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Basics and disturbances of genomic imprinting

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Abstract: Genomic imprinting ensures the parent-specific expression of either the maternal or the paternal allele, by different epigenetic processes (DNA methylation and histone modifications) that confer parent-specific marks (imprints) in the paternal and maternal germline, respectively. Most protein-coding imprinted genes are involved in embryonic growth, development, and behavior. They are usually organized in genomic domains that are regulated by differentially methylated regions (DMRs). Genomic imprints are erased in the primordial germ cells and then reset in a gene-specific manner according to the sex of the germline. The imprinted genes regulate and interact with other genes, consistent with the existence of an imprinted gene network. Defects of genomic imprinting result in syndromal imprinting disorders. To date a dozen congenital imprinting disorders are known. Usually, a given imprinting disorder can be caused by different types of defects, including point mutations, deletions/duplications, uniparental disomy, and epimutations. Causative trans-acting factors in imprinting disorders, including ZFP57 and the subcortical maternal complex (SCMC), have the potential to affect multiple DMRs across the genome, resulting in a multi-locus imprinting disturbance. There is evidence that mutations in components of the SCMC can confer an increased risk for imprinting disorders.

Keywords: genomic imprinting, differentially methylated regions, epigenetic reprogramming, imprinting cycle, subcortical maternal complex, (multi-locus) imprinting disturbances, maternal effect mutations

Introduction

Genomic imprinting utilizes parent-of-origin-specific epigenetic modifications (DNA methylation and histone modifications) to ensure the transcription of affected genes or ncRNAs from only one parental (maternal or paternal) allele. Roughly 150 genes and several hundred microRNAs are imprinted in humans. Most of the imprinted genes encode proteins, like the fetal growth factor IGF2, and some of them are non-coding RNAs, like the *H19* lncRNA or miRNAs. The imprinted miRNAs fine-tune the expression level of imprinted and non-imprinted target genes by binding to the respective mRNAs, and consequently inhibit the translation of these or induce the cleavage and degradation of the bound mRNA [1]. The imprinted genes also interact with and regulate other imprinted and some non-imprinted genes, similar to the imprinted miRNAs, thus suggesting the existence of an imprinted gene network (IGN) [2].

Imprinted genes are organized in genomic domains that can be several megabases in size; some encompass clusters of imprinted miRNAs, like the *MEG3* polycistronic gene that encodes 39 miRNAs (the miR-379/410 cluster, also called *MIRG*). The monoallelic expression of imprinted genes within these clusters is mainly regulated by differentially methylated regions (DMRs), which have been methylated in the gametes of one parent and not in the other [3]. When the DMR is methylated on the maternal copy it resides usually within a CpG-rich genomic region (CpG island). Currently 38 germline-derived DMRs are known and the majority of these imprinted DMRs (iDMRs) originate in the oocyte, i. e., they are maternally methylated [4]. Depending on their methylation status iDMRs are bound by protein complexes, like CTCF/cohesin, or riboprotein complexes, which results in allele-specific folding of the chromatin and further (epigenetic) modifications.

Most imprinted genes/ncRNAs are parent-specifically regulated by DNA methylation (canonical imprinting). Only recently it has been reported that imprinted expression can also be mediated by inherited histone modifications (non-canonical imprinting) [5]. Here H3K27me3 inherited from the oocyte is required to silence the maternal allele of the affected DNA domain. This repressive histone modification is gradually lost during preimplantation development. Following implantation the DNA of the maternal allele becomes methylated in extraembryonic cell lineages, thus creating secondary DMRs at the non-canonical sites [6]. In the embryonic cell lineages the DNA of both alleles becomes methylated, resulting in the loss of imprinting in the somatic tissues. A significant proportion of non-canonically imprinted domains overlaps with endogenous retroviral insertions that consequently may act as imprinted ncRNAs or function as imprinted enhancers for neighboring genes.

