Disturbed genomic imprinting and its relevance for human reproduction:

Causes and clinical consequences

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Abstract

Background: Human reproductive failure affecting fetal and maternal health is caused by numerous exogenous and endogenous factors, of which the latter undoubtedly include genetic changes. Pathogenic variants in either maternal or offspring DNA are associated with effects on the offspring including clinical disorders and nonviable outcomes. Conversely, both fetal and maternal factors can affect maternal health during pregnancy. Recently it has become evident that mammalian reproduction is influenced by genomic imprinting, an epigenetic phenomenon that regulates the expression of genes according to their parent from which they are inherited. About 1% of human genes are normally expressed from only the maternally or the paternally inherited gene copy. Since numerous imprinted genes are involved in (embryonic) growth and development, disturbance of their balanced expression can adversely affect these processes.

Objective and Rationale: This review summarizes current understanding of genomic imprinting in relation to human ontogenesis and pregnancy, and its relevance for reproductive medicine.

Outcome: A range of molecular changes to specific groups of imprinted genes are associated with imprinting disorders, syndromes with recognisable clinical features, which can include distinctive prenatal features. Whereas the majority of affected individuals exhibit alterations at single imprinted loci, some have multi-locus imprinting disturbance (MLID) with less predictable clinical features; and imprinting disturbance is also seen in some nonviable pregnancy outcomes, such as (recurrent) hydatidiform moles, which can therefore be regarded as a severe form of imprinting disorders. There is growing evidence that MLID can be caused by variants in the maternal genome altering the imprinting status of the oocyte and the embryo (maternal effect mutations). Pregnancies of women carrying maternal affect mutations can have different courses, ranging from miscarriages to birth of children with clinical features of various imprinting disorders.
Wider Implication: Increasing understanding of imprinting disturbances and their clinical consequences have significant impacts on diagnostics, counselling and management in context of human reproduction. Defining criteria for identifying pregnancies complicated by imprinting disorders facilitates early diagnosis and personalized management of both mother and offspring. Identifying the molecular lesions underlying imprinting disturbances (e.g. maternal effect mutations) allows targeted counselling of the family and focused medical care in further pregnancies.
Introduction

Genomic Imprinting

Human reproductive failure is caused by numerous factors, but it is out of question that molecular alterations play a major role in its etiology, affecting both fetal and maternal health. Pathogenic variants in the fetal genome cause a broad range of clinical features affecting embryonic and extraembryonic tissue, and result in fetal loss or congenital disorders in offspring. Maternal genetic determinants can also influence fetal health with even long-term consequences for the offspring, while conversely, both fetal and maternal factors can influence maternal health during pregnancy.

Until recently, these factors have been attributed to alterations of the DNA sequence itself (DNA sequence variants affecting single genes) or to chromosomal variants (numerical or structural chromosomal aberrations affecting multiple genes). However, in recent years it has become evident that mammalian reproduction is significantly influenced by genomic imprinting, an epigenetic phenomenon which does not alter the DNA itself but regulates the expression of specific genes. Genomic imprinting regulates the expression of 1-2% of human protein-coding genes in a parent-of-origin specific manner (for review: Patten et al., 2016). It consists of epigenetic marks which allow the cell to discriminate between the parental origin of alleles, resulting in the monoallelic expression either of the maternally or the paternally inherited gene copy (figure 1). Major epigenetic marks include methylation of cytosine in CpG islands localised in regulatory genomic regions (DMRs), interactions of non-coding RNAs, and histone modifications. These marks mediate and modify the accessibility of imprinted genes and their promoters for transcription factors.
Genomic imprinting is an epigenetically regulated process. Along with other somatic epigenetic marks, in the germline they are erased and then re-established in gametes according to the sex of the contributing parent. After fertilisation there is a second wave of general reprogramming where the epigenetic marks of the gametes are broadly removed so that those of early development may be applied. Imprinted DMRs are exempted from this post-fertilisation reprogramming and thus retain the epigenetic marks of their parent of origin. The imprinting signature of the currently known 38 germline-derived DMRs (Monk et al., 2018) is inherited from the parental gametes and is then maintained in the majority of somatic cells and tissues of an individual, making them unique among epigenetically regulated loci (figure 2).

Many genes regulated by genomic imprinting are found in clusters, i.e. imprinted loci often comprise multiple genes under a coordinated control of imprinting centers (ICs). In addition, interactions and co-regulations of different imprinted loci are obvious (Stelzer et al., 2014), thus the existence of an imprinted gene network (IGN) has been suggested (Varrault et al., 2006).

Evolution of genomic imprinting

Genomic imprinting has evolved in higher mammals (i.e. therians) and some seed plants. It emerged their evolution when the embryonic and early childhood nourishment of offspring became extensively tied to the maternal metabolism, at the expense of maternal resources. Therefore, it is not surprising that the majority of human imprinted genes are involved in (embryonic) growth and development, and disturbances of the balanced expression of imprinted factors are therefore often associated with altered growth and developmental delay.
Different hypotheses have been proposed to explain the evolutionary relevance of genomic imprinting as a complex regulative mechanism which is resource-consuming and prone to disturbances. Among others, these hypotheses refer to the observation that the majority of imprinted DMRs originate in the oocyte and accordingly the maternal gene copy is methylated (Monk et al., 2018b). In particular, two of these theories have attracted a broader attention:

According to the “parental conflict hypothesis” or kinship theory (Haig, 2004), the functional inequality of maternally and paternally imprinted genes reflects the differing interests of the parental genomes on the fitness of the kin. Whereas the paternal genes promote greater fitness of the offspring at the expense of the mother, the maternal imperative is to conserve resources for her own and for subsequent children. Accordingly, paternally expressed genes tend to be growth-promoting whereas maternally expressed genes are rather growth-limiting.

The “coadaption theory” (Wolf and Hager, 2006) suggests a coadaptive interaction between imprinted genes to improve fetal development and maternal nutrition and care. The theory is based on the observation that the sole expression of maternal gene copies is favoured, and this natural selection increases the adaptive integration of the maternal and offspring genomes.

However, there is currently no definitive for either of these hypotheses, and it can alternatively be suggested that imprinting has emerged due to different selective pressures on different imprinted factors.

**Disturbances of genomic imprinting**

The fine-tuned and co-regulated expression of imprinted genes is prone to endogenous and exogenous disturbances, and several clinical syndromes have been defined which are associated with altered imprinting patterns (the so-called imprinting disorders). Despite our limited understanding of the causes and consequences of imprinting errors, existing
knowledge informs current practice, and forms the basis for continuing improvement in
diagnostics and clinical management.

