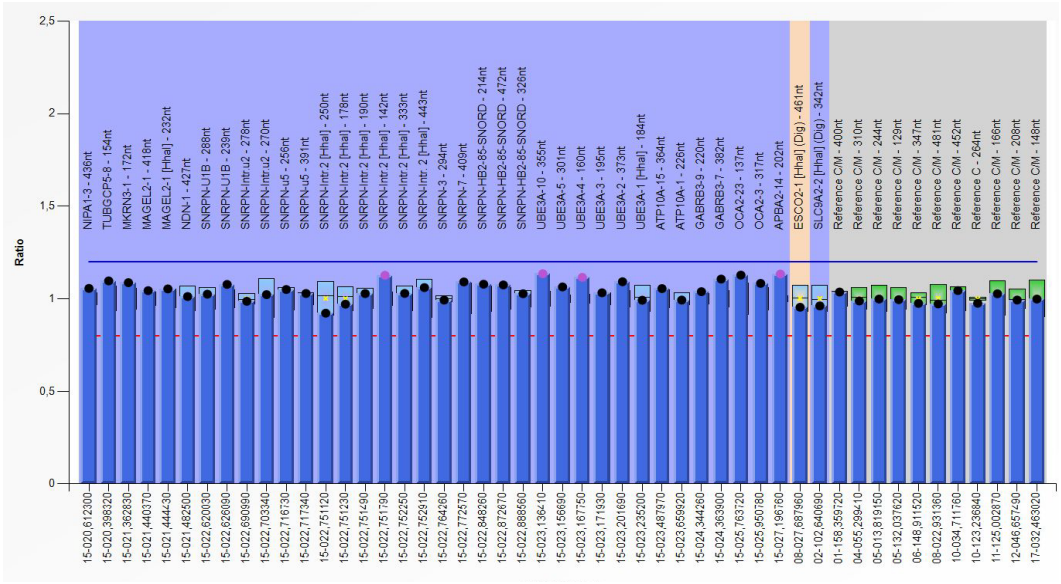
Referral to the Genetics Department of the Medical University of Graz was under the suspicion of PWS and "floppy infant". At this point, the boy was 8 days old. The phenotypic screening with WES and comparison with the parents' exome data did not reveal any disease-causing sequence or copy number alteration that is clearly associated with the phenotype.

Initial CNV analysis performed with MS MLPA revealed a normal CNV at the analysed regions, with no apparent deletion detected. Results of the analysis can be seen in Figure 5. The methylation analysis indicated an altered methylation status with hypermethylation of the *SNPRN* and *MAGEL2* locus in the PWCR, as seen in the

methylation results illustrated in Figure 6.
A normal CNV ratio and hypermethylation at these loci are consistent with matUPD or an imprinting defect in PWS [37].
Subsequent trio-genotype analysis of the index patient and both parents via SNP array revealed maternal uniparental heterodisomy as the molecular cause. Additionally, a low-grade (approximately 10%) mosaic trisomy 15 was detected in the blood sample. SNP array results are demonstrated in Figure 7, as well as in the close-up version of chromosome 15 in Figure 8.

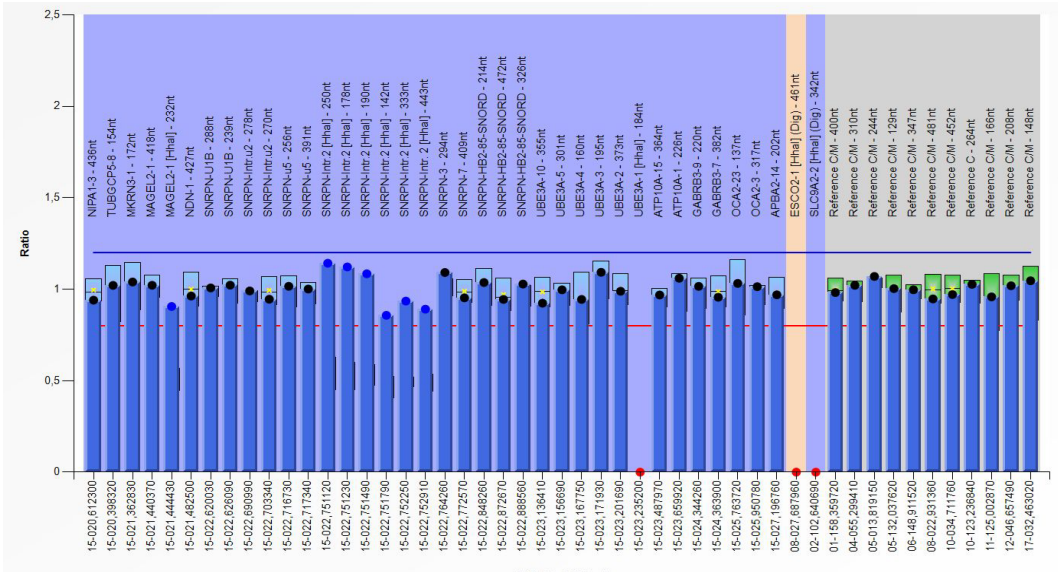
A follow-up cytogenetic study of the peripheral blood of the index patient at the age of 16 months showed an unremarkable male karyotype in all 14 metaphases without apparent mosaic trisomy, as seen in figure 9.

To date, this case presents the second live-born infant with matUPD in PWS and an additional mosaic trisomy 15 detected in a blood sample.




| D [nt] | Gene-Exon | Chr.band | hg18 loc. | Height | Area | Ratio[H] | Stdev | [REF] | Width | d[nt] | [Mut details] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 436 | NIPA1-3 | 15q11.2 | 15-020,612300 | 11445 | 57361 | 1,06 | 0,04 | = | 36 | 0,02 | - |
| 154 | TUBGCP5-8 | 15q11.2 | 15-020,398320 | 13522 | 49652 | 1,1 | 0,04 | = | 27 | 0,01 | - |
| 172 | MKRN3-1 | 15q11.2 | 15-021,362830 | 13562 | 52010 | 1,09 | 0,04 | = | 28 | 0,00 | - |
| 418 | MAGEL2-1 | 15q11.2 | 15-021,440370 | 9990 | 49846 | 1,04 | 0,04 | = | 37 | 0,14 | - |
| 232 | MAGEL2-1 [Hha | 15q11.2 | 15-021,444430 | 14520 | 58407 | 1,05 | 0,04 | = | 32 | 0,06 | - |
| 427 | NDN-1 | 15q11.2 | 15-021,482500 | 9413 | 47468 | 1,01 | 0,05 | = | 37 | 0,16 | - |
| 288 | SNRPN-U1B | 15q11.2 | 15-022,620030 | 13343 | 60194 | 1,02 | 0,04 | = | 41 | 0,07 | - |
| 239 | SNRPN-U1B | 15q11.2 | 15-022,626090 | 15222 | 58767 | 1,08 | 0,04 | = | 32 | 0,01 | - |
| 278 | SNRPN-Intr.u2 | 15q11.2 | 15-022,690990 | 12069 | 52041 | 0,99 | 0,03 | = | 38 | 0,05 | - |
| 270 | SNRPN-Intr.u2 | 15q11.2 | 15-022,703340 | 11198 | 46660 | 1,02 | 0,07 | = | 39 | 0,07 | - |
| 256 | SNRPN-u5 | 15q11.2 | 15-022,716730 | 13306 | 53012 | 1,05 | 0,05 | = | 29 | 0,00 | - |
| 391 | SNRPN-u5 | 15q11.2 | 15-022,717340 | 9567 | 44772 | 1,03 | 0,04 | = | 25 | 0,04 | - |
| 250 | SNRPN-Intr.2 | 15q11.2 | 15-022,751120 | 14886 | 59803 | 0,92 | 0,06 | = | 25 | 0,06 | - |
| 178 | SNRPN-Intr.2 | 15q11.2 | 15-022,751230 | 13391 | 53932 | 0,97 | 0,04 | = | 33 | 0,07 | - |
| 190 | SNRPN-Intr.2 | 15q11.2 | 15-022,751490 | 11878 | 43759 | 1,03 | 0,04 | = | 20 | 0,01 | - |
| 142 | SNRPN-Intr.2 | 15q11.2 | 15-022,751790 | 17262 | 65340 | *1,13* | 0,03 | >> | 27 | 0,01 | - |
| 333 | SNRPN-Intr.2 | 15q11.2 | 15-022,752250 | 11145 | 49299 | 1,03 | 0,05 | = | 30 | 0,03 | - |
| 443 | SNRPN-Intr. 2 | 15q11.2 | 15-022,752910 | 10251 | 51540 | 1,06 | 0,07 | = | 27 | 0,00 | - |
| 294 | SNRPN-3 | 15q11.2 | 15-022,764260 | 11168 | 48490 | 0,99 | 0,03 | = | 35 | 0,04 | - |
| 409 | SNRPN-7 | 15q11.2 | 15-022,772570 | 10583 | 51181 | 1,09 | 0,07 | = | 29 | 0,07 | - |
| 214 | SNRPN-HB2-85- | 15q11.2 | 15-022,848260 | 15549 | 58844 | 1,08 | 0,06 | = | 30 | 0,01 | - |
| 472 | SNRPN-HB2-85- | 15q11.2 | 15-022,872670 | 11530 | 61392 | 1,07 | 0,04 | = | 39 | 0,08 | - |
| 326 | SNRPN-HB2-85- | 15q11.2 | 15-022,888560 | 12283 | 53333 | 1,03 | 0,05 | = | 38 | 0,03 | - |
| 355 | UBE3A-10 | 15q11.2 | 15-023,136410 | 11009 | 48146 | *1,14* | 0,05 | >> | 33 | 0,03 | - |
| 301 | UBE3A-5 | 15q11.2 | 15-023,156690 | 11838 | 51729 | 1,06 | 0,04 | = | 38 | 0,00 | - |
| 160 | UBE3A-4 | 15q11.2 | 15-023,167750 | 16164 | 59887 | *1,12* | 0,04 | >> | 36 | 0,01 | - |
| 195 | UBE3A-3 | 15q11.2 | 15-023,171930 | 14744 | 61246 | 1,03 | 0,04 | = | 34 | 0,10 | - |
| 373 | UBE3A-2 | 15q11.2 | 15-023,201690 | 10701 | 48820 | 1,09 | 0,05 | = | 29 | 0,05 | - |
| 184 | UBE3A-1 [Hhal] | 15q11.2 | 15-023,235200 | 14027 | 52024 | 0,99 | 0,05 | = | 30 | 0,01 | - |
| 364 | ATP10A-15 | 15q12 | 15-023,487970 | 11257 | 52964 | 1,06 | 0,05 | = | 40 | 0,10 | - |
| 226 | ATP10A-1 | 15q12 | 15-023,659920 | 14112 | 57908 | 0,99 | 0,03 | = | 33 | 0,08 | - |
| 220 | GABRB3-9 | 15q12 | 15-024,344260 | 13882 | 54578 | 1,04 | 0,04 | = | 35 | 0,03 | - |
| 382 | GABRB3-7 | 15q12 | 15-024,363900 | 10665 | 50475 | 1,11 | 0,04 | = | 35 | 0,03 | - |
| 137 | OCA2-23 | 15q13.1 | 15-025,763720 | 14247 | 54188 | 1,13 | 0,05 | = | 31 | 0,00 | - |
| 317 | OCA2-3 | 15q13.1 | 15-025,950780 | 13039 | 57975 | 1,08 | 0,04 | = | 37 | 0,01 | - |
| 202 | APBA2-14 | 15q13.1 | 15-027,196760 | 14068 | 54854 | *1,13* | 0,04 | >> | 27 | 0,01 | - |
| 461 | ESCO2-1 [Hhal | 08p21.1 | 08-027,687960 | 6753 | 34706 | 0,95 | 0,05 | = | 27 | 0,12 | - |
| 342 | SLC9A2-2 [Hha | 02q12.1 | 02-102,640690 | 10270 | 45399 | 0,96 | 0,05 | = | 24 | 0,16 | - |
| 400 | Reference C/M | 01q23.2 | 01-158,359720 | 10357 | 48660 | 1,04 | 0,05 | = | 26 | 0,01 | - |
| 310 | Reference C/M | 04q12 | 04-055,299410 | 10878 | 45732 | 0,99 | 0,05 | = | 33 | 0,02 | - |
| 244 | Reference C/M | 05p15.2 | 05-013,819150 | 14367 | 55419 | 1 | 0,04 | = | 25 | 0,02 | - |
| 129 | Reference C/M | 05q31.1 | 05-132,037620 | 14631 | 56252 | 1 | 0,04 | = | 33 | 0,02 | - |
| 347 | Reference C/M | 06q24.3 | 06-148,911520 | 9587 | 41866 | 0,98 | 0,04 | = | 37 | 0,13 | - |
| 481 | Reference C/M | 08p21.3 | 08-022,931360 | 8615 | 45311 | 0,97 | 0,05 | = | 29 | 0,06 | - |
| 452 | Reference C/M | 10p11.21 | 10-034,711760 | 11173 | 58151 | 1,04 | 0,05 | = | 36 | 0,04 | - |
| 264 | Reference C | 10q26.13 | 10-123,236840 | 13032 | 50611 | 0,98 | 0,03 | = | 30 | 0,03 | - |
| 166 | Reference C/M | 11q24.2 | 11-125,002870 | 14227 | 52140 | 1,03 | 0,05 | = | 32 | 0,05 | - |
| 208 | Reference C/M | 12q13.11 | 12-046,657490 | 12651 | 47278 | 0,99 | 0,03 | = | 26 | 0,03 | - |
| 148 | Reference C/M | 17q12 | 17-032,463020 | 15522 | 57121 | 1 | 0,05 | = | 31 | 0,07 | - |
| **Median value all probe values:** | | | | **12283** | **52041** | **1,03** | **0,04** | | | **32** | **0,03** |

