

Genomic imprinting disorders: lessons on how genome, epigenome and environment interact

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Abstract |

Genomic imprinting, the monoallelic and parent-of-origin-dependent expression of a subset of genes, is required for normal development. Its disruption leads to human disease involving isolated or multi-locus epigenetic changes that can be traced back to alterations of *cis*-acting sequences or *trans*-acting factors controlling the establishment, maintenance and erasure of germline epigenetic imprints or may have no evident genetic cause. Recent insights into the dynamics of the epigenome including the effect of environmental factors suggest that the developmental outcomes and heritability of imprinting disorders are influenced by interactions between the genome, the epigenome and the environment in germ cells and early embryos. In this Review, we discuss the latest advances in the study of genomic imprinting, focusing on the imprinting life-cycle and its possible errors leading to human diseases. We discuss the modes of inheritance of imprinting defects and

evidences from humans and animal models that environmental factors may influence genomic imprinting. Finally, we highlight areas requiring additional research that could complete our understanding of imprinting disorders, as well as new technological advances that might correct imprinting errors.

Introduction

In **therian** mammals, a subset of autosomal genes is preferentially expressed from only one of the two parental chromosomes, some from the maternally inherited allele, others from the paternal allele¹. This parental origin-dependent expression results from differential epigenetic marking, primarily from methylated cytosine at CpG dinucleotides of genes during gametogenesis in the male and female germline. These genomic imprints endure for one generation, from their establishment in mature germ cells of an individual to their erasure in the gamete precursors of their progeny. Genomic imprinting thus represents a type of intergenerational epigenetic inheritance. Of note, parent-of-origin-dependent methylation differs from sequence-dependent allelic methylation, in which stochastic fluctuation between **epialleles [G]** is influenced by genetic variants².

In humans, approximately 100 imprinted genes have been identified³⁻⁵. Many imprinted genes have important roles during human development, and alteration of their expression and function can lead to imprinting disorders (Table 1), congenital conditions with a lifelong impact on health and in some cases increased cancer risk⁶. Molecular changes underlying imprinting disorders comprise genetic changes, such as copy number variants (CNVs), **uniparental disomy [G]** (UPD), and pathogenic gene sequence variants, or epigenetic changes that affect the regulation of imprinted loci (**epimutations [G]**). The frequency of the four types of molecular alterations varies markedly between different imprinting disorders, with the highest frequency of epimutations in the chromosome 11p15-associated disorders Beckwith–Wiedemann syndrome (BWS) and Silver–Russell syndrome (SRS)⁷. Epimutations that occur without detectable DNA sequence changes are referred to as primary epimutations and may represent random or environment-driven errors in the establishment or maintenance of an epigenetic programme. By contrast, secondary epimutations arise downstream from genetic changes that affect *cis*-acting elements or *trans*-acting

factors⁸. As normal imprinting marks once set persist throughout the life course of an organism, similarly, imprinting errors originating in the germline as primary or secondary epimutations are permanently maintained in somatic tissues, resulting in disease phenotypes later in development. Primary or secondary epimutations (as well as UPDs) that occur after fertilization can result in somatic mosaicism (Box 1). Although genetic alterations and epimutations differ in their nature and aetiology, they all disturb the fine-tuned balance of imprinted gene expression. In some cases, loss of methylation (LOM) and gain of methylation (GOM) of the same imprinting centre result in 'mirror' disorders that are broadly characterized by opposite clinical features and gene expression patterns, for example, in the case of BWS and SRS (Table 1 and Fig. 1)⁷.

Advances in whole-genome sequencing and single-cell genome-wide analysis are driving the study of imprinting disorders arising from pathogenic variants that disrupt key epigenetic reprogramming processes in early embryogenesis, shedding new light on the dynamics of the epigenome as it passes from parents, through gametes, to offspring. Furthermore, recent studies on the interaction between environment and the epigenomes of gametes and early embryos suggest mechanistic explanations for the sporadic occurrence of imprinting errors.