The monoallelic and parent-of-origin specific expression of imprinted genes can be disturbed
by four different types of molecular changes comprising both genomic variants affecting the
sequence and structure of imprinted genes and their regulatory regions, as well as altered
methylation of the respective differentially methylated regions (DMRs) (for review: Soellner
et al., 2017a)(figure 1). In the case of epimutations and uniparental disomies (UPDs), their
frequent post fertilization origin can result in a mosaic distribution which may elude detection
if the analysis is restricted to lymphocytes only (for example: Azzi et al., 2009).

Copy Number Variations (CNVs), Single Nucleotide Variants (SNVs)
Copy number variations (CNVs) and single nucleotide variants (SNVs) belong to the common
spectrum of genetic alterations in human diseases, and accordingly their inheritance
principally follows the Mendelian rules of inheritance. However, due to their parent-of-origin
dependent expression, the occurrence of clinical features is linked to the sex of the parent
contributing the affected allele. Examples are pathogenic variants in the maternally expressed
UBE3A gene and the paternally expressed IGF2 gene, where the carriers are affected by
Angelman syndrome (AS) or Silver-Russell syndrome (SRS) only in case of maternal or
paternal transmission, respectively (Begemann et al., 2015, Buiting et al., 2016).

Uniparental Disomy (UPD)
A group of variants typically associated with imprinting disorders are uniparental disomies (UPD), i.e. the inheritance of both chromosomes/chromosomal regions of a pair from only
one parent. Two subtypes of UPD can be discriminated: uniparental isodisomy (i.e.
inheritance of two identical copies of the same chromosome) and uniparental heterodisomy
(i.e. inheritance of both chromosomes from the same parent). UPDs can affect whole chromosomes, or only parts of a chromosome leading to segmental UPDs; if it affects an imprinted genomic region, either type of UPD can result in altered expression of respective gene(s).

Different modes of UPD formation have been postulated, all resulting from meiotic and/or mitotic nondisjunction mechanisms. These formation mechanisms also underlie the formation of numerical chromosomal aberrations, and therefore aneuploidy shares a mechanistic basis with UPD (Engel, 1993). In the same way, a common basis has been proposed for aneuploidy and altered imprinting (Deveault et al., 2011; Begemann et al., 2018).

Epimutations

In contrast to CNVs, SNVs and UPDs, the so-called epimutations alter DNA modifications rather than DNA sequence at the DMR. To distinguish epimutations without obvious molecular causes and those caused by genomic variants, the terms primary and secondary epimutations have been suggested (Horsthemke, 2010). In fact, the majority of epimutations in patients with imprinting disorders are supposed to be primary, which mean that they arise solely from epigenetic modifications themselves, but there is an increasing number of reports on secondary epimutations which are indirectly caused by genomic alterations, either to a DMR itself, (cis-acting) or to factors interacting with DMRs (trans-acting).

The most-recognised cis-acting variants are deletions within the imprinting control region 1 (IC1) on chromosome 11p15.5, which modify transcription factor binding and the chromatin organization of the DMR, altering its methylation, and thereby giving rise to Silver-Russell (SRS) or Beckwith-Wiedemann syndrome (BWS) (for review: Sparago et al., 2018). Another group of cis-acting factors comprise transcripts encoded by genes regulated by DMRs which are necessary for their proper imprinting setting (Lewis et al., 2019; Valente et al., 2019).
Trans-acting variants affect proteins involved in imprinting in the early embryo, including ZFP57, which targets DNA methylation to imprinted DNA (Quenneville et al., 2011), and maternally-encoded products expressed abundantly in the oocyte and sometimes referred to as the subcortical maternal complex (SCMC)(Monk et al., 2017).

Methods

Literature Databases (Pubmed, Medline) were thoroughly searched for the role of imprinting in human reproductive failure, in particular the terms “Multilocus Imprinting Disturbances, SCMC, NLPR/NALP, imprinting, reproduction” were used in various combinations.

Discussion

Clinical spectrum of imprinting disorders

Molecular disturbances of imprinted genes can have severe and life-long impacts on health of their carriers. Depending on the imprinted locus and the parental allele primarily affected in an individual, specific clinical pictures occur which have been summarised as imprinting disorders (table 1). Imprinting disorders belong to the group of rare diseases, with prevalences less than 1:10,000 among newborns each (for review: Soellner et al., 2017a). Up to date, twelve clinical disorders characterized by common underlying molecular mechanisms and overlapping or even contrary phenotypes have been defined (for review: Carli et al., 2019, Soellner et al., 2017a).

Many of the clinical features of imprinting disorders appear prenatally or in early childhood, and almost all persist during later life (for review: Soellner et al., 2017a).
The majority of phenotypic findings belongs to common groups of clinical symptoms, and include aberrant growth (aberrant pre- and/or postnatal growth retardation or growth acceleration), metabolic and endocrine disturbances (e.g. hypo- and hyperglycemia, pseudo-hypoparathyroism, precocious puberty), feeding difficulties and abnormal feeding behavior, learning difficulties and mental retardation.

In fact, it can be discussed whether temporal and spatial disturbed imprints might contribute to single symptoms associated with imprinting disorders. An example is aberrant imprinting of specific loci in the placenta which has been suggested to be linked to intrauterine growth retardation (Yamaguchi et al., 2019). However, the placental epigenome and its dynamics are far from being understood, thus requiring further studies to elucidate the link between placental imprinting and its consequences for prenatal and postnatal development (Monteagudo-Sanchez et al., 2019).

The majority of imprinting disorders show a disease-specific pattern of molecular alterations (table 1), e.g. deletions of the chromosomal region 15q11-q13 in Angelman syndrome (AS)/Prader-Willi syndrome (PWS), or loss of methylation (LOM) in the 11p15 imprinting center region 2 (IC2; alternative names: KCNQ1OT1:TSS-DMR, KvDMR, LIT; for recommendations for a standardized nomenclature see Monk et al., 2018) in Beckwith-Wiedemann syndrome (BWS). The specific diagnosis of an underlying imprinting disorder can be complicated by the variable expression of symptoms and the non-specificity of many of the phenotypic hallmarks of imprinting disorders. Furthermore, some key features are transient (e.g. hypotonia in PWS, facial gestalt in SRS) and other features may be subtle (e.g. asymmetry in some SRS patients). As a consequence, an unknown number of imprinting disorders patients are probably either mis- or undiagnosed. In order to overcome these difficulties, for some imprinting disorders consensus guidelines have been published, which mainly deal with scoring systems to define a threshold for the clinical diagnosis and give
recommendations for the medical care of affected patients (Wakeling et al., 2017; Brioude et al., 2018; Mantovani et al., 2018).