Figure 5: Results of CNV analysis using MS MLPA, performed on peripheral blood from the index patient. The probes show CNV ratios within the normal range, indicating the presence of two existing copies at the analysed regions. Low-grade mosaicism cannot be reliably detected using this method [84].




| D [nt] | Gene-Exon | Chr.band | hg18 loc. | Height | Area | Ratio[H] | Stdev | [REF] | Width | d[nt] | [Mut details] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 436 | NIPA1-3 | 15q11.2 | 15-020,612300 | 8600 | 44528 | 0,94 | 0,03 | = | 31 | 0,06 | - |
| 154 | TUBGCP5-8 | 15q11.2 | 15-020,398320 | 11034 | 41134 | 1,02 | 0,04 | = | 35 | 0,01 | - |
| 172 | MKRN3-1 | 15q11.2 | 15-021,362830 | 11267 | 43424 | 1,04 | 0,04 | = | 33 | 0,07 | - |
| 418 | MAGEL2-1 | 15q11.2 | 15-021,440370 | 8157 | 41425 | 1,02 | 0,03 | = | 34 | 0,05 | - |
| 232 | ***MAGEL2-1 [Hha*** | 15q11.2 | 15-021,444430 | 10509 | 44097 | ***0,91*** | 0,03 | >>* | 33 | 0,12 | - |
| 427 | NDN-1 | 15q11.2 | 15-021,482500 | 7242 | 37571 | 0,96 | 0,04 | = | 44 | 0,08 | - |
| 288 | SNRPN-U1B | 15q11.2 | 15-022,620030 | 10742 | 47547 | 1,01 | 0,04 | = | 41 | 0,14 | - |
| 239 | SNRPN-U1B | 15q11.2 | 15-022,626090 | 12437 | 50811 | 1,02 | 0,04 | = | 30 | 0,02 | - |
| 278 | SNRPN-Intr.u2 | 15q11.2 | 15-022,690990 | 9556 | 41310 | 0,99 | 0,03 | = | 35 | 0,00 | - |
| 270 | SNRPN-Intr.u2 | 15q11.2 | 15-022,703340 | 8461 | 35142 | 0,95 | 0,06 | = | 32 | 0,08 | - |
| 256 | SNRPN-u5 | 15q11.2 | 15-022,716730 | 10806 | 43415 | 1,02 | 0,04 | = | 26 | 0,07 | - |
| 391 | SNRPN-u5 | 15q11.2 | 15-022,717340 | 7654 | 35676 | 1 | 0,03 | = | 34 | 0,08 | - |
| 250 | ***SNRPN-Intr.2*** | 15q11.2 | 15-022,751120 | 13587 | 53501 | ***1,14*** | 0,05 | >>* | 30 | 0,04 | - |
| 178 | ***SNRPN-Intr.2*** | 15q11.2 | 15-022,751230 | 12007 | 48050 | ***1,12*** | 0,04 | >>* | 37 | 0,01 | - |
| 190 | ***SNRPN-Intr.2*** | 15q11.2 | 15-022,751490 | 10296 | 38901 | ***1,08*** | 0,04 | >>* | 26 | 0,02 | - |
| 142 | ***SNRPN-Intr.2*** | 15q11.2 | 15-022,751790 | 11828 | 46463 | ***0,86*** | 0,03 | >>* | 28 | 0,03 | - |
| 333 | ***SNRPN-Intr.2*** | 15q11.2 | 15-022,752250 | 8330 | 38941 | ***0,94*** | 0,04 | >>* | 54 | 0,05 | - |
| 443 | ***SNRPN-Intr. 2*** | 15q11.2 | 15-022,752910 | 7299 | 36962 | ***0,89*** | 0,06 | >>* | 35 | 0,02 | - |
| 294 | SNRPN-3 | 15q11.2 | 15-022,764260 | 9743 | 41748 | 1,09 | 0,03 | = | 42 | 0,06 | - |
| 409 | SNRPN-7 | 15q11.2 | 15-022,772570 | 8060 | 39977 | 0,95 | 0,05 | = | 44 | 0,09 | - |
| 214 | SNRPN-HB2-85- | 15q11.2 | 15-022,848260 | 12883 | 49898 | 1,04 | 0,04 | = | 35 | 0,09 | - |
| 472 | SNRPN-HB2-85- | 15q11.2 | 15-022,872670 | 8701 | 46204 | 0,94 | 0,04 | = | 53 | 0,03 | - |
| 326 | SNRPN-HB2-85- | 15q11.2 | 15-022,888560 | 10094 | 43850 | 1,03 | 0,04 | = | 42 | 0,04 | - |
| 355 | UBE3A-10 | 15q11.2 | 15-023,136410 | 8128 | 37832 | 0,92 | 0,03 | = | 50 | 0,12 | - |
| 301 | UBE3A-5 | 15q11.2 | 15-023,156690 | 9432 | 41782 | 1 | 0,03 | = | 40 | 0,03 | - |
| 160 | UBE3A-4 | 15q11.2 | 15-023,167750 | 12200 | 44540 | 0,94 | 0,03 | = | 32 | 0,01 | - |
| 195 | UBE3A-3 | 15q11.2 | 15-023,171930 | 12874 | 50984 | 1,09 | 0,03 | = | 31 | 0,09 | - |
| 373 | UBE3A-2 | 15q11.2 | 15-023,201690 | 8458 | 38306 | 0,99 | 0,03 | = | 37 | 0,03 | - |
| 184 | ***UBE3A-1 [Hhal]*** | 15q11.2 | 15-023,235200 | **0** | 0 | ***0*** | 0 | <<** | **0** | 0,00 | - |
| 364 | ATP10A-15 | 15q12 | 15-023,487970 | 8714 | 40555 | 0,97 | 0,04 | = | 47 | 0,10 | - |
| 226 | ATP10A-1 | 15q12 | 15-023,659920 | 11955 | 49052 | 1,06 | 0,03 | = | 30 | 0,12 | - |
| 220 | GABRB3-9 | 15q12 | 15-024,344260 | 11271 | 45946 | 1,02 | 0,03 | = | 34 | 0,05 | - |
| 382 | GABRB3-7 | 15q12 | 15-024,363900 | 8151 | 39236 | 0,96 | 0,03 | = | 31 | 0,02 | - |
| 137 | OCA2-23 | 15q13.1 | 15-025,763720 | 11750 | 43435 | 1,03 | 0,05 | = | 32 | 0,00 | - |
| 317 | OCA2-3 | 15q13.1 | 15-025,950780 | 10568 | 47018 | 1,01 | 0,04 | = | 42 | 0,05 | - |
| 202 | APBA2-14 | 15q13.1 | 15-027,196760 | 10900 | 43961 | 0,97 | 0,03 | = | 32 | 0,01 | - |
| 461 | ***ESCO2-1 [Hhal*** | 08p21.1 | 08-027,687960 | **0** | 0 | ***0*** | 0 | <<** | **0** | 0,00 | - |
| 342 | ***SLC9A2-2 [Hha*** | 02q12.1 | 02-102,640690 | **0** | 0 | ***0*** | 0 | <<** | **0** | 0,00 | - |
| 400 | Reference C/M | 01q23.2 | 01-158,359720 | 8129 | 39088 | 0,98 | 0,03 | = | 47 | 0,09 | - |
| 310 | Reference C/M | 04q12 | 04-055,299410 | 8880 | 39423 | 1,02 | 0,04 | = | 44 | 0,05 | - |
| 244 | Reference C/M | 05p15.2 | 05-013,819150 | 12286 | 48041 | 1,07 | 0,05 | = | 26 | 0,01 | - |
| 129 | Reference C/M | 05q31.1 | 05-132,037620 | 11733 | 43821 | 1 | 0,04 | = | 27 | 0,06 | - |
| 347 | Reference C/M | 06q24.3 | 06-148,911520 | 7639 | 34295 | 1 | 0,03 | = | 37 | 0,15 | - |
| 481 | Reference C/M | 08p21.3 | 08-022,931360 | 6520 | 35194 | 0,95 | 0,05 | = | 43 | 0,08 | - |
| 452 | Reference C/M | 10p11.21 | 10-034,711760 | 8671 | 45451 | 0,97 | 0,04 | = | 34 | 0,14 | - |
| 264 | Reference C | 10q26.13 | 10-123,236840 | 10694 | 43305 | 1,03 | 0,03 | = | 33 | 0,07 | - |
| 166 | Reference C/M | 11q24.2 | 11-125,002870 | 10897 | 39834 | 0,96 | 0,05 | = | 26 | 0,09 | - |
| 208 | Reference C/M | 12q13.11 | 12-046,657490 | 10305 | 41477 | 1,02 | 0,04 | = | 24 | 0,09 | - |
| 148 | Reference C/M | 17q12 | 17-032,463020 | 12982 | 46523 | 1,05 | 0,06 | = | 21 | 0,00 | - |
| **Median value all probe values:** | | | | **10300** | **43360** | **1** | **0,04** | | | **34** | **0,06** |