This Review focuses on imprints that effect essentially permanent and ubiquitous (rather than tissue-specific or transient (Box 2)) changes on gene expression potential at affected loci. We begin with a brief overview of the genomic basis of imprinting and its control, before reviewing the lifecycle of genomic imprinting and how disruption of the individual factors involved in the establishment, maintenance and erasure of imprints can result in disease. Finally, we discuss the heritability of imprinting defects and the role of environmental insults in imprinting disorders. For details on the evolutionary significance of genomic imprinting^{1,9}, the methods for imprinting analysis¹⁰, the physiological role of imprinted genes⁶ or the chromatin mechanisms in imprinting¹¹, the reader is referred to previous authoritative reviews.

[H1] The genomic basis of imprinting

The majority of imprinted genes are found in clusters, called imprinted domains, which enables coordination via shared regulatory elements such as long non-coding RNAs (lncRNAs) and differentially methylated regions (DMRs), where DNA methylation differs between the maternally derived and paternally derived alleles.

Each imprinted domain is controlled by an independent 'imprinting centre', which is generally characterized by a germline differentially methylated region (gDMR), also known as primary DMR (Fig. 2). About 35 gDMRs associated with imprinted loci have been identified in the human genome (Table 2)¹². gDMRs are also characterized by different chromatin configurations on parental chromosomes, with histone marks characteristic of closed chromatin (for example, histone 3 lysine 9 dimethylation (H3K9me2), trimethylation (H3K9me3) and histone 4 lysine 20 trimethylation (H4K20me3)) on the methylated allele, and histone marks characteristic of open chromatin (for example, H3K4me2 and H3K4me3) on the unmethylated allele (Fig. 2)^{4,11,13}. The methylated and unmethylated gDMR alleles are recognized by different transcription factors whose function is to direct differential epigenetic modification and imprinted expression of the locus (Fig. 2)¹⁴. Whereas maternally methylated gDMRs are more numerous, intragenic and generally correspond to promoters, often of lncRNAs, gDMRs methylated on the paternal chromosomes are intergenic and may function as insulators or enhancers (Table 2)^{1,15}. Of note, in multigenic imprinted domains, the imprinting centre often directs the expression of genes from both the chromosome on which is methylated and the opposite parental chromosome; this situation arises from the regulatory interactions between imprinting centres and the gene products, both coding and noncoding, under their control (Fig. 2).

[H2] Allele-specific expression in somatic cells

Imprinted genes can display monoallelic expression in most or all cell types, but for some genes imprinted expression is restricted to specific tissues (for example, *UBE3A*^{16,17}) or developmental windows (for example, *KCNQ1*¹⁸), or monoallelic expression and/or methylation can differ between individuals¹⁹⁻²¹. To control the allele-specific expression of imprinted genes in somatic cells, gDMRs direct the establishment of further allele-specific epigenetic features within the imprinted domain during development. These include secondary DMRs (also known as somatic DMRs), which correspond mostly to gene promoters and transcription factor binding sites (Table 2)²⁰, chromatin modifications and higher-order chromatin structures (possibly resulting from CTCF-cohesin interactions)^{22,23}, and lncRNAs with silencing capacity for flanking imprinted genes in cis²⁴ (Figs 1, 2a) (reviewed in REF. 1). In other cases, imprinted gDMRs direct alternative splicing, transcription

elongation or polyadenylation site usage, which results in allele-specific transcript isoforms^{20,25}. A minority of genes with parent of origin-dependent expression in somatic tissues have no evident DMR in their vicinity²⁰, and their allele-specific expression may possibly be controlled by epigenetic features other than DNA methylation²⁶.

Tandem repeats are a prominent feature of imprinting centres²⁷. Some repeats function to concentrate a high density of binding sites for transcription factors that regulate imprinted gene expression; for example, the tandem repeats in the *H19-IGF2* IG-DMR concentrate methylation restricted binding of ZFP57 and CTCF that are critical for imprinting (Fig. 2)^{28,29}. In this case, their recombination results in recurrent imprinting defects³⁰. On the other hand, deletion of a large array of repeats of long-interspersed elements (LINE-1) in the *Dlk1-Dio3* imprinting domain in mouse embryonic stem (ES) cells did not disrupt imprinting, or, apparently normal development of both maternal and paternal mutant mice³¹, which does not support a role for these repeats in imprinting.