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Evolution of imprinting in mammals

Most protein-coding imprinted genes are involved in embryonic growth, development, and behavior [7]. What is the evolutionary benefit of genomic imprinting? Genomic imprinting is a phenomenon that is observed in some plants and in higher (placental) mammals. Placental cells have diverse functions in pregnancy from invasion into the maternal uterus, fetal growth, and development, up to toleration and support of the pregnancy. These key functions are prone to selective pressures and might be a reason why genomic imprinting has evolved in placental mammals: The fact that paternally expressed genes commonly have a growth-promoting function, whereas maternally expressed genes tend to be growth-limiting suggests that the parents have different interest in the development of the next generation (parental conflict hypothesis [8]). According to this model, parent-specific gene expression of a gene could have evolved when firstly changes in the total level of expression of both alleles at the locus influence a trade-off between the fitness of the fetus and its mother. The fitness of the fetus could, e. g., be enhanced by increases in expression of the gene at the expense of the mother. Secondly individuals – on the population level – have different probabilities of carrying the individual maternal and paternal alleles. The different probabilities arise at the population level, since the individuals are more closely related regarding their maternal alleles (mother and fetus share the maternally derived allele of the fetus) than their paternal alleles (the mother usually does not carry an identical-by-descent copy of the paternally derived allele of the fetus and different offspring of a mother can have different fathers). As a consequence of both requirements, natural selection may favor alleles with effects that differ, depending on an allele's sex-of-origin in the previous generation [9].

An alternative explanation would be the adaptive integration of the maternal and offspring genomes, supported by the fact that the sole expression of maternal alleles is favored [10].

Life cycle of imprinting

There are two epigenetic reprogramming cycles, i. e., in the germline and after fertilization, which both involve genome-wide demethylation and remethylation waves (Fig. 1). In mouse the primordial germ cells (PGCs) undergo almost complete DNA demethylation in the two phases [11]. Global DNA methylation is erased during PGC expansion and migration, whereas imprinted and meiotic

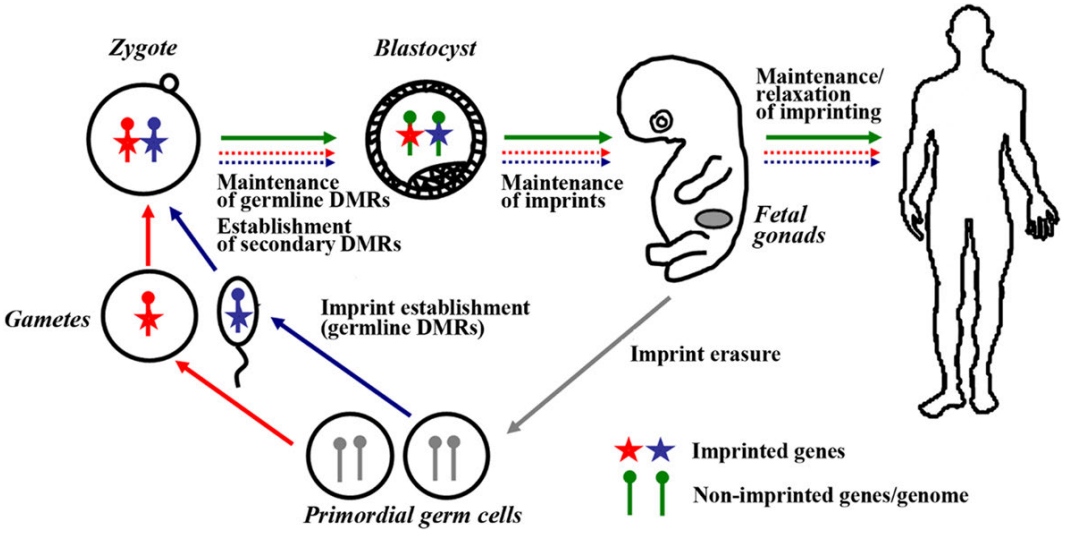

Figure 1: Imprinting cycle. Two epigenetic reprogramming cycles, each involving genome-wide demethylation and remethylation waves, occur in the germline and after fertilization, respectively. In the fetal germline essentially all epigenetic information is erased (gray lines) and then, depending on the sex of the germline, replaced by male (blue) or female germline-specific (red) methylation patterns. The two germ-cell genomes that are combined at fertilization undergo postzygotic genome reprogramming, during which most germline patterns are erased again. Somatic patterns (green), which are identical on both parental chromosomes, are established *de novo*. Only imprinted genes maintain their germline patterns during development and differentiation. A decay of the methylation landscape including relaxation or loss of imprinting may occur during ontogenesis and ageing of the individual.