In rare cases, the testing results may indicate an alteration opposite to the initial clinical diagnosis (e.g. SRS-associated molecular finding in a patient with BWS features and vice versa)(Mackay et al., 2019). Furthermore, a considerable number of individuals with imprinting disorders have altered DNA methylation at multiple imprinted loci across the genome; this situation is referred to as multi-locus imprinting disturbance or MLID (Sanchez-Delgado et al., 2016). The MLID patients often show a specific “classical” imprinting disorders phenotype, but some patients with MLID develop a mixture of symptoms of different imprinting disorders (e.g. in case of TNDM, Mackay et al., 2006, 2008). Further efforts are needed to understand the exact mechanisms of (epi-) genotype/phenotype correlation in imprinting disorders.

The contribution of imprinting disturbances to the clinical phenotype is not only assessed by (epi)genotype-phenotype correlation studies, but valuable insights have already been provided by mouse models. Due to the complexity and species-specific imprinting marks even at the same loci, the findings from mouse models cannot be transferred one-to-one to the respective human imprinted locus (for review: Hanna et al., 2018). However, several examples show that they will help to understand specific clinical features in imprinting disorders, like growth disturbances, body fat composition or behavior (e.g. McNamara et al., 2016; van de Pette et al., 2016; Tunster et al., 2018).

Pre- and perinatal findings in imprinting disorders

Prenatal manifestations of imprinting disorders have not yet been surveyed systematically, but at least for the more frequent entities information on prenatal findings are available (table 1b).
In fact, abnormal fetal growth and fetal defects such as abdominal wall defects are the major common prenatal symptoms of imprinting disorders, but these symptoms are nonspecific and variable.

**Imprinting disorders with intrauterine growth retardation (IUGR)**

Intrauterine growth retardation (IUGR) is a frequent prenatal finding in several imprinting disorders (i.e. SRS, PWS, TNDM, Temple syndrome / TS14). IUGR and relative macrocephaly occur typically in the third trimester in Silver-Russell-syndrome (SRS), but relative macrocephaly is also a typical finding observed in BWS in the second trimester (Kagan et al., 2015) The molecular basis of these ultrasound findings and the prognosis on the further course of the pregnancy are often unclear. Induction of labor or caesarean section is often discussed in pregnancies with intrauterine growth disturbances, but it is controversially discussed if in context of an imprinting disorders in the fetus, induction is indicated (Eggermann et al., 2016). In rare cases of SRS and PWS, additional malformations such as cleft lip/palate, hypospadias and cardiac defects have been reported prenatally and might complicate the clinical classification as imprinting disorders. Most patients with TS14 show an IUGR phenotype overlapping with those of SRS and PWS patients, therefore TS14 should be discussed in the differential diagnostic workup of these diseases (for review: Beygo et al., 2017).

**Imprinting disorders with intrauterine overgrowth and/or abdominal wall defects**

Syndromes with overgrowth, especially Beckwith–Wiedemann syndrome (BWS), can reveal macrosomia even in utero. Additional findings of BWS are abdominal wall defects (omphalocele), macroglossia, visceromegaly, polyhydramnios, placental mesenchymal dysplasia/placentomegaly, and a long umbilical cord (Gaillot-Durant et al., 2018; Kagan et al., 2015; Eggermann et al., 2016).
In BWS and other overgrowth syndromes there is an elevated cancer risk, which is generally not associated with the prenatal diagnosis of an embryonic tumour; however, there are rare cases with the prenatal detection of a tumour (Longardt et al., 2014). In BWS patients (epi-)
genotype / phenotype correlations have been established, especially in the context of hemihypertrophy (strongly associated with upd(11)pat), abdominal wall defects (higher in IC2 LOM and CDKN1C mutations) and tumour risks (higher in upd(11)pat, IC1 GOM)(table 1). In addition to these clinical features, BWS pregnancies have an elevated risk for preeclampsia and eclampsia. In a series of 12 pregnancies affected by BWS, six ended in severe preeclampsia (Kagan et al., 2015).

Abdominal wall defects and macroglossia, often in combination with normal or reduced growth, are the first symptoms of TNDM, which should be kept in mind as differential diagnosis of BWS. Most neonates with TNDM present with hyperglycemia, with insulin required for an average of the first three months of life (Temple and Shield, 2010); therefore, prompt molecular testing for TNDM is required to distinguish it from other monogenic diabetes and stratify treatment appropriately (deFranco et al., 2015). Another differential diagnosis in pregnancies complicated by polyhydramnios and abdominal wall defects is Kagami-Ogata syndrome (KOS14), in which a small, bell-shaped thorax with so-called “coat-hanger ribs” is the most characteristic finding. Many patients with KOS14 have been reported to die in-utero or in the early newborn period, but children who survive this critical period seem to have a more favourable outcome (Ogata and Kagami, 2016).

**Imprinting and hydatidiform moles**

Finally, imprinting disorders courses can be so severe that they are incompatible with life and then lead to an increased rate of miscarriages. The most severe phenotype of an imprinting disorders in humans are familiar or recurrent hydatidiform moles. Whereas molecular disturbances at specific loci appear to be associated with viable prenatal phenotypes and more
or less specific symptoms, imprinting defects in familiar or recurrent hydatidiform moles are genome wide defects.

The term hydatidiform mole describes an aberrant human pregnancy with a placental overgrowth but severely abnormal or absent embryonic development. By histopathology, complete hydatidiform mole and partial hydatidiform mole are distinguishable by the residual embryonic tissue in the latter, but in both cases the placenta is characterized by edematous swelling of chorionic villi and trophoblastic hyperplasia (for review: van den Veyer et al., 2006; Kalogiannidis et al., 2018). The incidence of hydatidiform mole is estimated as 1 in 600-1000 pregnancies in Western countries and an overall recurrence risk of 1 - 9 % has been estimated (recurrent hydatidiform mole) (for review: Nguyen et al., 2018a, b).

The vast majority of complete hydatidiform moles (80-90%) have a diploid but androgenetic genome (for review: Candelier, 2016). Among them, 80-90% are the result of a monospermic fertilisation of an empty oocyte with subsequently endoduplication of the haploid paternal genome, whereas 10-20% are caused by a dispermic fertilisation. In contrast to the diploid complement status of complete hydatidiform mole, partial hydatidiform moles is mostly triploid, with one maternal and two paternal genomes. In rare cases, both parental genomes can be identified in complete hydatidiform mole (biparental complete mole), and interestingly this type of complete hydatidiform mole is observed in families with recurrent molar pregnancies. In biparental complete hydatidiform mole a complete loss of maternal imprinting marks was observed (El-Maarri et al., 2003).