Figure 6: Results of the methylation analysis using MS MLPA performed on peripheral blood from the index patient. HhaI digested probes in *MAGEL2* and *SNRPN* revealed a methylation ratio close to 1.0, concordant with hypermethylation of the analysed loci (blue dots). Note: the methylation-specific probe in *UBE3A* is located in a CPG island which is always unmethylated in normal blood-derived DNA. The methylation-sensitive probes in *ESCO2* and *SLC9A2* serve as digestion control probes and are also always unmethylated (red dots).

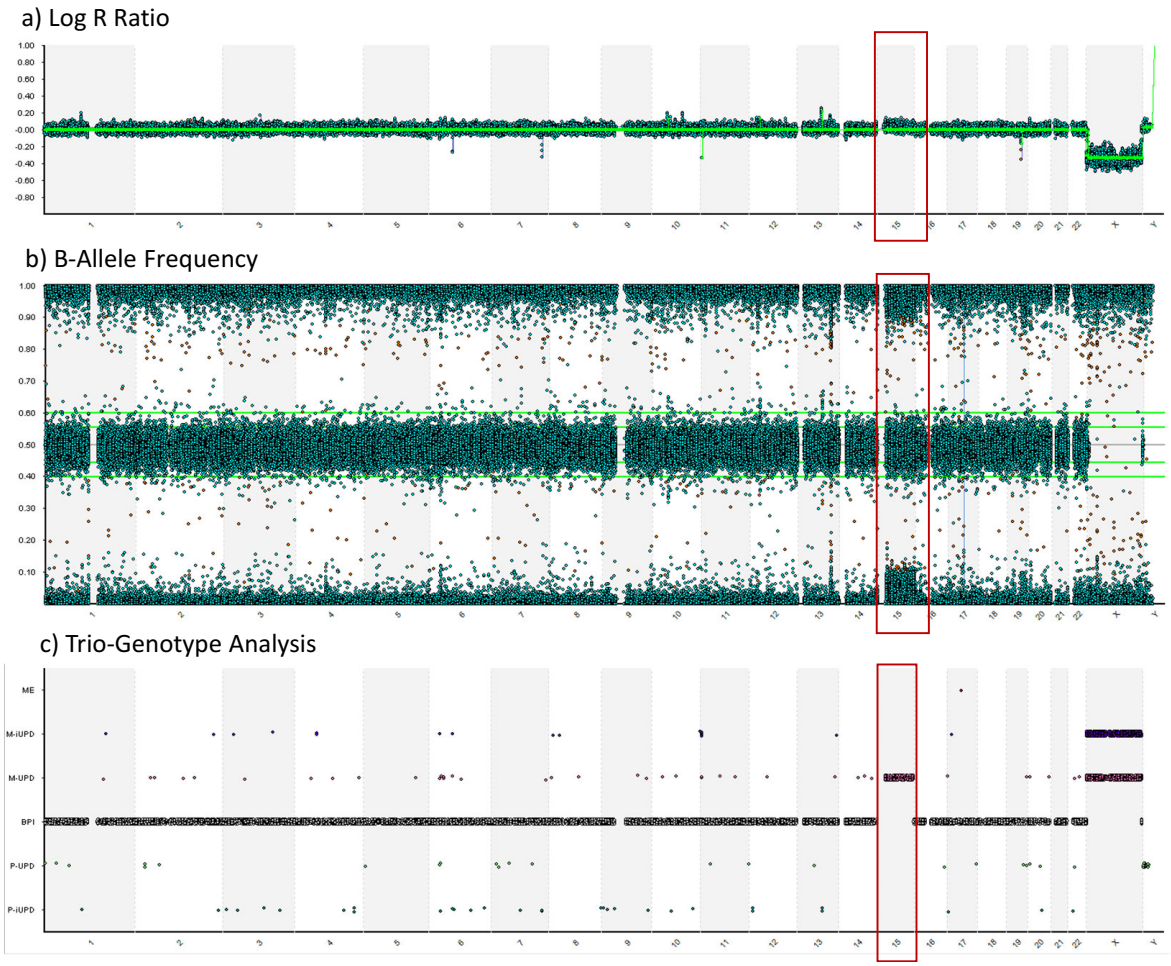



Figure 7: SNP array results of the index patient (a),(b) and trio-genotype analysis including the parents (c).
(a) The Log R ratio reflects the relative amount of DNA at the SNP loci across the entire genome. A normalised signal intensity of approximately 0.0 indicates two copies. On chromosome 15, a slight increase in the log R ratio can be observed, indicating a possible mosaic gain. (b) Visual indication of MT15 is confirmed by taking the B-allele frequency into account. The B-allele frequency represents the proportions of B alleles in relation to A alleles, thus giving information about the genotype at the SNP loci (0.0 = AA, 0.5 = AB, 1.0 = BB). At chromosome 15, the outer B-Allele clusters are extended (frequencies of approximately 0.0 to 0.1 and 0.9 to 1.0, respectively), which, based on the study by Conlin et al. [61], indicates a mosaic fraction of about 10% including an additional third haplotype. (c) Trio-genotype analysis revealed a matUPD of chromosome 15, as indicated by the pink SNP cluster (M-UPD). The remaining autosomes show BPI.

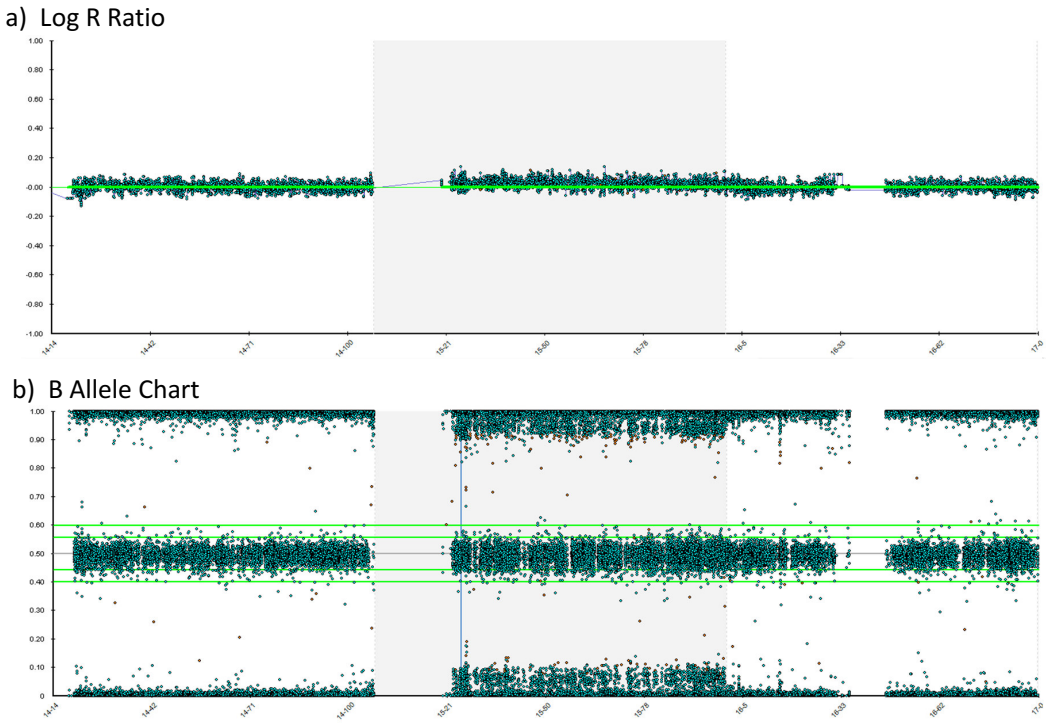



Figure 8: (a) Shows a close up of the Log R Ratio of chromosome 15, where a slight increase in signal intensity can be observed. (b) In the close-up version of the B Allele chart, a heterozygotic genotype of chromosome 15, as well as a third haplotype, can be observed. No homozygous regions are present on chromosome 15.