genes become only demethylated upon their entry in the genital ridge [12]. Removal of most or all epigenetic information from previous generation(s) ensures an equivalent epigenetic state of PGCs prior to male versus female germline differentiation. Human PGCs retain less than 10 % of methylation at week 11, with remethylation starting at week 19 of fetal development [13]. Mouse data suggest that in the male germline *de novo* methylation is initiated prenatally in mitotically arrested pro-spermatogonia and completed postnatally in a gene/sequence-dependent manner until the pachytene spermatocyte stage [14]. In marmoset, which more closely resembles human spermatogenesis, remethylation occurs progressively during postnatal and pubertal development, continuing until adulthood [15]. In the female germline, maternal methylation imprints are also established in a gene-specific manner; however, this occurs during later stages of oocyte growth [16]. Transcriptional read-through from an oocyte-specific upstream promoter is required for *de novo* iDMR methylation. Most likely, transcription leads to deposition of H3K36me3, which then recruits Dnmt3a and its cofactor Dnmt3l [17–19]. In the mouse model, maternal imprints appear to be set by meiotic metaphase II, whereas in humans some maternal imprints may not be completed until after fertilization shortly before pronuclear fusion [20].

In the zygote and early embryo, genome-wide DNA demethylation erases most germline methylation patterns, followed by *de novo* methylation and establishment of somatic methylation patterns around the time of implantation [21]. Postzygotic reprogramming appears to be a dynamic balance between global demethylation and region-specific remethylation. In primate eight-cell embryos, remethylation becomes more pronounced than demethylation, consistent with an overall increase in DNA methylation level [22] and activation of the embryonic genome [23]. Only the 100–200 imprinted genes among our approximately 21,000 genes escape this methylation reprogramming after fertilization and maintain their germline-specific methylation and activity patterns with the help of DNMT1 [24] and proteins like DPPA3/STELLA [25] and ZFP57 [26]. Despite species differences, in both humans [27] and mice [28], demethylation of the paternal genome after fertilization is much faster and more complete than that of the maternal genome. Therefore, it may be more difficult to protect paternal methylation patterns from postzygotic demethylation than maternal methylation patterns, explaining the abundance of maternal methylation imprints [4].

Loss of imprinting

Contrary to common belief, iDMR methylation in differentiated cell types/tissues is not black and white. The non-imprinted and to a lesser extent the imprinted parental allele of a given gene can show considerable methylation variation between individuals [29, 30]. This may be due to genetic variation or environmental and stochastic factors. The percentage of methylated non-imprinted alleles and unmethylated imprinted alleles of a given gene can range from several percent to more than 50 % in blood samples from normal individuals. This relaxation of imprinting implies that in a percentage of normal body cells both alleles of an imprinted gene are methylated (and by extrapolation functionally silenced) or unmethylated (functionally active). Indeed, biallelic expression of *IGF2* was observed in 10–20 % of blood samples from healthy individuals [31]. In normal colonic tissue, this relaxation of imprinting is thought to predispose to colon cancer [32]. Loss of imprinting (somatic epimutations) is frequently observed in tumor or tumor precursor cells and contributes to the multi-step process of tumorigenesis [33].

Defect-types of genomic imprinting

The observation that imprinted genes do not seem to function uncoupled [34] from an IGN illustrates the complexity of clinical phenotypes that arise early in life when the genomic imprinting is disturbed. To date a dozen syndromal imprinting disorders are known. In principal four different major types of defects have been observed in imprinting disorders: point mutations (single nucleotide variants [SNVs]), deletions/duplications (copy number variants [CNVs]), uniparental disomies (UPDs), and epimutations (e. g., gain of methylation [GOM] and loss of methylation [LOM]) (Fig. 2).

Point mutations affecting imprinted genes are inherited according to the Mendelian rules, but only have functional consequences when they affect the active parental allele. Examples are mutations in the maternally expressed negative cell-cycle regulator *CDKN1C* that cause Beckwith–Wiedemann syndrome (BWS) as loss-of-function mutations and Silver–Russell syndrome (SRS) syndrome as gain-of-function mutations [35].

Imprinted gene deletions have similar effects as the loss-of-function mutations, when inherited with the active locus. For example, deletions on the maternal chromosome 15q11-q13 are responsible for roughly 75 % of Angelman syndrome cases [36], whereas deletions on the