Imprinted gene products intensify their exquisite regulation by co-operation in a network (Imprinted gene network, IGN)^{32,33}. For example, the transcription factor PLAGL1³² and the *H19* lncRNA³³ have been shown to regulate the mRNA level of several members of the IGN in a DNA methylation-independent manner, in mouse tissues. The human lncRNA *IPW*, which resides within the Prader–Willi syndrome (PWS) locus on chromosome 15, is able to regulate the expression of *MEG3* on chromosome 14 by targeting the EHMT2 H3K9 histone methyltransferase (also known as G9a) to its imprinting centre³⁴. Furthermore, many imprinted gene clusters encode microRNAs (miRNAs) and small nucleolar RNAs (snoRNAs), which may be involved in the post-transcriptional control of imprinted genes³⁵. **These interactions may explain some of the overlaps observed in the phenotypes of imprinting disorders (Table 1).**

[H2] Multilocus imprinting disturbances

A subset of patients with imprinting defects exhibits multilocus imprinting disturbances (MLID), that is, imprinting disruption at multiple loci across the genome. MLID is confined to epimutation subgroups of imprinting disorders (Table 1) and involves loci associated with known imprinting disorders as well as those not currently linked with specific phenotypes^{36,37}. To date, most patients with MLID have

shown clinical features characteristic of one imprinting disorder, notably BWS, SRS or transient neonatal diabetes mellitus (TNDM), which is probably due to the high frequency of epimutations in these imprinting disorders. However, epigenotype–phenotype correlations are not always obvious, possibly because of the spectrum of epimutations involved or their mosaic nature (Box 1)³⁷⁻³⁹.

[H1] The imprinting life-cycle and disease

Throughout their generational lifespan (Fig. 3), genomic imprints must be maintained and preserved from epigenetic reprogramming in somatic cells. Many factors involved in these complex processes and their DNA binding sites can be targets of mutations that cause human imprinting disorders (Table 3).

[H2] Imprinting centre methylation dynamics in germ cells

Of the ubiquitous gDMRs present in somatic tissues, all but two originate from the oocyte (Table 2)^{5,12}. This disparity reflects fundamental differences in the mechanisms of methylation acquisition in the female and male germlines, and in the treatment of parent-of-origin-derived methylation in the zygote (Fig. 3)²¹. In primordial germ cells (PGCs), the precursors of sperm and oocytes, germline specification requires remodelling of the epigenome as a pre-requisite for gametogenesis. Our knowledge of these processes comes chiefly from studies in mice^{40,41}, and the characterization of human PGCs has revealed subtle interspecies differences, but overall the global erasure of methylation is comparable⁴²⁻⁴⁴.

A hallmark of PGC remodelling is imprint erasure. Genome-wide de-methylation of 5-methylcytosine (5mC) is a passive process during PGC expansion that results from diminished protein levels of the *de novo* DNA methyltransferase DNMT3A and UHRF1, the recruitment factor of the maintenance DNA methyltransferase DNMT1. Reprogramming of imprinted methylation follows slower kinetics. In mice, it is associated with oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) by the ten-eleven translocation 5mC dioxygenase 1 (TET1) and TET2; this modification is not recognized by the maintenance methylation machinery and therefore promotes passive demethylation^{40,41,44,45}.

Errors in the erasure process have been observed in patients with rare, sporadic imprinting disorders. In the case of GOM of the PWS/AS imprinting centre (also

known as *SNURF:TSS-DMR*), grandmaternal methylation is not erased in paternal PGCs, and as a result the paternal allele retains this maternal imprint (Fig. 4a)⁴⁶. Similarly, the hypermethylation of imprinting centres in sperm from subfertile individuals is consistent with incomplete erasure of imprints⁴⁷.