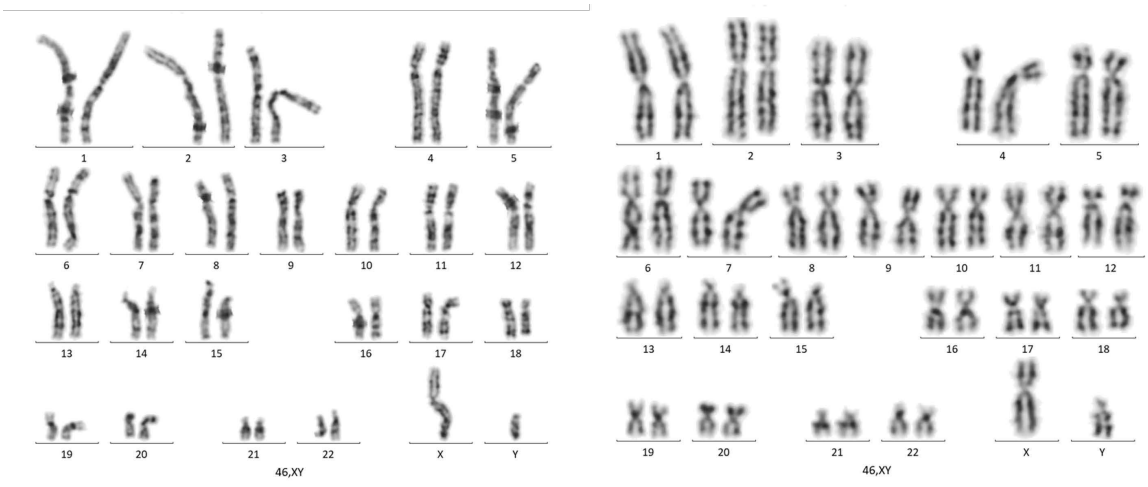



Figure 9: Karyogram of the index patient obtained from the cytogenetic analysis performed on cultured lymphocytes at approximately 16 months of age. The karyogram reveals a normal male karyotype[46, XY] with no indication of trisomy 15 in 14 metaphases examined.

## 3.2 Results of the retrospective study

The retrospective study included 4 probands (case no. 1-4), three with matUPD in PWS and one with patUPD in AS. An overview of the retrospective study is shown in Table 4.

Table 4: Results of the retrospective study including the index patient (0) from the case report.

| Case no. | Age at time of study (years) | UPD mat/pat | UPD type | Cytogenetics | SNP Array (MT15 present) |
|---|---|---|---|---|---|
| 0 (index patient) | 1.5 | matUPD | heterodisomy | normal male karyotype (14 MP; age 16 months) | MT15 ≈ 10% (age 7 days) no indication of MT15 |
| 1 | 2.5 | matUPD | mixed iso-/ heterodisomy | not performed | not detected |
| 2 | 25 | matUPD | mixed iso-/ heterodisomy | normal male karyotype (30 MP) | not detected |
| 3 | 17 | matUPD | heterodisomy | normal male karyotype (20 MP) | not detected |
| 4 | 12 | patUPD | isodisomy | normal male karyotype (12 MP) | not detected |

*Abbreviations: metaphases (MP)*

**Proband no. 1** was 2.5 years old at the time of study and presented with a mixed iso-/heterodisomic UPD. Cytogenetics were not performed and no trisomic cells could be detected in the SNP array.

Since the proband came to the institute accompanied by his mother, an evaluation of his phenotypic appearance and clinical findings could be performed. The boy presented with an IUGR during the pregnancy, as well as hypotonia at birth. Later, he also had low suction while feeding and problems swallowing. According to his mother, his development is approximately 6 months delayed in contrast to his older siblings. At the time of evaluation, he could stand up with assistance.

He spent around 120 days in the hospital after his birth and also received high-flow oxygen therapy. As far as clinical findings are concerned, the boy has cryptorchidism, but no further congenital anomalies. He also expresses a mild strabismus from time to

time.
Facial features observed phenotypically included almond-shaped eyes with bilateral epicanthic folds, overall flat facial features, a thinner upper lip, and a high forehead. According to the mother, these features may, in part, also resemble those of the father, who is of African descent. Additionally, the boy had relatively short hands and feet, as well as joint hypermobility, which may also be attributed to the overall hypotonia observed in the proband. The proband already receives GH treatment.

**Proband no. 2** was 25 years old at the time of study and presented with a mixed iso-/heterodisomic UPD. Cytogenetics from blood at the age of 25 were unremarkable and in the SNP array, no trisomic cells were detected. The male proband was only diagnosed with PWS at the age of 12 years at the institute. Before the diagnosis, he was considered to have feeding, growth, and regulatory difficulties. At the visit of the proband and his family to the institute, an evaluation of his clinical features and phenotypical appearance was performed.
According to the family, there was low fetal movement during pregnancy and he was born with a low birth weight and hypotonia. He expressed reduced sucking strength and required a nasogastric tube for feeding.
Developmental delay is also present in the proband. According to his parents, he began walking at around 18 months of age. He also had a delayed speech development. Now at the age of 25, he works at a workshop for disabled people and can count to ten, write simple words and letters and express himself in full sentences.
As for clinical diagnosis, he does have a congenital heart defect as well as cryptorchidism, with the right testis already being removed surgically. He also had club feet at birth and a hypospadia grade I. Pulmonary hypertension and intestinal neuronal dysplasia were described in the institute's documents. According to the family, he also had an inguinal hernia. Strabismus is also present.
The facial features included almond-shaped eyes with bilateral epicanthic folds and a small mouth with thin lips. His height is approximately 160cm with the use of GH and he has relatively smaller hands and feet. At the blood drawing, a thicker skin was also noticed.

**Proband no. 3** was 17 years old at the time of study and presented with a heterodisomic matUPD. Cytogenetics and SNP array detected no trisomic cells.

**Proband no. 4** was 12 years old at the time of study and was the only proband with patUPD in AS. The UPD was isodisomic. Cytogenetics and SNP array detected no trisomic cells.

# Chapter 4

# Discussion

## 4.1 Diagnostics

The case presented in this thesis is of particular interest, as, to the current knowledge, this is only the second time MT15 was detected in the peripheral blood of a liveborn infant diagnosed with matUPD in PWS. The level of mosaicism, being approximately 10%, is about the same as in the first described case in literature by Izumi et al. [49]. They proposed the level of mosaicism to be around 5-10%. Both times, in the current case report and in the case of Izumi et al. [49], the level of mosaicism was assessed by SNP array.

The genetic analysis performed in this case report, therefore, also gives an interesting insight into the detection of MT15, as most cases published so far [76–81] have primarily used classical karyotyping (G-banding) on cultured tissue for the detection of possible mosaicism. Only the two latest cases described by Izumi et al. [49] and Aparicio et al. [82] also performed FISH analysis for a higher resolution. Izumi et al. [49] have already proposed that traditional chromosome analysis, used for detecting mosaicism, is not optimal, as it may not detect low-level mosaicism, which may lead to the underrecognition of low-level mosaicism in combination with UPD. Discrepancies in the level of detection of mosaicism between cytogenetics and SNP array have been described before [61, 72], and are also present in patient 2 of Izumi et al. [49].

This discrepancy is also present in the current case, as the cytogenetics of 14 cultured metaphase lymphocytes did not yield any trisomic cell lines.

The selective growth of certain cell lines in cell cultures may be the reason for the discrepancies in culture-based studies versus non-culture-based studies. It has been shown that even a low level of mosaic cell lines (2%) can selectively grow in CVS cultures, overtaking the other cell lines and thereby heavily influence the outcome [85]. Taking this into consideration, in the present case, the non-trisomic cell lines may have overtaken the trisomic cell lines, therefore leading to the discrepancy between the non-culture-based array analysis and culture-based cytogenetics. Chen et al. [70] further provided a case in which the level of MT15 differed between uncultured (approximately 20%) and cultured (no mosaicism) amniocytes analysed with traditional cytogenetics and aCGH. This led to them hypothesising that abnormal cell lines may disappear after long-term culture, which is an interesting aspect, as long-term cultures of lymphocytes may also be interpreted in a similar manner.

It is also important to acknowledge the potential influence of the level of mosaicism on the observed outcomes and the likelihood of trisomic cells occurring in the analysed sample cells. There may have been no trisomic cells present in the 14 metaphases analysed in cytogenetics, even though mosaic cell lines are present. Statistically, with the level of mosaicism being 10% according to the SNP array, one metaphase of the 14 metaphases should present with MT15. A small deviation from this statistical probability can therefore easily distort the result. As the level of mosaicism is relatively low in our case, it can be deduced that a larger sample size may have been necessary for the detection of MT15 in cytogenetics in accordance with the SNP array results.

Furthermore, it should be noted that the follow-up cytogenetic study was performed more than a year after the SNP array analysis, suggesting that a shift in the proportion of trisomy 15 cells in the blood is conceivable.

As for molecular genetic analysis, the initially performed MLPA is not able to detect low-grade mosaicism [84], which is why the MT15 detected in the SNP array is not apparent in the MLPA results. However, individual probes even show a slightly elevated signal as seen in Figure 5. These probes are marked in column [REF] with "»". However, a deletion in the PWCR as the cause of PWS in the patient can be reliably ruled out with the performed MLPA.

The SNP array results and trio-genotype analysis of the index patient and the parents yielded a heterodisomic matUPD for chromosome 15 of the index patient, as well as the presence of a third haplotype.

The finding of the heterodisomic matUPD in trio-genotype analysis corresponds to the SNPs array, where heterozygotic SNPs were found across the entire chromosome 15, as seen in image b) in Figure 7 and Figure 8. The B-Allele frequency of 0.5% hereby demonstrates the presence of two different genotypes at the SNP loci. In the case of isodisomy, the whole chromosome 15 would present with homozygosity.

Since the present case presented with a complete heterodisomic matUPD across the entire chromosome 15, this is indicative of meiosis I non-disjunction without previous recombination. For visual clarification, this would correspond to the pathway described in part b) of meiotic errors leading to matUPD in Figure 3. The third haplotype is indicative of the presence of MT15 with a mosaic fraction of about 10%, according to the study by Conlin et al. [61].

This therefore provides further evidence for the theory of a trisomic rescue event occurring to eliminate the trisomic cell lines that arise due to the error in meiosis. It also provides further evidence that the SNP array is a useful method for detecting MT15 and may be performed more frequently in conjunction with MS MLPA in the diagnosis of PWS and AS. This may lead to the discovery of additional mosaic trisomic cell lines and increase our knowledge about possible phenotype-genotype correlations in affected individuals.