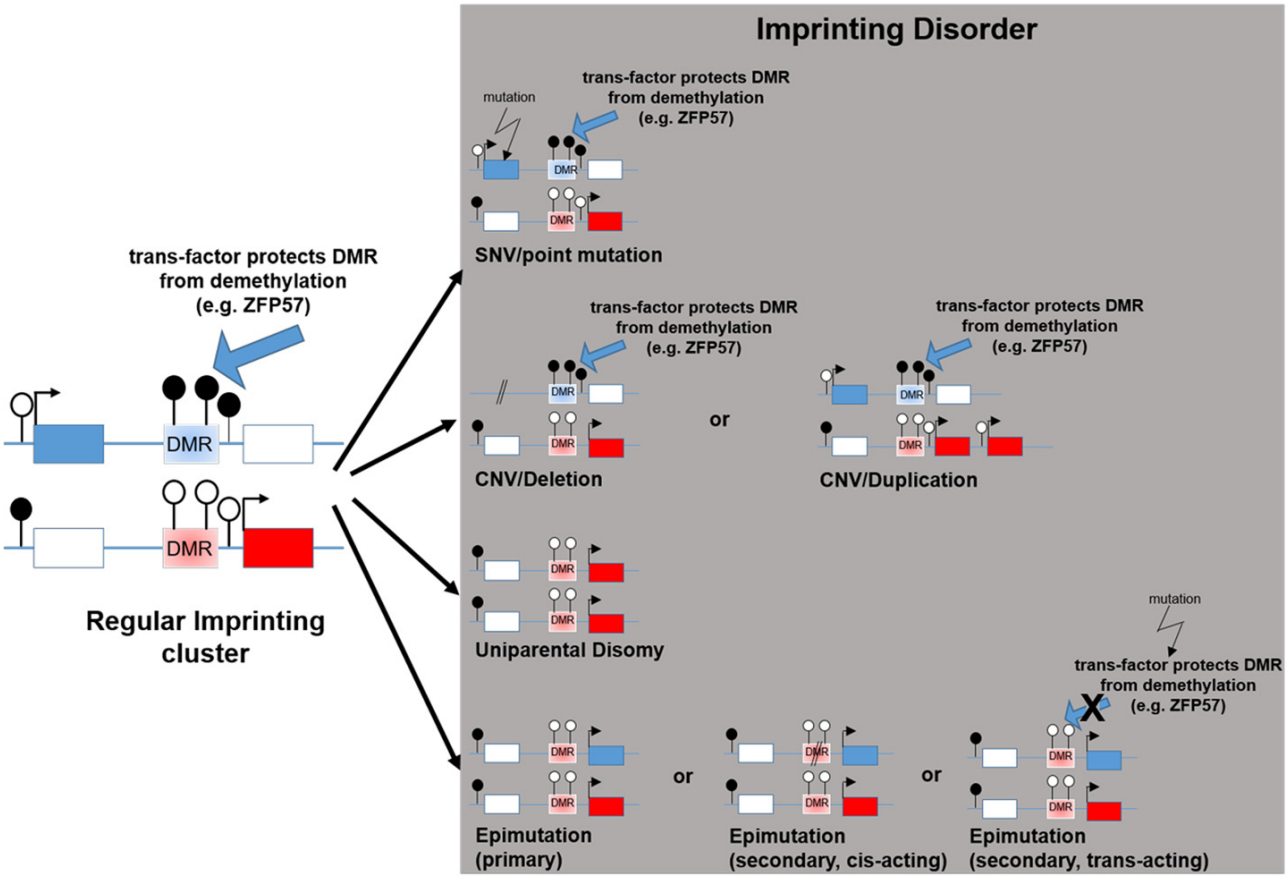

Figure 2: Known types of genomic imprinting disturbances. DMRs regulate the monoallelic expression of imprinted genes (blue = paternal, red = maternal). They are methylated in a parent-of-origin-specific manner (filled lollipops = methylated, white lollipops = unmethylated), resulting in activation or inactivation of associated imprinted genes within the controlled domain by additional epigenetic marks like promoter methylations. Note that this is an exemplary illustration of imprinting mechanisms and *in vivo* mechanisms are more complex (e. g., DNA methylation is not always coupled with silencing). Methylated DMRs are protected from demethylation during embryogenesis by trans-factors like ZFP57. A SNV/point mutation in the active copy of an imprinted gene leads to loss of function or gain of function of the affected gene. CNVs can be deletions or duplications that, as long as they affect the active copy of an imprinted gene, result either in a reduction (deletion) or in an increase (duplication) of effective gene dosage. Uniparental disomy can be either maternal or paternal (only two maternal or paternal chromosomes are present), resulting in potential expression of both copies of the parentally active genes and silencing of the imprinted genes within the affected domains that are normally expressed from the other parental allele. The figure depicts a maternal UPD, with increased expression of maternally active genes and loss of paternally expressed imprinted genes. Epimutations can also cause an imprinting disorder. DMR LOM as an example of a primary epimutation resembles the effects of a UPD, with the resulting dosage alterations of affected imprinted genes. Secondary epimutations alter the regulatory effect of the DMR by disturbing an interaction with a trans-factor (like ZFP57) that is essential for mediating the methylation specific effects on imprinted genes of the cluster. Secondary epimutations are mutations within a DMR that affect trans-factor binding, or are mutations in a trans-factor itself.

paternal chromosome 15 account for roughly 70 % of Prader–Willi syndrome.