Though the causes of the majority of hydatidiform mole cases are currently unknown, there is an increase of knowledge about the pathoetiology of specific subgroups. In women suffering uncommon recurrent androgenetic complete hydatidiform mole which have hitherto been thought to arise by chance, deleterious variants of meiotic factors have been identified (Nguyen et al., 2018a, b). In the rare finding of familial hydatidiform mole with a biparental genetic contribution and a complete loss of maternal imprinting marks the familiarity and the
inheritance pattern in affected pedigrees let to the assumption of an underlying monogenetic disorder in the pregnant women themselves (consanguinity of parents of the women, recurrent hydatidiform mole in different partnerships of the same woman). Meanwhile, more than 110 pathogenic maternal effect variants affecting components of the SCMC have been identified in women suffering recurrent hydatidiform mole (Nguyen et al., 2018a, b). Furthermore, the first maternal effect mutation associated with a multilocus imprinting disturbance (MLID) has been identified in these families as well (Murdoch et al., 2006).

In summary, in pregnancies with abnormal fetal growth, poly- or oligoamnios and further fetal and placental abnormalities (table 1b) altered imprinting should be considered, and in these situations the initiation of appropriate molecular diagnostic tests might be discussed.

Prenatal testing in imprinting disorders

In addition to ultrasound findings and clinical features as indications, prenatal genetic testing should generally be considered in case of a family history of imprinting disorders (including SNVs/CNVs affecting imprinted genes) of familial chromosomal alterations (e.g. chromosomal rearrangements predisposing to CNVs or UPDs) or prenatal detection of chromosomal disturbances which might result in an imprinting disorder (trisomy resulting in UPD, CNVs).

In any case, the laboratory offering genetic tests and the referring clinicians should be aware of the limitations of invasive prenatal molecular diagnostics of imprinting disorders (for review: Eggermann et al., 2016). These include the timing of sample drawing and the methylation status of the DMR of interest at that time, as some DMRs are not finally established during the time of chorionic villi sampling. In particular, placental cells have a unique epigenetic profile (Monk, 2015). Methylation-specific testing at this early stage can thus lead to false results, as can mosaicism of epimutations or UPD. Due to the complexity of
molecular alterations at imprinted loci and the differences of information values of the
different testing methods, every test for imprinted loci requires an in-house validation, and the
laboratory has to report precisely on the applied assay and its technical limitations. These
challenges and limitations have also to be considered in the future when non-invasive prenatal
diagnosis (NIPD) based on cell-free fetal DNA might be implemented in prenatal testing of
imprinting disorders (Wang et al., 2017). Finally, the handling of both negative and positive
results require expert genetic counseling.

Multilocus Imprinting Disturbances (MLID) and maternal effect mutations

Multilocus Imprinting Disturbances (MLID)
As already described, ‘classic’ imprinting disorders are associated with specific genetic loci,
and may have underlying characteristic epimutations or genetic variation at these loci (e.g.
deletions/duplications or pathogenic sequence changes acting in cis)(figure 1, table 1); by
contrast, MLID affects loci across the genome, and therefore should result from global
disruption of epigenetic processes, and if there is any underlying genetic variation it must be
trans-acting. MLID is frequently mosaic (affecting some but not all somatic cells), suggesting
that it arise in a fraction of embryonic cells early in development.
MLID is almost exclusive to individuals with epigenetic errors, rather than UPD or CNV.
Perhaps because of this, it is mainly recognized in imprinting disorders where epigenetic
errors cause the majority of cases. For example, in AS and PWS, where copy number changes
and UPD are overwhelmingly causative, MLID is extremely rare; in BWS, SRS, TNDM and
pseudohypoparathyroidism Ib (PHP Ib), where a significant fraction of cases are epigenetic,
MLID is present in 10-50% of epigenetic cases (table 1).
However, the true prevalence of MLID is currently unknown and almost certainly
underestimated. Firstly, studies to date have tested limited subsets of imprinted genes in
research cohorts (e.g. Blié et al., 2009; Poole et al., 2013; Azzi et al., 2014; Bens et al., 2016; Kagami et al., 2017; Fontana et al., 2018); more comprehensive and sensitive testing would probably reveal higher rates of MLID. Secondly, most MLID cases present with a ‘primary’ imprinting disorder recognised by a clinical geneticist, but MLID can by definition cause clinical features that would not fit with any imprinting disorder (e.g. Baple et al. 2011; Caliebe et al., 2013; Docherty et al., 2015); this suggests that some cases may go unrecognised. Thirdly, epimutations in MLID are generally mosaic, and mosaicism at low levels or mosaicism confined to tissues unavailable for testing can elude detection (Azzi et al., 2014).

Embryonic causes of MLID
MLID was first identified in patients with TNDM (Arima et al., 2005; Mackay et al., 2006) and then subsequently in most classical imprinting syndromes (e.g. Blié et al., 2009; Azzi et al., 2014; Kagami et al., 2017; Rochtus et al., 2016). Initially assumed to be a stochastic, non-heritable phenomenon, MLID was described in siblings (Boonen et al., 2008); subsequently recessive mutations of ZFP57 were identified in patients with TNDM (Mackay et al., 2008). In several cases, unaffected parents of affected children were shown to have heterozygous pathogenic variants of ZFP57, indicating classical autosomal recessive inheritance. ZFP57 associates with a CG-rich hexameric motif, with a strong preference for the methylated DNA allele at imprinted loci, and acts to recruit a multi-protein complex including the scaffold factor TRIM28 and the methyltransferase DNMT1. It therefore enables imprinted DNA to maintain its high methylation during early development, when the genome as a whole becomes hypomethylated due to DNA replication without methylation replacement. Further key factors are involved in imprinting maintenance, including TRIM28 (Messerschmidt et al., 2012) and the recently-identified ZNF445 (Takahashi et al., 2019), but
disease-causing variants have not been identified in humans, presumably because severe hypomorphism is incompatible with life.