## 4.2 Comparison of the current case report to the literature

As only a few cases with the simultaneous presence of matUPD 15 and MT15 detected in tissue or blood have been described so far (see Table 2 and 3), it is of interest to classify the clinical and phenotypic findings in accordance with existing literature. This is further underscored by the fact that the present case, as the case presented by Izumi et al. [49], are a liveborn infant with a rather mild phenotype in contrast to many of the cases that have been described before with matUPD and MT15.

Neither the present patient nor patient 2 from Izumi et al. [49] exhibited the more severe phenotype that was previously associated with individuals with matUPD and additional MT15. Olander et al. [81] suggested a common phenotypic class for individuals with matUPD and mosaic trisomy 15, as, according to them, they express the most severe phenotype associated with a high incidence of congenital heart disease,

linking the more severe phenotype to the presence of trisomic cell lines. Even though the current case presents with congenital heart disease, it was not as severe as in other cases. Among the previously published cases, especially ventricular septal defect (VSD) and ASD stand out [76, 79, 81, 82]. Echocardiography of the index patient yielded an initial ASD of the secondary type, which was later described as a PFO at discharge from the hospital. To the current knowledge, the boy had no further complications or limitations concerning the PFO, which would not be congruent with a severe congenital heart disease.

Regarding congenital anomalies, the current case presented with hydronephrosis (grade 1-2) on both kidneys. Anomalies of the kidneys and renal system have been described in two of the cases in the literature before, one with horseshoe kidneys with vesico ureteral reflux [79] and one with vesico ureteral reflux grade II [82].

Alongside the index patient, undescended testes or cryptorchidism were present in two further case reports [81, 82]. Although this clinical finding may be more associated with PWS and less with the additional MT15, as cryptorchidism is described to be present in about 80-90% of males with hypogonadism in PWS [3].

Prenatally IUGR has been described before [49, 76, 80], but was not found in the present case, even though the boy was SGA at birth. Except for the ablation of a vanishing twin in the early pregnancy, no further abnormal prenatal findings were present in the current case. Previously, a breech presentation of the fetus has been described in two cases, but was not present in the current case [49, 81].

Phenotypically, a variety of facial features have been described before, with the most common ones being upslanted palpebral fissures, epicanthal folds, low-set posteriorly angulated or dysplastic rotated ears and a downturned mouth [49, 76, 79, 81, 82]. The only facial features described in the index patient were a fleeing chin and low-set ears right after birth, which is not directly in alignment with the previous reports. Even though phenotypical features of the face were discussed with the parents, no specific clinical evaluation of the facial features was performed. Therefore, no relevant allocation to a distinct type of facial features can be made for the index patient. Adding to this, many of the features described in previous cases may also fit facial features typically found in PWS and may therefore not be directly associated with MT15 and UPD 15 in PWS. For example, a downturned mouth and almond-shaped palpebral fissures have also been described as part of distinct facial features in the classic presentation of PWS [4], which was also observed in the phenotypical evaluation

of two probands in the retrospective study. On both numbers 1 and 2, almond-shaped eyes were noted at the phenotypical presentation. Neither showed any MT15 in genetic analysis, only matUPD.

As in most cases described in the literature, advanced maternal age is also involved in the current case, with the mother of the index patient being 38 years old at birth. Of the previous cases described in Table 3 and Table 2, maternal age at birth was between 32 and 43 years. This adds to the association between advanced maternal age and a higher likelihood of formation of chromosomal aneuploidies in women who are over 35 years at birth, also including non-disjunction errors in meiosis [86].

The relevance and difficulties of testing for matUPD 15 after the detection of MT15 in prenatal diagnostics has been discussed, especially if trisomic cell lines have been detected in amniocentesis [70, 72]. The present case demonstrates that infants with MT15 and matUPD found in peripheral blood may not present with a more severe phenotype and congenital anomalies as suggested before [81]. This may also influence the relevance of the finding of MT15 cells in conjunction with matUPD15 in prenatal diagnostics and further decision-making regarding a continuation of the pregnancy versus termination. At the same time, it cannot be conducted if the MT15 in the case report may have been present only in blood lymphocytes and not in other tissues, therefore not leading to a more severe phenotype in this case.

An interesting aspect in the case report is also that a vanishing twin was ablated during the second trimester. Trisomic rescue was discussed as a possible mechanism in monozygotic twins presenting with discordant phenotypes (trisomy 13 in one twin and mosaic-trisomy 13 in the other) [87, 88]. This phenomenon has also been described for trisomy 18 [89] and trisomy 21 [90]. To the best knowledge no case involving trisomy 15 has been described before. However, it is possible that a similar mechanism could have been present in the current case report. It can be hypothesised that the ablated twin in the second trimester may also have presented with a full trisomy 15, making it non-viable. It is evident that further information regarding the ablation and twin is required in order to draw any conclusion. But this could be an interesting aspect for further research in similar cases.

## 4.3 Retrospective study

As the mechanism behind the arising of matUPD 15 in PWS is thought to be commonly due to trisomic rescue, it is possible that more PWS patients with UPD 15 would show either CPM of MT15 or MT15 in tissue or blood. This is especially the case in heterodisomic UPD and mixed iso-/heterodisomy. In the case of complete isodisomy, a monosomic rescue mechanism would also be possible [61].

In the retrospective study, no MT15 was found in either of the individuals with PWS and AS. This outcome was not entirely unexpected, as only four probands were included in the study. No difference between results in cytogenetics and SNP array was found in three of the probands' samples, with one proband only being analysed with SNP array.

It is noteworthy that the result of the study can not exclude mosaicism in the analysed probands with certainty, as the level of mosaicism may have been too low for detection with SNP array. Additionally, a possible mosaicism may not be present in the lymphocytes at the time of probe sampling but could be present in other tissues or have been present at an earlier stage in life.

In order to make a valid statement about whether MT15is present and whether there is a difference between the cytogenetic results and the array results, various tissues from the affected individuals would have had to be examined. However, this is not feasible in practice. In addition, samples from CVS and amniocentesis would also be of great interest for further studies, allowing a comparison of prenatal and postnatal results concerning MT15.

## 4.4 Limitations

The limitations in the case report are primarily in the diagnostics and follow-up of the index patient. Cytogenetics was only performed at the age of 16 months, whereas the MS MLPA and SNP array was performed on blood obtained from the patient at 8 days of age. Therefore, a direct comparison between the results of the analysis, as mentioned earlier, should be taken with caution, as there is a time discrepancy between the probe materials. The level of mosaicism in blood may have changed during that timeframe, leading to no trisomic cells being detected in cytogenetics. In addition,

only a DNA taken from lymphocytes was analysed and no other tissues were studied. In many of the other cases described in Table 2, skin and/or placental tissue was also analysed. Especially, analysis of placental tissue would have been interesting to determine whether trisomic cell lines were present. This was not possible to conduct, since CVS was not performed during pregnancy. In conclusion, this means that MT15 could only be determined in blood in this study and not in other tissues.

The time of probe sampling is also a limitation in the retrospective study. The blood used for the SNP array was obtained from the blood samples of when the probands were first diagnosed with PWS or AS. This had been at different ages for each proband and was rarely conducted directly after birth, with proband no. 2 only being diagnosed with PWS at the age of 12 years.
Possible mosaicism in blood lymphocytes may have been reduced over time due to the selective disadvantage of trisomic cells, making it undetectable by SNP array analysis. This also adds to an overall detection limit of the used diagnostic tools, with the SNP array having a detection limit of mosaicism of around 5% as demonstrated by Conlin et al.[61]. Mosaicism levels lower than 5% may not have been detected in the retrospective study. Ideally, the SNP array as well as cytogenetics should have been performed on samples taken directly after birth, which was not possible in the retrospective study.

Additionally, only four probands were able to be recruited for the study, as PWS and AS are relatively rare syndromes, and only probands with abnormal methylation results from the institute were contacted. Therefore, the results of the retrospective study should be interpreted with caution, as the study did not contain a sufficient number of probands to draw a significant conclusion from it.

The case reports described in this thesis in Table 2 were found via a scoped literature review. No systematic search was conducted. Therefore, no guarantee of completeness can be given.

## 4.5 Conclusion and Outlook

Even though no additional cases with MT15 in UPD have been found in the retrospective study performed in this thesis, it is still expected that more individuals do, in fact, have mosaicism for trisomic cell lines in accordance with the trisomy rescue theory. An

additional future use of SNP array in the diagnosis of PWS and AS may yield a higher detection rate of MT15 in individuals with UPD and lead to more detected cases.

At the present time, phenotypic features and congenital anomalies in published case reports contradict each other, so that no clear statement can be drawn for the simultaneous presence of MT15 and matUPD in PWS. More case reports and studies are needed to possibly delineate a clear phenotype of individuals with PWS and MT15.

The present case report of the index patient demonstrates that phenotypic changes do not need to be more severe because of the trisomic cell lines.

# Bibliography


[1] Prader A., Labhart A., and Willi H. Ein syndrom von adipositas, kleinwuchs, kryptorchismus und oligophrenie nach myatonieartigem zustand im neugeborenenalter. *Schweizerische Medizinische Wochenschrift*, (86):1260–1261, 1956.

[2] Lawson H. A., Cheverud J. M., and Wolf J. B. Genomic imprinting and parent-of-origin effects on complex traits. *Nat Rev Genet.*, 14(9):609–617, 9 2013. doi: 10.1038/nrg3543.

[3] Driscoll D. J., Miller J. L., Cassidy S. . B., Adam M. P., Feldman J., and Mirzaa G. M. Prader-Willi Syndrome. In Adam M., Bick S., Mirzaa G., and et al. , editors, *GeneReviews®*. University of Washington, Seattle, Seattle (WA), 1998. URL https://www.ncbi.nlm.nih.gov/books/NBK1330/.

[4] Cassidy S. B., Schwartz S., Miller J. L., and Driscoll D. J. Prader-Willi syndrome. *Genetics in Medicine*, 14(1):10–26, 2012. ISSN 15300366. doi: 10.1038/gim.0b013e31822bead0.

[5] Miller J. L., Lynn C. H., Driscoll D. C., Goldstone A. P., Gold J.-A., Kimonis V., Dykens E., Butler M. G., Shuster J. J., and Driscoll D. J. Nutritional phases in Prader-Willi syndrome. *American journal of medical genetics. Part A*, 155A(5): 1040–9, 5 2011. ISSN 1552-4833. doi: 10.1002/ajmg.a.33951.