UPDs originate from meiotic non-disjunction (maternal UPDs) prior to fertilization or mitotic segregation defects (non-disjunction defects) early in development. Maternal UPDs always have alternating segments of iso- and heterodisomy because of the cross-overs in meiosis. Paternal UPDs are usually the result of the duplication of a single parental chromosome (uniparental isodisomy), originating from a monosomy rescue. Theoretically, they may also result from trisomy rescue of an embryo fertilized by a disomic sperm or complementation of a nullisomic oocyte and a disomic sperm (uniparental heterodisomy). The affected cells and their progeny have whole chromosomes (UPDs) or parts of chromosomes (segmental UPDs) inherited from only one parent. When the genomic region covered by a UPD contains imprinted genes, this leads to aberrant transcriptional dosages of the affected genes. This can cause complex molecular defects with syndromal clinical phenotypes. BWS and overgrowth are associated with pa-

ternal UPD 11p15.5, whereas SRS and growth retardation are associated with maternal UPD 11p15.5. Most imprinting disorders have a significant proportion of UPDs as the underlying molecular defect. The earlier in development the UPD arises, the higher the percentage of affected cells. The clinical appearance of the resulting imprinting disorders varies, since the patients are mosaic for the underlying defects. For this reason the first international consensus on BWS suggested to extend the definition of BWS to BWS-spectrum (BWSp) [37]. The mosaic nature of patients with UPDs (to our knowledge no case with 100 % UPD cells has been reported) also can be a problem for the molecular diagnosis of imprinting disorders. Cells in easily accessible tissues (e. g., blood leucocytes) might not contain the imprinting defect, which will be discussed in another section of this journal issue. Since UPDs arise early in development, they are neither inherited nor passed on to the next generation, since the parental imprints on the affected chromosomes are reestablished in gametogenesis.

The fourth type of defect in imprinting disorders are epigenetic alterations (epimutations) in the iDMRs marking the parental origin of imprinted genes. A defect in the appropriate DMR modification (normally methylation) can dysregulate the associated imprinted genes. Epimutations can arise through incorrect erasure or reestablishment of the appropriate iDMR methylation patterns during germline reprogramming and, thus, are transmitted to all cells of the resulting embryo/organism. In contrast to germline epimutations, imprinting defects which arise during preimplantation development affect only a subset of embryonal cells, leading to somatic mosaicism (as described for UPDs). Such constitutive [38] epimutations are present in a varying percentage of cells in different body tissues. Primary epimutations are not associated with any alterations of the DMR DNA sequence, but arise solely through perturbations of the epigenetic modification itself. This is the majority of epimutations to date described in patients with imprinting disorders. Secondary epimutations are due to genomic alterations within the iDMR (cis-acting) [39], like microdeletions within the maternal *H19*/*IGF2*:IG-DMR (ICR1) on chromosome 11p15.5, that are associated with a GOM of the DMR and consequently BWS [40]. Alternatively, mutations in factors interacting with the DMR (trans-acting) can also cause imprinting disorders [39]. The probably best-known trans-acting factor in imprinting disorders is ZFP57, which protects DNA methylation at most iDMRs [41]. Recessive mutations of ZFP57 associated with DMR LOM can be observed in patients with transient neonatal diabetes mellitus (TNDM) [42].

Multi-locus imprinting disturbance

Causative trans-acting factors in imprinting disorders have the potential to globally dysregulate epigenetic processes, thus affecting multiple DMRs across the genome. This is called a multi-locus imprinting disturbance (MLID). MLID is detected in over 10 % of patients with imprinting disorders such as TNDM, BWS, and SRS, which are frequently caused by epigenetic errors [43]. It is often observed only in a fraction of somatic cells. Similar to UPDs, the mosaic nature of MLID argues for an origin of the underlying defect early in development.