Maternal effect mutations

Genetic variants causing imprinting disorders are not confined to affected individuals, but have been detected in their mothers. Homozygous or compound heterozygous pathogenic variants, deletions or rearrangements in NLRP7 were detected in more than 50% of women with recurrent hydatidiform mole (Murdoch et al., 2006). Women with biallelic inactivation of NLRP7 have normal genomic methylation themselves, but fail to establish imprints in oocytes, leading to hydatidiform mole and reproductive wastage. This description was the first example of a so called maternal effect mutation leading to an imprinting disorder, and contrasts with ZFP57, whose biallelic inactivation causes MLID in affected individuals themselves. Women affected by NLRP7 mutation suffer repeated molar pregnancies, though healthy children have been reported rarely (Akoury et al., 2015). The severity of the recurrent hydatidiform mole phenotype suggested that complete maternal NLRP7 inactivation compromised the oocyte itself (Sanchez-Delgado et al., 2015), in contrast with hypomorphic variants of other components of the subcortical maternal complex (SCMC), which seem to mosaically compromise the cleavage-stage embryo (Mahadevan et al., 2017). However, several recent reports suggest a more nuanced picture, with different SCMC mutations giving rise to recurrent hydatidiform mole, recurrent imprinting disorders or other reproductive outcomes (table 2, table 2). Meyer et al. (2009) reported in 2009 a homozygous frameshift mutation in NRLP2 in the mother of two children with BWS caused by epimutations at the IC2 and in 2011 KHDC3L was identified as second gene for recurrent hydatidiform mole explaining another 5% of cases (Parry et al., 2011).
The SCMC (figure 2 C) is a large multimeric protein complex formed in the mature mammalian oocyte and localized at its periphery. After fertilization, it is preserved in the outermost cells of the preimplantation embryo, but its expression declines in the inner cell mass and is excluded from regions with cell-cell contacts (Li et al., 2008; Bebbere et al., 2016). Disruption of components of the SCMC in mice is associated with an developmental arrest at the two- or four-cell stage (Tashiro et al., 2010, Tong et al., 2004).

The SCMC components are exclusively expressed from the maternal genome in oocytes and early embryos, but then degraded in further embryonic development without compensation by the embryonal genome. Several functions in oocyte and early embryo progression have been assigned to the different SCMC proteins (for review: Monk et al., 2017)(table 2), including meiotic spindle formation and epigenetic reprogramming of the zygote (for review: Bebbere et al., 2016). It is therefore conceivable that maternal-effect mutations in genes encoding SCMC components might cause aneuploidy and disturbed imprinting in the offspring, with a life-long impact on the imprinting status of an individual.

At least three of the NLRP gene family (NLRP2, NLRP5, NLRP7) are highly expressed in growing oocytes, where they and presumably other proteins in the SCMC (including KHDC3L, PADI6 and OOEP) are required for epigenetic reprogramming and maintenance of ploidy in the early embryo (Tong et al., 2000; Li et al., 2008; Yurttas et al., 2008; Zheng et al., 2009; Fernandes et al., 2012; Zhao et al., 2016; Mahadevan et al., 2017; for review: Monk et al., 2017). Pathogenic variants in these proteins have been found in one cohort in 50% of mothers of children with MLID who experienced reproductive problems like recurrent miscarriages and hydatidiform mole, as well (Caliebe et al., 2013; Docherty et al., 2015; Soellner et al., 2017; Begemann et al., 2018). Furthermore, a growing number of reports describe pathogenic variation in the same genes in women with adverse pregnancy outcomes.
and no liveborn offspring (e.g. Alazami et al., 2015; Xu et al., 2016; Maddirevula et al. 2017; Qian et al., 2007, 2011, 2018; Wang et al., 2018, Mu et al., 19).

Pregnancies of women who carry maternal affect mutations therefore have different courses and can reflect the broad spectrum of reproductive complications in context of imprinting disorders. Figure 3 shows an example of a pedigree of a family with a maternal effect mutation (adopted from Soellner et al., 2017b). The first pregnancy had been complicated by severe preeclampsia, elevated hCG (human chorionic gonadotropin) and polyhydramnios. Prenatal ultrasound showed macroglossia and placental cysts, suggestive of mesenchymal dysplasia. The pregnancy was terminated, and molecular testing resulted in aberrant imprinting at different loci thus confirming a MLID status. The second pregnancy ended in a spontaneous abortion with no further medical documentation. In the third pregnancy, elevated βHCG levels and large multicystic ovaries were detected whereas placental and fetal ultrasound investigations were normal. Methylation-specific testing in amniotic fluid revealed MLID as well. Spontaneous preterm labor occurred at 24 weeks and the neonate died due to ruptures of the lungs. In the mother, a heterozygous nonsense 2-bp deletion in exon 5 of the NLRP7 gene was identified.

This example contributes to the hypothesis that homozygous and heterozygous variants in maternal effect genes cause aberrant imprinting in the offspring and lead to recurrent reproductive failure. Due to the fulminant courses of the pregnancies in this family, specific screening and close prenatal monitoring of women carrying NLRP7 and other maternal effect variants is proposed. Furthermore, egg donation might be the strategy to facilitate successful pregnancies in women with maternal effect mutations (Akoury et al., 2015; Soellner et al., 2017b)(figure 3).

Is there a causal link between disturbed imprinting and aneuploidy?
Several studies show that chromosomal aneuploidy, imprinting errors or both are associated with molar pregnancy and/or MLID (Deveault et al., 2009; Soellner et al., 2017b; Begemann et al., 2018). Interestingly, two MLID cases with proven NLRP2 or NLRP7 mutations have aneuploidy (45,X (Soellner et al., 2017); 47,XXY (Begemann et al., 2018)). This co-occurrence is in agreement with the observations of Deveault and coworkers (2009) that maternal-effect variants in NLPR7 might be associated with early cleavage abnormalities in early embryogenesis. The contribution of SCMC components to euploidy of mammalian oocytes could also be shown for the factor Filia in mice, the orthologue of the human recurrent hydatidiform mole and MLID associated KHDC3L gene. Zheng et al. (2012) demonstrated that depletion of the maternally expressed oocyte factor Filia disturbed oocyte progression by aneuploidy resulting from abnormal spindle assembly, chromosomal misalignment, and spindle assembly checkpoint inactivation.

A functional link between disturbed imprinting and chromosomal nondisjunction is suggested by two reports of individuals with both imprinting disturbance and UPD (Arima et al., 2005; Begemann et al., 2012). In fact, the rate of uniparental disomy rises with maternal age, being about 1% of that of aneuploidy; this is presumably because UPD corrects the numerical error of aneuploidy, but only in a minority of cases. UPD is a known cause of imprinting disorders, but is also found in the general population at a rate that may be higher than commonly thought (Nakka et al., 2019). It is interesting to wonder whether UPD, currently perceived as the most stochastic cause of imprinting disorders, may in fact be a consequence of a disturbance of the oocyte. This question will only be resolved by genomic analysis for couples affected by reproductive difficulties, excluding those with patent alternative issues and focusing on those with recurrent problems.
Environmental factors and assisted reproduction affecting imprinting marks

The majority of patients with imprinting disorders carrying epimutations have a negative family history, and causative genomic alterations in cis or trans causing aberrant imprinting (secondary epimutations) are rarely identified. The apparent preponderance of primary epimutations, i.e. aberrant methylation marks without known genomic correlate, and their sporadic occurrence, indicate a role of environmental interferences in the pathoetiology of disturbed imprinting (for review: Monk et al., 2019). In fact, preimplantation and early postimplantation are particularly vulnerable stages in human reproduction, as epigenetic modifications are re-established during this period. Several environmental factors, such as parental nutritional and metabolic status, drug abusus and endocrine-disrupting substances like bisphenol A (for review: Kitsiou-Tzeli et al., 2017; Dunford and Sangster, 2017; Murphy et al., 2018; Monk et al., 2019; Xavier et al., 2019), have been proposed to affect epigenetic programming during development, with consequences throughout the lifecourse and potentially over subsequent generations (Drobna et al., 2018). It can therefore be asked whether a proportion of primary epimutations is attributed to environmental exposures in early development or even in preceding generations. In fact, the complex interaction between environment and (epi)genomics is difficult to assess, and current data are mainly based on studies in mice. In humans, comprehensive epidemiological surveys have already been conducted indicating a transgenerational inheritance of epigenetic information (for review: Xavier et al., 2019), but further studies are needed.