[6] Muscogiuri G., Barrea L., Faggiano F., Maiorino M. I., Parrillo M., Pugliese G., Ruggeri R. M., Scarano E., Savastano S., Colao A., and RESTARE . Obesity in Prader-Willi syndrome: physiopathological mechanisms, nutritional and pharmacological approaches. *Journal of endocrinological investigation*, 44(10):2057–2070, 10 2021. ISSN 1720-8386. doi: 10.1007/s40618-021-01574-9.

[7] Tuysuz B., Kartal N., Erener-Ercan T., Guclu-Geyik F., Vural M., Perk Y., Erçal D., and Erginel-Unaltuna N. Prevalence of Prader–Willi Syndrome among Infants

with Hypotonia. *The Journal of Pediatrics*, 164(5):1064–1067, 5 2014. ISSN 00223476. doi: 10.1016/j.jpeds.2014.01.039.

[8] Tauber M. and Hoybye C. Endocrine disorders in Prader-Willi syndrome: a model to understand and treat hypothalamic dysfunction. *The Lancet Diabetes & Endocrinology*, 9(4):235–246, 4 2021. ISSN 22138587. doi: 10.1016/S2213-8587(21)00002-4.

[9] Cassidy S. B. Prader-Willi syndrome. *Journal of Medical Genetics*, 34(11):917–923, 11 1997. ISSN 1468-6244. doi: 10.1136/jmg.34.11.917.

[10] Jin Y.-Y. and Luo F.-H. Early psychomotor development and growth hormone therapy in children with Prader-Willi syndrome: a review. *European Journal of Pediatrics*, 183(3):1021–1036, 11 2023. ISSN 1432-1076. doi: 10.1007/s00431-023-05327-z.

[11] Whittington J. and Holland A. Cognition in people with Prader-Willi syndrome: Insights into genetic influences on cognitive and social development. *Neuroscience & Biobehavioral Reviews*, 72:153–167, 1 2017. ISSN 01497634. doi: 10.1016/j.neubiorev.2016.09.013.

[12] Schwartz L., Caixàs A., Dimitropoulos A., Dykens E., Duis J., Einfeld S., Gallagher L., Holland A., Rice L., Roof E., Salehi P., Strong T., Taylor B., and Woodcock K. Behavioral features in Prader-Willi syndrome (PWS): consensus paper from the International PWS Clinical Trial Consortium. *Journal of Neurodevelopmental Disorders*, 13(1):25, 12 2021. ISSN 1866-1947. doi: 10.1186/s11689-021-09373-2.

[13] Shelkowitz E., Gantz M. G., Ridenour T. A., Scheimann A. O., Strong T., Bohonowych J., and Duis J. Neuropsychiatric features of Prader–Willi syndrome. *American Journal of Medical Genetics Part A*, 188(5):1457–1463, 5 2022. ISSN 1552-4825. doi: 10.1002/ajmg.a.62662.

[14] Shriki-Tal L., Avrahamy H., Pollak Y., Gross-Tsur V., Genstil L., Hirsch H., and Benarroch F. Psychiatric disorders in a cohort of individuals with Prader–Willi syndrome. *European Psychiatry*, 44:47–52, 7 2017. ISSN 0924-9338. doi: 10.1016/j.eurpsy.2017.03.007.

[15] Mao S., Yang L., Gao Y., and Zou C. Genotype–phenotype correlation in Prader-Willi syndrome: A large-sample analysis in China. *Clinical Genetics*, 105(4): 415–422, 4 2024. ISSN 0009-9163. doi: 10.1111/cge.14477.

[16] Pascual-Morena C., Martínez-Vizcaíno V., Cavero-Redondo I., Álvarez-Bueno C., Martínez-García I., Rodríguez-Gutiérrez E., Otero-Luis I., Saz-Lara del A., and Saz-Lara A. Prevalence and genotypic associations of epilepsy in Prader-Willi Syndrome: A systematic review and meta-analysis. *Epilepsy & Behavior*, 155: 109803, 6 2024. ISSN 15255050. doi: 10.1016/j.yebeh.2024.109803.

[17] Dimitropoulos A., Ferranti A., and Lemler M. Expressive and receptive language in Prader–Willi syndrome: Report on genetic subtype differences. *Journal of Communication Disorders*, 46(2):193–201, 3 2013. ISSN 00219924. doi: 10.1016/j.jcomdis.2012.12.001.

[18] Eggermann T., Nanclares Perez de G., Maher E. R., Temple I. K., Tümer Z., Monk D., Mackay D. J. G., Grønskov K., Riccio A., Linglart A., and Netchine I. Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clinical Epigenetics*, 7(1):123, 12 2015. ISSN 1868-7075. doi: 10.1186/s13148-015-0143-8.

[19] Walter J. Epigenetische Kontrolle biologischer Prozesse. In Nordheim A. and Knippers R., editors, *Molekulare Genetik*, chapter Genomische Prägung, pages 467–471. Georg Thieme Verlag, Stuttgart, 11 edition, 2018. ISBN 9783132426375. doi: 10.1055/b-006-149922.

[20] Tucci V., Isles A. R., Kelsey G., Ferguson-Smith A. C., Tucci V., Bartolomei M. S., Benvenisty N., Bourc'his D., Charalambous M., Dulac C., Feil R., Glaser J., Huelsmann L., John R. M., McNamara G. I., Moorwood K., Muscatelli F., Sasaki H., Strassmann B. I., Vincenz C., Wilkins J., Isles A. R., Kelsey G., and Ferguson-Smith A. C. Genomic Imprinting and Physiological Processes in Mammals. *Cell*, 176(5):952–965, 2 2019. ISSN 00928674. doi: 10.1016/j.cell.2019.01.043.

[21] Elhamamsy A. R. Role of DNA methylation in imprinting disorders: an updated review. *Journal of Assisted Reproduction and Genetics*, 34(5):549–562, 5 2017. ISSN 1058-0468. doi: 10.1007/s10815-017-0895-5.

[22] Bieth E., Eddiry S., Gaston V., Lorenzini F., Buffet A., Conte Auriol F., Molinas C., Cailley D., Rooryck C., Arveiler B., Cavaillé J., Salles J. P., and Tauber M.

Highly restricted deletion of the SNORD116 region is implicated in Prader–Willi Syndrome. *European Journal of Human Genetics*, 23(2):252–255, 2 2015. ISSN 1018-4813. doi: 10.1038/ejhg.2014.103.

[23] Butler M. G., Hartin S. N., Hossain W. A., Manzardo A. M., Kimonis V., Dykens E., Gold J. A., Kim S.-J., Weisensel N., Tamura R., Miller J. L., and Driscoll D. J. Molecular genetic classification in Prader-Willi syndrome: a multisite cohort study. *Journal of Medical Genetics*, 56(3):149–153, 3 2019. ISSN 0022-2593. doi: 10.1136/jmedgenet-2018-105301.

[24] Amos-Landgraf J. M., Ji Y., Gottlieb W., Depinet T., Wandstrat A. E., Cassidy S. B., Driscoll D. J., Rogan P. K., Schwartz S., and Nicholls R. D. Chromosome Breakage in the Prader-Willi and Angelman Syndromes Involves Recombination between Large, Transcribed Repeats at Proximal and Distal Breakpoints. *The American Journal of Human Genetics*, 65(2):370–386, 8 1999. ISSN 00029297. doi: 10.1086/302510.

[25] Gu W., Zhang F., and Lupski J. R. Mechanisms for human genomic rearrangements. *PathoGenetics*, 1(1):4, 12 2008. ISSN 1755-8417. doi: 10.1186/1755-8417-1-4.

[26] Butler M. G., Bittel D. C., Kibiryeva N., Talebizadeh Z., and Thompson T. Behavioral Differences Among Subjects With Prader-Willi Syndrome and Type I or Type II Deletion and Maternal Disomy. *Pediatrics*, 113(3):565–573, 3 2004. ISSN 0031-4005. doi: 10.1542/peds.113.3.565.

[27] Engel E. A new genetic concept: Uniparental disomy and its potential effect, isodisomy. *American Journal of Medical Genetics*, 6(2):137–143, 1 1980. ISSN 0148-7299. doi: 10.1002/ajmg.1320060207.

[28] Nicholls R. D., Knoll J. H. M., Butler M. G., Karam S., and Lalande M. Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. *Nature*, 342(6247):281–285, 11 1989. ISSN 0028-0836. doi: 10.1038/342281a0.

[29] Benn P. Uniparental disomy: Origin, frequency, and clinical significance. *Prenatal Diagnosis*, 41(5):564–572, 4 2021. ISSN 0197-3851. doi: 10.1002/pd.5837.

[30] Robinson W. P. Mechanisms leading to uniparental disomy and their clinical consequences. *BioEssays*, 22(5):452–459, 4 2000. ISSN 02659247. doi: 10.1002/(SICI)1521-1878(200005)22:5<452::AID-BIES7>3.0.CO;2-K.

[31] Yamazawa K., Ogata T., and Ferguson-Smith A. C. Uniparental disomy and human disease: An overview. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, 154C(3):329–334, 8 2010. ISSN 1552-4868. doi: 10.1002/ajmg.c.30270.

[32] Hassan S. and Tatton-Brown K. Prader-Willi syndrome, 2023. URL https://www.genomicseducation.hee.nhs.uk/genotes/knowledge-hub/prader-willi-syndrome/.

[33] Ohta T., Gray T., Rogan P., Buiting K., Gabriel J., Saitoh S., Muralidhar B., Bilienska B., Krajewska-Walasek M., Driscoll D., Horsthemke B., Butler M., and Nicholls R. Imprinting-Mutation Mechanisms in Prader-Willi Syndrome. *The American Journal of Human Genetics*, 64(2):397–413, 2 1999. ISSN 00029297. doi: 10.1086/302233.

[34] Buiting K., Färber C., Kroisel P., Wagner K., Brueton L., Robertson M., Lich C., and Horsthemke B. Imprinting centre deletions in two PWS families: implications for diagnostic testing and genetic counselling. *Clinical Genetics*, 58(4):284–290, 10 2000. ISSN 0009-9163. doi: 10.1034/j.1399-0004.2000.580406.x.

[35] Holm V. A., Cassidy S. B., Butler M. G., Hanchett J. M., Greenswag L. R., Whitman B. Y., and Greenberg F. Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics*, 91(2):398–402, 2 1993. ISSN 0031-4005.