Re-methylation and imprint acquisition occur asynchronously between the sexes, with *de novo* methylation in the male germline occurring before birth and maintained through many cycles of mitotic division before entry into meiosis, whilst female germ cells remain hypomethylated until maturation (Fig. 3). *De novo* methylation and imprint acquisition initiate in meiotically arrested (at prophase I) mouse oocytes following birth, and are largely completed by the germinal vesicle stage of development and resumption of meiosis^{42,48-51}. Such dynamics have not been extensively studied in human oocytes⁵²; however, in humans, meiosis II oocytes and the first and second polar bodies have comparable methylation, including at imprinted maternally methylated gDMRs⁴⁹ (D. M., unpublished observations), which suggests the timings are similar to mouse.

On the basis of data derived from mouse models, the majority of methylation is deposited in oocytes by DNMT3A and its obligate, catalytically inert, cofactor DNMT3L^{51,53,54}, whereas both DNMT3A and DNMT3B contribute in male germ cells⁵⁵. DNMT1 has an auxiliary role ensuring symmetric methylation of CpG sites in oocytes⁵⁶. Transcription and underlying chromatin signature are important factors determining methylation acquisition⁵⁰. Transcription in oocytes is required for methylation at numerous gDMRs⁵⁷, an act that may render the chromatin more accessible to the *de novo* methylation machinery and/or be associated with specific chromatin changes. The co-transcriptional histone H3K36me3 mark is deposited at intragenic CpG islands and subsequently recognized by DNMT3A and DNMT3B^{58,59}. Successive removal of dimethylation and trimethylation of histone H3K4 by KDM1A or KDM1B (known previously as AOF1 and LSD1, respectively) allows for direct interaction with DNMT3L⁶⁰⁻⁶². Despite being a generic methylase in mouse oocytes, DNMT3L is not detectable by expression profiling in human oocytes between germinal vesicle phase and meiosis II⁶³, suggesting that it is not required for *de novo* methylation in the human female germline. In mouse male germ cells, transcriptional read-through is involved in acquisition of imprinting centre methylation, whereas histone H3K4 methylation and promoter activity are present at maternal imprinting centres that are protected from *de novo* methylation⁶⁴.

Failure to establish imprints during gametogenesis can result in imprinting disorders. Establishment of gDMRs involves several enzymatic steps, any of which may be prone to stochastic errors. In oocytes, deficient transcription through CpG islands destined to be gDMRs can result in failure to establish maternal imprints⁵⁷; in such cases, there would be no mosaicism (Fig. 4b)⁶⁵. Genetic mutations affecting transcription through the gDMR have been identified in rare patients with BWS with complete and isolated lack of methylation at the imprinting centre 2 (IC2), the imprinting centre of the centromeric domain of the BWS/SRS locus (also known as *KCNQ1OT1:TSS-DMR*) (REF⁶⁶ and A. R., unpublished observations), and disruption of germline transcription is probably also present in Angelman Syndrome (AS) cases with non-mosaic LOM of the *SNURF:TSS-DMR* and deletions of the AS smallest region of deletion overlap (AS-SRO)⁶⁷.

[H2] Imprinting centre methylation dynamics in the early embryo

The divergent DNA methylation patterns of oocyte and sperm are harmonized by the time the embryo reaches the blastocyst stage^{51,68}, as part of the extensive epigenetic reprogramming that underpins zygotic genome activation [G] (ZGA; Box 3 and reviewed in REF⁶⁹) and is required first to acquire totipotency and subsequently to initiate differentiation. The murine paternal genome is demethylated early in the first cell cycle, in part by TET3-induced oxidation of 5mC^{5,49,68,70}, whereas maternal demethylation is predominantly passive, by replicative dilution during cleavage-stage divisions, possibly through the restricted activity or localization of DNMT1 and its accessory factors (Fig. 3)^{71,72}. Studies in human systems are currently limited but indicate differences in abundance and roles of DNMTs in the oocyte and embryo⁶³. However, in both humans and mice, whereas most gDMRs lose DNA methylation in pre-implantation stages^{49,51}, imprinting centres evade the embryonic wave of epigenetic reprogramming, and studies in both mouse models and human patients with rare imprinting disorders suggest they do so through interaction with critical factors expressed in the oocyte and early embryo.