Numerous studies have demonstrated an association between increased risk of imprinting disorders and assisted reproductive technologies (ART), and a recent systematic review and metaanalysis of more than 351 publications confirmed an increase of children with imprinting disorders in children conceived by ART (Lazaraviciute et al., 2014). However, due to the heterogeneity of the indications for ART and the ART protocols (infertility treatment as well
as in-vivo fertilisation and culture protocols) the specific cause(s) for the link of ART and imprinting disorders are unclear. For further details about this complex subject we kindly refer the readers to the excellent papers which have already been published in Human Reproductive Update (van Montfoort et al., 2012; Anckaert et al., 2013; Lazaraviciute et al., 2014). In addition to ART as a possible risk factor for aberrant imprinting, the role of male infertility on sperm methylation is also under discussion. Although the functional link between them is currently unclear, a meta-analytic approach demonstrated that there is an association (with altered sperm methylation at H19, MEST, and SNRPN. Although its role in infertility remains unclear, sperm DNA methylation (Santi et al., 2017) could be associated with the epigenetic risk in ART. However, as the identification of two children with MLID after oocyte donation shows (own unpublished data), the discrimination between genetic and environmental causes of disturbed imprinting is difficult. In these cases it remains unclear for now whether their altered imprinting marks originate from maternal effect mutations in the oocyte donors, or whether they are linked to the cause of infertility in a couple or to a component of the ART protocol. Taken together, the current knowledge suggests that maternal genetic variation, as well as environmental factors, can affect the constitution of the oocyte and its capacity for development. In the most severe cases the oocyte itself is compromised because its epigenome is either lost before fertilisation or incapable of reprogramming, giving rise to either molar pregnancy or functional infertility. Less severely-affected oocytes sustain fertilisation, but have reduced competence for epigenetic reprogramming or ploidy maintenance in individual cells of the cleavage-stage embryo. If development is severely compromised or delayed the embryo dies. If it survives to blastulation, subsequent
development may overwrite early epigenetic errors (except for imprinting errors, which cannot be corrected in somatic cells) and confine aneuploidy to the placenta. Thus, oocyte compromise may give rise to infertility, molar pregnancy, pregnancy loss, and liveborn offspring with imprinting and ploidy errors that may or may not be clinically discerned.

**Translational use of the current knowledge on imprinting for reproductive management**

The increasing knowledge about the pathobiology of disturbed imprinting and its clinical consequences has significant impacts on diagnostics and management in human reproductive medicine. Interestingly, the central role of genomic imprinting in human reproduction and embryonic development was clear from the inception of imprinting research, but molecular diagnosis and clinical management is mainly focused on patients with a clinical suspicion of imprinting disorders.

Based on recent studies on the causes of disturbed imprinting marks and their relevance for human reproduction, several conclusions can be drawn and translated into molecular diagnostics, reproductive and genetic counselling.

*Molecular genetic testing*

Molecular genetic testing of disturbed imprinting is challenging due its molecular heterogeneity and the occurrence of mosaicism. Therefore, a negative testing result does not exclude the possibility of an undetected (epi)mutation, and this particularly applies to prenatal testing for imprinting alterations. Thus, both false negative and false prenatal testing results can be obtained, and these limitations should be considered in the course of prenatal management. In fact, the management should rather be based on clinical parameters (e.g.
ultrasound findings) than on molecular testing results, the latter should only be used to confirm a suspicious diagnosis.

Reproductive medicine aspects

Altered imprinting and its molecular causes have a relevant influence on human reproduction. There is growing evidence that disturbance of human germ cell maturation can cause not only aneuploidy but also imprinting defects (e.g. Filia/KHDC3L). It is currently unclear whether the association between ART and imprinting disturbances are attributable to the parental issue underlying infertility, or the ART protocol itself. In particular, the effect of cell culture on the imprinting status is in the focus of discussion (e.g. Anckaert et al., 2013). Further studies are needed to answer these questions, but these aspects should be discussed with couples during reproductive counselling.

The strong association between maternal-effect mutations in components of the SCMC and miscarriages including (recurrent) hydatidiform mole is meanwhile well established (table 2), but it has recently turned out that women carrying these genetic variants are at risk for pregnancies with imprinting disturbances. Thus, for these patients the molecular identification of the basic causes is urgently required to predict the outcome of pregnancies and to adapt the reproductive management. In case of spontaneously obtained pregnancies, the risks of miscarriages and aneuploidies as well as of altered imprinting have to be addressed. The latter is indeed difficult to estimate as it cannot be predicted which imprinted locus might be disturbed (Soellner et al., 2017b). Even in case an invasive prenatal testing for the currently known imprinting disorders loci is performed, the prediction of the clinical outcome is hardly possible in case of a positive testing result.

To avoid these uncertainties and burdens for the families, oocyte donation has been suggested as an appropriate treatment for women carrying maternal effect mutations. In fact, the first
births of healthy children in these families after oocyte donation has been reported (Akoury et al., 2015)(figure 3). These reports corroborate the hypothesis that the disturbed imprinting regulation in the oocyte is responsible for the reproductive failure in these families. However, this treatment does not prevent imprinting disturbances in any case (see 3.5).

Antenatal care in families with maternal-effect mutations should not only be focused on the fetus. A careful maternal monitoring is also necessary due to the increased risk for preeclampsia and preterm delivery (Kagan et al., 2015; Soellner et al., 2017b).

**Genetic Counselling**

Genetic counselling of families with imprinting defects is challenging due to the molecular and clinical heterogeneity, and the non-Mendelian mode of inheritance of some of their molecular causes.