[36] Gunay-Aygun M., Schwartz S., Heeger S., O'Riordan M. A., and Cassidy S. B. The Changing Purpose of Prader-Willi Syndrome Clinical Diagnostic Criteria and Proposed Revised Criteria. *Pediatrics*, 108(5):e92–e92, 11 2001. ISSN 1098-4275. doi: 10.1542/peds.108.5.e92.

[37] Beygo J., Buiting K., Ramsden S. C., Ellis R., Clayton-Smith J., and Kanber D. Update of the EMQN/ACGS best practice guidelines for molecular analysis of Prader-Willi and Angelman syndromes. *European Journal of Human Genetics*, 27(9):1326–1340, 9 2019. ISSN 1018-4813. doi: 10.1038/s41431-019-0435-0.

[38] Schouten J. P. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Research*, 30(12):57e–57, 6 2002. ISSN 13624962. doi: 10.1093/nar/gnf056.

[39] Moelans C. B., Atanesyan L., Savola S. P., and Diest van P. J. Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA). pages 537–549. 2018. doi: 10.1007/978-1-4939-7481-8{_}27.

[40] Robinson W. P., Barrett I. J., Bernard L., Telenius A., Bernasconi F., Wilson R. D., Best R. G., Howard-Peebles P. N., Langlois S., and Kalousek D. K. Meiotic origin of trisomy in confined placental mosaicism is correlated with presence of fetal uniparental disomy, high levels of trisomy in trophoblast, and increased risk of fetal intrauterine growth restriction. *American journal of human genetics*, 60 (4):917–27, 4 1997. ISSN 0002-9297.

[41] Cassidy S. B., Lai L. W., Erickson R. P., Magnuson L., Thomas E., Gendron R., and Herrmann J. Trisomy 15 with loss of the paternal 15 as a cause of Prader-Willi syndrome due to maternal disomy. *American journal of human genetics*, 51(4):701–8, 10 1992. ISSN 0002-9297. URL http://www.ncbi.nlm.nih.gov/pubmed/1357962http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1682792.

[42] Purvis-Smith SG , Saville T , Manass S , Yip MY , Lam-Po-Tang PR , Duffy B , Johnston H , Leigh D , and McDonald B . Uniparental disomy 15 resulting from correction of an initial trisomy 15. *Am J Hum Genet.*, 50(6):1348–1350, 5 1992.

[43] Taylor T. H., Gitlin S. A., Patrick J. L., Crain J. L., Wilson J. M., and Griffin D. K. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. *Human Reproduction Update*, 20(4):571–581, 7 2014. ISSN 1355-4786. doi: 10.1093/humupd/dmu016.

[44] Hassold T. and Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nature Reviews Genetics*, 2(4):280–291, 4 2001. ISSN 1471-0056. doi: 10.1038/35066065.

[45] Schaaf C. P. and Zschocke J. *Basiswissen Humangenetik*. Springer-Lehrbuch. Springer Berlin Heidelberg, Berlin, Heidelberg, 2013. ISBN 978-3-642-28906-4. doi: 10.1007/978-3-642-28907-1. URL http://link.springer.com/10.1007/978-3-642-28907-1.

[46] Mascari M. J., Gottlieb W., Rogan P. K., Butler M. G., Waller D. A., Armour J. A., Jeffreys A. J., Ladda R. L., and Nicholls R. D. The Frequency of Uniparental

Disomy in Prader-Willi Syndrome. *New England Journal of Medicine*, 326(24): 1599–1607, 6 1992. ISSN 0028-4793. doi: 10.1056/NEJM199206113262404.

[47] Kovaleva N. V. and Cotter P. D. Mosaicism for Autosomal Trisomies: A Comprehensive Analysis of 1266 Published Cases Focusing on Maternal Age and Reproductive History. *Genes*, 15(6):778, 6 2024. ISSN 2073-4425. doi: 10.3390/genes15060778.

[48] Fridman C. and Koiffmann C. P. Origin of uniparental disomy 15 in patients with Prader-Willi or Angelman syndrome. *American Journal of Medical Genetics*, 94(3):249–253, 9 2000. ISSN 0148-7299. doi: 10.1002/1096-8628(20000918)94:3<249::AID-AJMG12>3.0.CO;2-X. URL https://onlinelibrary.wiley.com/doi/10.1002/1096-8628(20000918)94:3<249::AID-AJMG12>3.0.CO;2-X.

[49] Izumi K., Santani A. B., Deardorff M. A., Feret H. A., Tischler T., Thiel B. D., Mulchandani S., Stolle C. A., Spinner N. B., Zackai E. H., and Conlin L. K. Mosaic maternal uniparental disomy of chromosome 15 in Prader–Willi syndrome: Utility of genome-wide SNP array. *American Journal of Medical Genetics Part A*, 161(1):166–171, 1 2013. ISSN 1552-4825. doi: 10.1002/ajmg.a.35625. URL https://onlinelibrary.wiley.com/doi/10.1002/ajmg.a.35625.

[50] Morandi A., Bonnefond A., Lobbens S., Carotenuto M., Giudice del E. M., Froguel P., and Maffeis C. A girl with incomplete Prader–Willi syndrome and negative MS-PCR, found to have mosaic maternal UPD-15 at SNP array. *American Journal of Medical Genetics Part A*, 167(11):2720–2726, 11 2015. ISSN 1552-4825. doi: 10.1002/ajmg.a.37222.

[51] Kalousek D. K. and Dill F. J. Chromosomal Mosaicism Confined to the Placenta in Human Conceptions. *Science*, 221(4611):665–667, 8 1983. ISSN 0036-8075. doi: 10.1126/science.6867735.

[52] Eggenhuizen G. M., Go A., Koster M. P. H., Baart E. B., and Galjaard R. J. Confined placental mosaicism and the association with pregnancy outcome and fetal growth: a review of the literature. *Human Reproduction Update*, 27(5):885–903, 8 2021. ISSN 1355-4786. doi: 10.1093/humupd/dmab009.

[53] Echten-Arends van J., Mastenbroek S., Sikkema-Raddatz B., Korevaar J. C., Heineman M. J., Veen van der F., and Repping S. Chromosomal mosaicism in hu-

man preimplantation embryos: a systematic review. *Human Reproduction Update*, 17(5):620–627, 9 2011. ISSN 1460-2369. doi: 10.1093/humupd/dmr014.

[54] Schaaf C. P. and Zschocke J. Pränataldiagnostik. In *Basiswissen Humangenetik*, pages 162–168. Springer-Verlag Berlin Heidelberg 2013, 2. auflage edition, 2013. doi: 10.1007/978-3-642-28907-1{_}17.

[55] Jelin A. C., Sagaser K. G., and Wilkins-Haug L. Prenatal Genetic Testing Options. *Pediatric Clinics of North America*, 66(2):281–293, 4 2019. ISSN 00313955. doi: 10.1016/j.pcl.2018.12.016.

[56] Levy B., Hoffmann E. R., McCoy R. C., and Grati F. R. Chromosomal mosaicism: Origins and clinical implications in preimplantation and prenatal diagnosis. *Prenatal Diagnosis*, 41(5):631–641, 4 2021. ISSN 0197-3851. doi: 10.1002/pd.5931.

[57] Schaaf C. P. and Zschocke J. Genetische Labordiagnostik. In *Basiswissen Humangenetik*, pages 141–155. Springer-Verlag Berlin Heidelberg 2013, 2. auflage edition, 2013. doi: 10.1007/978-3-642-28907-1{_}15.

[58] Wallace S. E. and Bean L. J. Educational Materials — Genetic Testing: Current Approaches. In Adam M., Feldman J., and Mirzaa GM , editors, *GeneReviews® [Internet]*. University of Washington, Seattle, Seattle (WA), 2017. URL https://www.ncbi.nlm.nih.gov/books/NBK279899/.

[59] Shao L., Akkari Y., Cooley L. D., Miller D. T., Seifert B. A., Wolff D. J., and Mikhail F. M. Chromosomal microarray analysis, including constitutional and neoplastic disease applications, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, 23(10):1818–1829, 10 2021. ISSN 10983600. doi: 10.1038/s41436-021-01214-w.

[60] Schaaf C. P. and Zschocke J. Pathomechanismen genetischer Krankheiten. In *Basiswissen Humangenetik*, pages 51–71. 2013. doi: 10.1007/978-3-642-28907-1{_}4.

[61] Conlin L. K., Thiel B. D., Bonnemann C. G., Medne L., Ernst L. M., Zackai E. H., Deardorff M. A., Krantz I. D., Hakonarson H., and Spinner N. B. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Human Molecular Genetics*, 19(7):1263–1275, 4 2010. ISSN 0964-6906. doi: 10.1093/hmg/ddq003.

[62] Schaaf C. P. and Zschocke J. Mutationen und genetische Variabilität. In *Basiswissen Humangenetik*, pages 31–50. Springer-Verlag Berlin Heidelberg 2013, 2. auflage edition, 2013. doi: 10.1007/978-3-642-28907-1{_}17.

[63] Grati F. Chromosomal Mosaicism in Human Feto-Placental Development: Implications for Prenatal Diagnosis. *Journal of Clinical Medicine*, 3(3):809–837, 7 2014. ISSN 2077-0383. doi: 10.3390/jcm3030809.

[64] Matuszewska K. E., Bukowska-Olech E., Piechota M., Staniek-Łacna K., Drews K., Więckowska B., Koczyk G., Popiel D., Dawidziuk A., Kochalska N., Milanowska K., Białek-Prościńska A., Skrzypczak J., Hirschfeld A. S., Wnuk-Kłosińska A., Wiśniewska M., Jamsheer A., and Latos-Bieleńska A. From chromosomal aberrations to mutations in individual genes – the significance of genetic studies of chorions after miscarriage in the search for causes of miscarriages. *The Journal of Maternal-Fetal & Neonatal Medicine*, 37(1), 1 2024. ISSN 1476-7058. doi: 10.1080/14767058.2024.2364249.

[65] Kuller J. and Laifer S. Trisomy 15 Associated with Nonimmune Hydrops. *American Journal of Perinatology*, 8(01):39–40, 1 1991. ISSN 0735-1631. doi: 10.1055/s-2007-999337.

[66] Coldwell S., Fitzgerald B., Semmens J. M., Ede R., and Bateman C. A case of trisomy of chromosome 15. *Journal of Medical Genetics*, 18(2):146–148, 4 1981. ISSN 1468-6244. doi: 10.1136/jmg.18.2.146.