[H2] Oocyte factors

DPPA3 (also known as Stella or PGC7) is required for the maintenance of DNA methylation in the early mouse embryo and protects 5mC from conversion to 5hmC

in the maternal pronucleus, by associating with chromatin marked by H3K9me2⁷³. *DPPA3* is a maternal-effect gene: concepti of maternal null mice rarely progress beyond the two-cell stage, and their genomes are severely demethylated⁷⁴.

Maternal-effect variants in NLRP proteins and associated factors have been implicated in pregnancy outcomes including hydatidiform mole [G] and infertility, as well as monozygotic twinning, pregnancy loss and MLID (Fig. 4c-d)^{37,39,75,76}. Women with biallelic inactivating *NLRP7* mutations are affected by familial hydatidiform mole (FHM)⁷⁷, where nonviable products of conception have normal biparental genomic constitution but complete loss of maternal imprinting marks. The high penetrance [G] of FHM suggests that *NLRP7* is involved in oocyte-specific imprint establishment (Fig. 4c)⁷⁸, but hypomorphic maternal *NLRP7* variants have been associated with MLID⁷⁹.

In mouse models, NLRP5 and its associated proteins are referred to as the subcortical maternal complex (SCMC)⁸⁰. They are highly expressed in the oocyte, but their mRNA and protein abundance decline to undetectable levels by blastulation⁸¹. In mouse models, maternal ablation of SCMC gene function compromises embryo development, with frequent demise between the 2-cell and blastula stage, and disruption of processes including maintenance of genome integrity, euploidy, mitochondrial function, and gene transcription and translation⁸²⁻⁸⁴. A mouse model of maternal *Nlrp2* deficiency shows severe reproductive compromise with embryo demise at all developmental stages and mosaic loss and gain of methylation at imprinted loci, indicating that abnormal subcellular localization of DNMT1 and or SCMC members may cause early embryonic loss and imprinting defects⁸⁵.

The effects of maternal SCMC variants suggest a link between DNA methylation, genome integrity and developmental competence in the early embryo. If the embryo's competence is severely compromised, both ploidy and DNA methylation may be intolerably affected, leading to embryo demise. If errors in ploidy and/or methylation are tolerated, the embryo may survive blastulation and continue development, with ongoing differentiation overwriting early epigenetic errors — except for imprints, which are indelible in somatic cells. Evidence for this comes from reports of preimplantation genetic diagnosis of embryos with maternal-effect *NLRP7* mutations in which all cleavage-stage embryos arrested and had various maternal aneuploidies⁸⁶. Arguably, if an embryo had presented with a normal chromosome

complement it would have likely developed into a molar pregnancy or severe MLID due to disturbed maternal imprints. Hence, MLID may be no more or less than evidence of embryonic crises during the critical window encompassing epigenetic reprogramming and ZGA, with an ascertainment bias for live birth and normal ploidy. Mothers with maternal-effect variants have children with variable disturbance of both paternally- and maternally-methylated imprinting centres (Fig. 4d), and a spectrum of reproductive outcomes including apparent infertility, fetal loss, hydatidiform mole, liveborn children with MLID who exhibit clinical symptoms, and liveborn children with MLID and no clinical phenotypes^{37,39}. The only consistent feature of offspring is MLID itself.