Until recently, genetic counselling in imprinting disorders was focused on families with chromosomal copy number variants and point mutations in imprinting disorders causing genes (e.g. **UBE3A** in AS and **CDKN1C** in BWS). Their inheritance is autosomal-dominant, but the penetrance in the offspring depends on the sex of the parent from which the variant is transmitted. Examples are **IGF2** variants which result in SRS only in case of paternal transmission due to the monoallelic **IGF2** expression from the paternal allele. Likewise, in families with chromosomal structural variants, the parental sex has impact on the phenotype of the child; in rare familial cases, opposite clinical pictures can occur depending whether the variant is transmitted from the mother or from the father (Jurkiewicz et al., 2017).

Additionally, in case of chromosomal disturbances their size and gene content have to be determined as they can significantly alter and aggravate the clinical picture of its carrier, thereby masking the imprinting disorders which is linked to the chromosomal region. Finally, some common familial chromosomal translocations can predispose the formation of UPD. In
particular, Robertsonian translocation carriers (i.e. translocations between the acrocentric chromosomes; frequency of 1/1000 among newborns (Gardner et al., 2012)) are at risk for UPD formation. Thus, in imprinting disorders patients with UPD of chromosomes 15 and 14 chromosomal analysis of the parents should be considered (Beygo et al., 2019).

With the identification of maternal effect mutations, new aspects have to be considered in genetic counselling. Based on a careful documentation of family history, including miscarriages, pregnancy complications, aneuploidies and features suggestive of imprinting disturbances, pathogenic variants in components of the SCMC and further factors properly mediating the imprinting cycle of life have to be taken into account. Though the elucidation of these and other factors in the pathobiology of human reproductive failure is in its infancy, the current knowledge has already been translated into genetic and reproductive counselling.

**Conclusions and Perspectives**

Several gaps remain in understanding these complex mechanisms, but the development and implementation of next generation sequencing-based assays will continue to transform the genetic diagnosis of germline and somatic genetic diseases. Future testing strategies will not only target DNA to identify genetic and epigenetic causes of diseases, but they will include the parallel analysis of RNA to determine the functional relevance of the molecular disturbance. However, genetic testing is already an indispensable prerequisite for precise clinical managements of patients with congenital anomalies (for review: Kamps et al., 2017; Xu et al., 2015). In fact, these improvements do not only affect diagnostics of patients themselves, but offer improved counselling and (prenatal) management of the parents and families. In particular, the validated use of high resolution and throughput assays (microarrays, next generation sequencing) targeting nearly all types of mutations (SNVs, CNVs, epimutations) has revolutionized the molecular testing strategies. Thereby profound
insights in the etiology of human diseases as well as new diagnostic tools as the bases for a self-determined decision on health of patients and their families are provided.

In summary, this increasing knowledge helps to further define diagnostic and clinical criteria for the identification of persons at risk for imprinting disorders and related pregnancy complications. Thus, an early diagnosis will become possible, enabling a personalised clinical management of the imprinting disorders patients themselves and a counselling of their families, and avoiding diagnostic odysseys.

**Author’s roles**

ME and TE have suggested the structure of the paper, and all authors have contributed to the different topics according to their expertise (ME: clinical genetics, OK: reproductive aspects, DJM: MLID and basic imprinting mechanisms, MB: molecular aspects, TE: general aspects). All authors have critical discussed, checked and finally approved the paper.
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Conflict of Interest statement

The authors declare no conflict of interest.
**Figures and Tables**

**Figure 1:** Genomic imprinting and its currently known four types of disturbances. (A) Whereas in non-imprinted genes (filled boxes) the sex of the contributing parent (red: maternal gene copy, blue: paternal copy) does not influence the level of expression (arrows indicate expression, numbers indicate the dosage of gene expression), DNA methylation of the paternal allele (filled lollipop) results in monoallelic expression of only the maternal gene copy, and vice versa. (striped boxes: maternally expressed genes; dotted boxes: paternally expressed alleles). (B) In case of maternal UPD (only two maternal chromosomes are present), both copies of maternally expressed genes are expressed. In contrast, the paternally expressed gene is silenced in that disturbance, and therefore the resulting dosage is reduced. When a paternally expressed gene is deleted, the result is the same as for UPD as the maternal gene copy is silenced and does not compensate the loss of the paternal copy. Hypermethylation as an example for an epimutation silences the active allele, here also illustrated for the paternally expressed allele. In case of hypomethylation (not shown), an overexpression of the affected factor can be assumed. Sequence variations affecting the genomic sequence of an imprinted gene reveal the same functional consequences as the other three types, again illustrated for a paternally inherited active allele. Please note that this is an exemplary illustration of imprinting mechanisms, but the in-vivo mechanisms are much more complex (e.g. DNA methylation is not always coupled with silencing, and gene expression does not consist of simple switch-off/on regulation). It should also be noted that, since the normal expression of imprinted genes is hemizygous, pathogenesis may result from either reductions or increases of effective gene dosage, depending on the function of the genes affected.
Figure 2: The life cycle of imprinting during gametogenesis and early embryonic development. (A, B) In early primordial germ cells (PGCs) epigenetic marks including DNA methylation are erased; the subsequent germ cell maturation comprises the differentially setting of sex-specific imprinting marks. After fertilisation, these marks are maintained in a tissue- and isoform-specific manner. (C) Critical to imprinting maintenance is the SCMC, whose maternally-encoded components are deposited in the ooplasm and are prerequisites for cleavage-stage embryo development and the maternal to zygote transition. (for explanation of part (A) see Figure 1.)

Figure 3: Pedigree of a family with a maternal-effect NLRP7 variant ((NM_001127255.1:c.2010_2011del, p.(Phe671Glnfs*18) and reproductive histories of miscarriages (small black circle; IV/3, IV/6) and miscarriages affected by MLID (black squares; IV/2, IV/4). It should be noted that miscarriages only occur when the woman carries the variant (III/3, III/5), whereas paternal transmission is not associated with reproductive failure (II/2; probably II/4). Interestingly, it appears that patient III/2 could circumvent the reproductive failure by egg donation (IV/1).(Family members with proven carriership for the variant are marked by a dot, the others have not been tested). For further description of the family see Soellner et al., 2017b. (The pedigree is modified from Soellner et al., 2017b).
Table 1: Overview on the currently known twelve imprinting disorders, their major (a) clinical and (b) prenatal findings, and molecular alterations. For the standardised names of the imprinted loci see Monk et al. (2018) (Chr chromosome, IUGR intrauterine growth retardation, PNGR postnatal growth retardation, hCG human chorionic gonadotropin, PTH parathormone, DMR differentially methylated region, LOM loss of methylation, GOM gain of methylation, CNV copy number variation (deletion, duplication); *mainly in the third trimester)

Table 2: Genes encoding components of the SubCortical Maternal Complex (SCMC) in which maternal effect mutations have been identified, and associated clinical and molecular findings. (NA not assessed; NR not reported; hom homozygous, het heterozygous, comphet compound heterozygous; for abbreviations of the associated phenotypes see abbreviations; * only leucocyte DNA samples were analysed)


Arima T, Kamikihara T, Hayashida T, Kato K, Inoue T, Shirayoshi Y, Oshimura M, Soejima H, Mukai T, Wake N. ZAC, LIT1 (KCNQ1OT1) and p57KIP2 (CDKN1C) are in an imprinted gene network that may play a role in Beckwith-Wiedemann syndrome. *Nucleic Acids Res* 2005:33; 2650-60.