The first trans-acting factor reported for causing MLID was ZFP57 [42]. Biallelic mutations in *ZFP57* cause LOM at multiple maternally methylated DMRs regulating among others *PLAGL1*, *GRB10*, *PEG3*, and *KCNQ1OT1* and are observed in nearly half of TNDM patients [44]. Together with DNMT1, KAP1, and SETDB1, ZFP57 protects iDMRs that contain a methylated hexanucleotide motif (T/GGCC^{met}GC) from demethylation (of DNA and H3K9me3) during preimplantation reprogramming as a multi-protein complex (reviewed in [44]). This fits in the proposed scenario for MLID development in early embryogenesis with consecutive occurrence of the defect in a mosaic state. On the phenotypic level the TNDM patients with MLID have only subtle features deviating from the presentation of classical TNDM patients, like macroglossia, umbilical hernia, congenital heart defects, or learning difficulties, that are rather associated with other imprinting disorders. However, *ZFP57* mutations were subsequently described in most other imprinting disorders as well. In SRS and BWS patients also combined LOM of different DMRs can be observed, e. g., at the *H19*/*IGF2*:IG- and *KCNQ1OT1*-IG DMRs. Why similar methylation defects underlie opposite imprinting disorders is unclear. The phenotype probably reflects the extent of LOM at the affected DMRs, the most severe LOM, or the most important affected DMR in a certain organ dominating the clinical appearance [45]. Interestingly, to date no variants of other components of the ZFP57 multi-protein complex have been reported to be causative for MLID; they probably result in too severe epigenetic alterations that might be incompatible with life.

Other known trans-factors that induce MLID belong to the subcortical maternal complex (SCMC) [46]. The SCMC is a multi-protein complex that forms subcortically in the mature oocyte from proteins that are translated from the maternal genome in the oocyte and early embryo. For this reason, pathologic variants of SCMC components are called “maternal effect mutations” (reviewed in [44]).

Among other functions the SCMC ensures developmental progress during the two-cell stage by ensuring correct epigenetic reprogramming of the zygote and preventing aneuploidy. Three genes of the *NLRP* gene family (*NLRP2*, *NLRP5*, and *NLRP7*) encode proteins that belong to the SCMC and were reported to lead to imprinting disorders (TNDM, SRS, BWS) with MLID [47, 48]. Pathogenic mutations in these *NLRP* genes occur in the mothers of MLID patients that generally experience reproductive problems (e. g., recurrent miscarriages and hydatidiform moles). Other variants of genes of the SCMC have also been identified as maternal effect mutations in imprinting disorders with MLID (*PAD16*, *OOEP*, *UHRF1*, and *ZAR1*) [47]. Here also the clinical phenotypes of TNDM, SRS, and BWS have been observed.

Imprinting defects in reproduction

Accumulating evidence suggests an association between maternal effect mutations in components of the SCMC and miscarriages including (recurrent) hydatidiform mole and an increased risk for pregnancies with imprinting disturbances. However, epigenetic marks can be influenced not only by genetic factors, like the SCMC components, but also by environmental factors. Therefore, a proportion of primary epimutations may be caused by environmental exposures in early development [49]. So far, no increased risk of imprinting disorders that could be connected to environmental factors in preceding generations has been reported. BWS and maybe some other imprinting defects appear to occur more frequently in children conceived by assisted reproductive technologies (ARTs) [50]. Ovulation induction by hormones or *in vitro* maturation of oocytes may interfere with imprint establishment and/or maintenance of maternal methylation imprints during late stages of oocyte development [51, 52]. Mosaic methylation errors in some ART children with imprinting disorders [53] are consistent with the view that underlying epimutations occur during the first cell divisions in several-day-old embryo cultures. Numerous studies have described aberrant methylation patterns of imprinted genes, in particular *MEST*, *SNRPN*, and *H19*, in infertile men [54]; however, these associations have to be interpreted with caution and may be at least partially due to somatic cell contamination of oligozoospermic samples [55].

Conclusions for research and clinical practice

- Genomic imprinting ensures parent-of-origin-specific expression of affected genes by DNA methylation and histone modifications.
- Imprinted genes are usually organized in genomic domains that are regulated by differentially methylated regions (DMRs).
- There are two genome-wide DNA demethylation and remethylation cycles, i. e., in the germline and during early embryogenesis; only the imprinted genes escape postzygotic epigenetic reprogramming, maintaining their germline-specific methylation imprints and activities.
- Defects of genomic imprinting, which can be caused by genetic mutations, deletions/duplications, uniparental disomy, and epimutations, can result in one of 12 known syndromal imprinting disorders.
- Aberrations in trans-acting factors like ZFP57 and the subcortical maternal complex (SCMC) can affect multiple DMRs and result in a multi-locus imprinting disturbance (MLID).

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