[H2] Zygotic factors

The KRAB zinc-finger protein (KRAB ZFP) ZFP57 acts as the focus for a multi-protein complex that protects imprinting centres from both passive and active demethylation⁸⁷⁻⁸⁹. ZFP57 recognizes a hexameric motif enriched in all maternally and paternally methylated imprinting centres in mouse^{90,91}. KRAB ZFPs are a large, expanding family; their rapid evolution seems to keep pace with the endogenous retroviruses (ERVs) whose expression they suppress through DNA hypermethylation⁹². Besides repressing retroviral transcription, ZFP57 protects DNA methylation of imprinted loci in early development. In mice, *Zfp57* is a maternal-zygotic-effect gene the ablation of which is incompatible with full offspring survival⁸⁷. Human *ZFP57* acts zygotically, with recessive mutations associated with the imprinting disorders TNDM and a specific pattern of MLID⁹³, although this may represent ascertainment bias of mutation patterns that are compatible with life (Fig. 4e). It is probable that additional KRAB ZFPs expressed in the oocyte, acting via maternal-effect, are involved in earlier imprinting centre maintenance in humans with a degree of redundancy between ZFPs in recruiting the KRAB repressor complex to specific gDMRs.

Human mutations have rarely been identified in other zygotic factors implicated in methylation maintenance, presumably because complete ablation would be incompatible with life, as seen in mouse models (for example, *Trim28*⁹⁴, *Uhrf1*⁹⁵ and *Dnmt1*⁷¹). Nonetheless, haploinsufficiency of *TRIM28/KAP1* has been associated with polyphenism, obesity and reduced expression of imprinted genes in mice and humans⁹⁶, and haploinsufficiencies of *DNMT1* and *UHRF1* have been described in

association with BWS and MLID, respectively^{39,97}. *Cis*-acting genetic variants have been identified in imprinting centre sequences, and study of these variants can help identify the zygotic factors that act on them to perpetuate the imprinted status (Fig. 4f-g). Inherited microdeletions in IC1 (which controls the imprinted expression of *IGF2* and *H19* at chromosome 11p15.5) mostly derived from recombination between repeats have been described in individuals with BWS³⁰ and SRS⁹⁸, but interestingly their effects are seen only in maternal and paternal inheritance, respectively, and the methylation defects are generally mosaic, once again suggesting that imprinted states acquired in the early embryo are faithfully perpetuated in subsequent development. In patients with SRS, no maternal transmissions have yet been reported, but it is suggested that loss of ZFP57 binding sites may result in postzygotic attrition of methylation (Fig. 4f)²⁹; in patients with BWS, erroneous GOM of the maternal allele is thought to result from loss of SOX2 and POU5F1 binding or weakening of CTCF binding^{28,99} (Fig. 4g). Murine models demonstrate that CTCF and ZFP57-binding sequences are involved in embryonic maintenance of IC1 imprinting^{90,100}, although some differences may exist between human and mouse species¹⁰¹.

In summary, it seems that imprinting centre sequences have characteristics that support allele-specific gene expression, chromatin organization and DNA methylation in the early embryo, enabling these patterns to evade early-embryonic reprogramming and subsequently persist in somatic tissues.

[H2] Intergenerational inheritance of imprinting defects

Most imprinting disorders caused by epimutations occur in individuals with no relevant family history (primary epimutation). In such cases the underlying molecular cause may be associated with an environmental insult or stochastic error, and the risk of further cases in the family is minimal. Consistent with the hypothesis of non-heritability of primary epimutations, a methylation defect shown to have originated in an individual with SRS was subsequently abolished in his germline (Fig. 5a)¹⁰². However, a subset of isolated cases may have an underlying genetic cause even in the absence of a family history (secondary epimutation). Multiple genetic causes of secondary epimutations have been identified, providing important information on the *cis*-acting elements and *trans*-acting factors involved in imprinting control. Often, these cases are autosomal dominantly inherited, with parent-of-origin effects on