[67] Isikay S. and Carman K. B. An infant with trisomy 15 mosaicism. *Clinical Dysmorphology*, 22(4):172–174, 10 2013. ISSN 0962-8827. doi: 10.1097/MCD.0000000000000012.

[68] McPadden J., Helm B. M., Spangler B. B., Ross L. P., Boles D. B., and Schrier Vergano S. A. Mosaic trisomy 15 in a liveborn infant. *American Journal of Medical Genetics Part A*, 167(4):821–825, 4 2015. ISSN 1552-4825. doi: 10.1002/ajmg.a.36958.

[69] Prontera P., Buldrini B., Aiello V., Gruppioni R., Bonfatti A., Venti G., Ferlini A., Sensi A., Calzolari E., and Donti E. Trisomy 15 mosaicism owing to familial reciprocal translocation t(1;15): implication for prenatal diagnosis. *Prenatal Diagnosis*, 26(6):571–576, 6 2006. ISSN 0197-3851. doi: 10.1002/pd.1463.

[70] Chen C. P., Chern S. R., Chen Y. N., Wu P. S., Yang C. W., Chen L. F., and Wang W. Mosaic trisomy 15 at amniocentesis: Prenatal diagnosis, molecular genetic analysis and literature review. *Taiwanese Journal of Obstetrics and Gynecology*, 54(4):426–431, 8 2015. ISSN 18756263. doi: 10.1016/j.tjog.2015.06.002.

[71] Zhang M., Liang Y., Li H., and Xu F. Use of the MS-MLPA assay in prenatal diagnosis of Prader–Willi syndrome with mosaic trisomy 15. *Taiwanese Journal of Obstetrics and Gynecology*, 63(1):81–84, 1 2024. ISSN 10284559. doi: 10.1016/j.tjog.2023.09.022.

[72] Silva M., Alves C., Pedro S., Marques B., Ferreira C., Furtado J., Martins A. T., Fernandes R., Correia J., and Correia H. Trisomy 15 mosaicism: Challenges in prenatal diagnosis. *American Journal of Medical Genetics Part A*, 167(11):2847–2850, 11 2015. ISSN 1552-4825. doi: 10.1002/ajmg.a.37229.

[73] Surh L. C., Wang H., and Hunter A. G. Deletion and Uniparental Disomy Involving the Same Maternal Chromosome 15. *New England Journal of Medicine*, 330 (8):572–573, 2 1994. ISSN 0028-4793. doi: 10.1056/NEJM199402243300813.

[74] Roberts E., Stevenson K., Cole T., Redford D. H. A., and Davison E. V. Prospective Prenatal Diagnosis of Prader–Willi Syndrome due to Maternal Disomy for Chromosome 15 following Trisomic Zygote Rescue. *Prenatal Diagnosis*, 17(8): 780–783, 8 1997. ISSN 01973851. doi: 10.1002/(SICI)1097-0223(199708)17:8<780::AID-PD133>3.0.CO;2-Z. URL https://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-0223(199708)17:8<780::AID-PD133>3.0.CO;2-Z.

[75] Walczak C., Enders H., Grissinger K., and Dufke A. Retrospective diagnosis of trisomy 15 in formalin-fixed, paraffin-embedded placental tissue in a newborn girl with Prader-Willi syndrome. *Prenatal Diagnosis*, 20(11):914–916, 2000. ISSN 01973851. doi: 10.1002/1097-0223(200011)20:11<914::AID-PD939>3.0.CO;2-6.

[76] Milunsky J. M., Wyandt H. E., Huang X. L., Kang X. Z., Elias E. R., and Milunsky A. Trisomy 15 mosaicism and uniparental disomy (UPD) in a liveborn infant. *American Journal of Medical Genetics*, 61(3):269–273, 1 1996. ISSN 01487299. doi: 10.1002/(SICI)1096-8628(19960122)61:3<269::AID-AJMG12>3.0.CO;2-R.

[77] Slater H. R., Vaux C., Pertile M., Burgess T., and Petrovic V. Prenatal diagnosis of Prader-Willi syndrome using PW71 methylation analysis–uniparental disomy and the significance of residual trisomy 15. *Prenatal diagnosis*, 17(2):109–13, 2 1997.

ISSN 0197-3851. doi: 10.1002/(SICI)1097-0223(199702)17:2<109::AID-PD36>3.0.CO;2-2. URL http://www.ncbi.nlm.nih.gov/pubmed/9061757.

[78] Christian S. L., Smith A. C., Macha M., Black S. H., Elder F. F., Johnson J. M., Resta R. G., Surti U., Suslak L., Verp M. S., and Ledbetter D. H. Prenatal diagnosis of uniparental disomy 15 following trisomy 15 mosaicism. *Prenatal diagnosis*, 16(4):323–32, 4 1996. ISSN 0197-3851. doi: 10.1002/(SICI)1097-0223(199604)16:4<323::AID-PD856>3.0.CO;2-5. URL http://www.ncbi.nlm.nih.gov/pubmed/8734806.

[79] Devriendt K., Matthijs G., Claes S., Legius E., Proesmans W., Cassiman J. J., and Fryns J. P. Prader-Willi syndrome in a child with mosaic trisomy 15 and mosaic triplo-X: a molecular analysis. *Journal of Medical Genetics*, 34(4):318–322, 4 1997. ISSN 1468-6244. doi: 10.1136/jmg.34.4.318. URL https://jmg.bmj.com/lookup/doi/10.1136/jmg.34.4.318.

[80] Hsu L. Y., Yu M. T., Neu R. L., Van Dyke D. L., Benn P. A., Bradshaw C. L., Shaffer L. G., Higgins R. R., Khodr G. S., Morton C. C., Wang H., Brothman A. R., Chadwick D., Disteche C. M., Jenkins L. S., Kalousek D. K., Pantzar T. J., and Wyatt P. Rare trisomy mosaicism diagnosed in amniocytes, involving an autosome other than chromosomes 13, 18, 20, and 21: Karyotype/phenotype correlations. *Prenatal Diagnosis*, 17(3):201–242, 3 1997. ISSN 01973851. doi: 10.1002/(SICI)1097-0223(199703)17:3<201::AID-PD56>3.0.CO;2-H.

[81] Olander E., Stamberg J., Steinberg L., and Wulfsberg E. A. Third Prader-Willi syndrome phenotype due to maternal uniparental disomy 15 with mosaic trisomy 15. *American Journal of Medical Genetics*, 93(3):215–218, 7 2000. ISSN 0148-7299. doi: 10.1002/1096-8628(20000731)93:3<215::AID-AJMG11>3.0.CO;2-K. URL https://onlinelibrary.wiley.com/doi/10.1002/1096-8628(20000731)93:3<215::AID-AJMG11>3.0.CO;2-K.

[82] Aparicio M. V., Seidel V., Orera Clemente M. A., Caballero S. M., and Luna M. S. Mosaic trisomy 15 and prenatal genetic counselling: a case of Prader-Willi syndrome due to maternal uniparental disomy. *Case Reports in Perinatal Medicine*, 8(1), 3 2019. ISSN 2192-8959. doi: 10.1515/crpm-2018-0028. URL https://www.degruyter.com/document/doi/10.1515/crpm-2018-0028/html.

[83] Rocklin M., Elder F., Ledbetter D., Christian S., Huang B., Rosenberg H., Sheppard C., and Wilkins I. True fetal trisomy 15 mosaicism with maternal uniparental disomy. *Am.J.Hum.Genet.*, 55(Suppl.):1677–1677, 1994.

[84] Veghel-Plandsoen van M. M., Wouters C. H., Kromosoeto J. N. R., Ridder-Klünnen den M. C., Halley D. J. J., and Ouweland van den A. M. W. Multiplex ligation-depending probe amplification is not suitable for detection of low-grade mosaicism. *European Journal of Human Genetics*, 19(9):1009–1012, 9 2011. ISSN 1018-4813. doi: 10.1038/ejhg.2011.60.

[85] Zhang Y., Lei Q., Liu J., Lin M., Luo L., Li T., Wang Q., and Zhou C. Selective growth of mosaic cells in chromosomal analysis of chorionic villi by conventional karyotyping. *Molecular and Cellular Probes*, 51:101532, 6 2020. ISSN 08908508. doi: 10.1016/j.mcp.2020.101532.

[86] Verdyck P., Altarescu G., Santos-Ribeiro S., Vrettou C., Koehler U., Griesinger G., Goossens V., Magli C., Albanese C., Parriego M., Coll L., Ron-El R., Sermon K., and Traeger-Synodinos J. Aneuploidy in oocytes from women of advanced maternal age: analysis of the causal meiotic errors and impact on embryo development. *Human Reproduction*, 38(12):2526–2535, 12 2023. ISSN 0268-1161. doi: 10.1093/humrep/dead201.

[87] McFadden P., Smithson S., Massaro R., Huang J., Prado G. T., and Shertz W. Monozygotic Twins Discordant for Trisomy 13. *Pediatric and Developmental Pathology*, 20(4):340–347, 7 2017. ISSN 1093-5266. doi: 10.1177/1093526616686471.

[88] Ramsey K. W., Slavin T. P., Graham G., Hirata G. I., Balaraman V., and Seaver L. H. Monozygotic twins discordant for trisomy 13. *Journal of Perinatology*, 32 (4):306–308, 4 2012. ISSN 0743-8346. doi: 10.1038/jp.2011.123.

[89] Reuss A., Gerlach H., Bedow W., Landt S., Kuhn U., Stein A., Reschke M., Albrecht B., Westrich V., Trawicki W., and Eiben B. Monozygotic twins discordant for trisomy 18. *Ultrasound in Obstetrics & Gynecology*, 38(6):727–728, 12 2011. ISSN 0960-7692. doi: 10.1002/uog.8978.

[90] Dahoun S., Gagos S., Gagnebin M., Gehrig C., Burgi C., Simon F., Vieux C., Extermann P., Lyle R., Morris M. A., Antonarakis S. E., Béna F., and Blouin J. Monozygotic twins discordant for trisomy 21 and maternal 21q inheritance: A

complex series of events. *American Journal of Medical Genetics Part A*, 146A (16):2086–2093, 8 2008. ISSN 1552-4825. doi: 10.1002/ajmg.a.32431.