Expression and DNA Methylation of Imprinted Genes in Brain. *Endocrinology* 2018: 159; 132-144.


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


<table>
<thead>
<tr>
<th>Imprinting disorder OMIM</th>
<th>Prevalence</th>
<th>Chromosome</th>
<th>Molecular defect (frequency)</th>
<th>MLID</th>
<th>Main clinical features</th>
<th>Refs</th>
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<tr>
<td>Transient neonatal diabetes mellitus (TNDM) 601410</td>
<td>1/300.000</td>
<td>6q24</td>
<td>- upd(6)pat: 41% - Paternal duplications: 29% - <em>PLAG1</em>:alt-TSS-DMR LOM</td>
<td>30%</td>
<td>IUGR, transient diabetes mellitus, hyperglycaemia without ketoacidosis, macroglossia, abdominal wall defects</td>
<td>Docherty et al., 2013</td>
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<td>Silver-Russell syndrome (SRS) 180860</td>
<td>1/75.000-1/100.000</td>
<td>Chr 7</td>
<td>- upd(7)mat: 5-10% - upd(11p15)mat - 11p15 CNVs (&lt;1%) - <em>H19</em>/IGF2:IG:DMR LOM: 30-60% - <em>CDKN1C</em>, <em>IGF2</em>, <em>HMGA2</em>, <em>PLAG1</em> point mutations</td>
<td>7-10%</td>
<td>IUGR, PNGR, relative macrocephaly at birth, body asymmetry, prominent forehead, feeding difficulties</td>
<td>Wakeling et al., 2017, Eggermann et al., 2016</td>
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<td>unknown</td>
<td>Chr 8q24.3</td>
<td>- <em>KCNK9</em> point mutations</td>
<td></td>
<td>Intellectual disability, hypotonia, dysmorphism</td>
<td>Barel et al., 2008</td>
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<td>Beckwith–Wiedemann syndrome (BWS) 130650</td>
<td>1/15.000</td>
<td>Chr 11p15</td>
<td>- upd(11p15)pat: 20% - 11p15 CNVs: 2-4% - <em>H19</em>/IGF2:IG:DMR GOM: 5% - <em>KCNQ1OT1</em>:TSS-DMR LOM: 50% - <em>CDKN1C</em> point mutations: 5% sporadic; 40-50% in families</td>
<td>25%</td>
<td>Macroglossia, exomphalos, lateralized overgrowth, Wilms tumour or nephroblastomatosis, hyperinsulinism, adrenal cortex cytomegaly, placental mesenchymal dysplasia, pancreatic adenomatosis</td>
<td>Brioude et al., 2018, Kagan et al., 2015, Eggermann et al., 2016</td>
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<td>Temple syndrome (TS14) 616222</td>
<td>unknown</td>
<td>Chr 14q32</td>
<td>- upd(14)mat: 29%* - 14q32 paternal deletion: 10%* - <em>MEG3</em>/DLK1:IG-DMR LOM: 61%*</td>
<td>?</td>
<td>IUGR, PNGR, neonatal hypotonia, feeding difficulties in infancy, truncal obesity, scoliosis, precocious puberty, small feet and hands</td>
<td>Ionnanidis et al., 2014, Gillessen et al., 2018</td>
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<td>Angelman syndrome</td>
<td>1/20.000 -1/12.000</td>
<td>Chr 15q11–q13</td>
<td>- Maternal deletion: 70-75% - upd(15)pat: 3-7%</td>
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<td>Severe intellectual disability, microcephaly, no speech, unmotivated laughing, ataxia, seizures, scoliosis</td>
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<td>Disease Name</td>
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<td>Central precocious puberty 2 (CPPB2) 615356</td>
<td>Unknown Chr 15q11.2</td>
<td>-SNURF:TSS-DMR LOM: 2-3% -UBE3A point mutations: 10-%</td>
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<td>??</td>
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<td>-MKRN3 point mutations</td>
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<td>Abreu et al., 2013</td>
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<td>Pseudohypoparathyroidism 1B (PHP1B) 603233</td>
<td>Unknown Chr 20q13</td>
<td>-20q13 maternal deletion: 8.5% -GNAS DMRs LOM: 42.5% - upd(20)pat: 2.5% -20q13 point mutations: 46.5%</td>
<td>Resistance to PTH and other hormones, Albright hereditary osteodystrophy, subcutaneous ossifications, feeding behaviour anomalies, abnormal growth patterns</td>
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<td>-upd(20)mat</td>
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<td>Mulchandani et al., 2016</td>
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<td>Macroglossia</td>
<td>Polyamnion/oligoamnion</td>
<td>ImpDis specific prenatal features</td>
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<td>Yes</td>
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<td>SRS</td>
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<td>Oligoamnion</td>
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<td>BWS</td>
<td>Macrosomia</td>
<td>Yes</td>
<td>Placentomegaly, placental mesenchymal dysplasia</td>
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<td>KOS14</td>
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<td>Yes</td>
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<td>Yes</td>
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<td>associated phenotype in the offspring</td>
<td>Molecular MLID</td>
<td>maternal zyosity</td>
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<td>Yes</td>
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<td>KHDC3L (c6orf221) (611687)</td>
<td>Filia</td>
<td>HYDM</td>
<td>Yes</td>
<td>hom/comphet</td>
<td>Yes</td>
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<td>PADI6 (610363)</td>
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<td>Yes</td>
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</table>
IMPRINTING REGULATION AND ITS DISTURBANCES

A

MATERNAL CHROMOSOME

PATERNAL CHROMOSOME

2x

1x

1x

B

UNIPARENTAL DISOMY (UPD) MATERNAL

DELETION PATERNAL

EPIMUTATION HYPERMETHYLATION OF PATERNAL ALLELE

POINT MUTATION ON PATERNAL ALLELE
INHERITANCE OF MATERNAL EFFECT VARIANTS AND CLINICAL CONSEQUENCES

[Genetic diagram showing inheritance patterns]