penetrance, such that the epimutation and clinical phenotype only appear on maternal or paternal transmission (for example, familial PWS with paternally-inherited imprinting centre mutations (Fig. 5b)⁴⁶, and familial BWS associated with maternally-inherited IC1 microdeletions (Fig. 5c)³⁰ and POU5F1-binding sites variants⁹⁹). However, autosomal recessively inherited TNDM is caused by pathogenic variants in *ZFP57* (Fig. 5d)⁹³, and maternal pathogenic variants in maternal-effect genes (*NLRP2*, *NLRP5*, *NLRP7*, *PADI6* and *OOEP*) (Fig. 5e)³⁹ are associated with MLID in offspring. In the case of maternal-effect variants, the recurrence risk after an affected pregnancy can be up to 100% (recurrence may be avoided by oocyte donation), although even in the most severe forms penetrance may be incomplete (for example, *NLRP7* familial hydatidiform moles) and there may be variable phenotypic expression^{37,39}.

Genetic variants associated with imprinting centre epimutations can demonstrate variable clinical presentation and incomplete penetrance²⁸ or apparent anticipation [G] with increased clinical severity over multiple generations¹⁰³. These findings suggest that whereas highly-penetrant variants, such as those disrupting transcription factor binding, exhibit patent and penetrant phenotypes, genomic variants with lower penetrance may need to be identified by comprehensive sequencing efforts. Consistent with this hypothesis, a recent study demonstrated that frequent sequence variants have subtle effects on imprinted methylation, expression and phenotype²⁰, suggesting that imprinting is a more quantitative than categorical phenomenon.

[H1] Environmental influences on imprinting

In addition to genetic causes of imprinting centre epimutations, environmental factors may also influence the imprinting process. In humans, evidence for this phenomenon derives from assisted reproductive technologies (ART)¹⁰⁴. Other environmental influences on imprinting centres may include nutritional status or exposure to chemical pollutants in utero¹⁰⁵. In many cases, changes in methylation represent increased variability on the methylated allele, likely relating to a failure of maintenance, or an adaptive response to the external stimuli.

[H2] Assisted reproductive technologies

ART is usually performed for male and/or female infertility and includes procedures such as ovarian hyperstimulation to obtain multiple oocytes for retrieval, in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and embryo culture and transfer, all of which coincide with critical events in epigenome reprogramming. Reports of ART-conceived children with rare imprinting disorders (for example, AS and BWS) first suggested a potential link with the occurrence of epimutations at imprinting centres (Fig. 6)¹⁰⁶⁻¹⁰⁸. Epidemiological studies have provided further evidence for an increased risk of having children with BWS, AS or SRS when using ART^{104,109}, although the absolute risk is very small (for example, for BWS up to 10-fold increased risk compared to no ART, and <0.1% of all children conceived with ART help)¹⁰⁹. ART has also been associated with an increased frequency of MLID, but this has not been a universal finding^{110,111}. MLID and Large Offspring syndrome (a condition with similarities with BWS) have been observed in bovine fetuses conceived by IVF¹¹². In pigs, global genomic DNA methylation and/or gene expression, including imprinted loci and genes involved in epigenetic reprogramming, were altered in blastocysts produced by IVF and partially restored with addition of natural reproductive fluids¹¹³. Furthermore, superovulation and embryo transfer induced developmental defects and imprinting centre epimutations in the placenta of mouse models¹¹⁴.

In addition to ART-related procedures, infertility per se has been linked to the pathogenesis of imprinting disorders (Fig. 6). The frequency of AS with epimutations was shown to be increased in subfertile couples, independent of IVF, ICSI or embryo culture¹¹⁵. More recently, impaired methylation of imprinting centres was reported in sperm of subfertile men⁴⁷. Furthermore, unrecognized ART-associated epigenetic alterations may play a role in the increased risk of low birthweight and congenital anomalies that have been reported in ART-conceived children¹¹⁶ and animal models¹¹⁷. Maternal age and delayed ovulation or fertilization are associated with depletion of oocyte proteins and RNA stores and altered developmental fitness of embryos¹¹⁸⁻¹²⁰, suggesting that maternal effect genes may be critically vulnerable to these or other challenges that occur during ART (Fig. 6).

Disentangling the effects of infertility and ART in the aetiology of ART-associated imprinting disorders in humans is difficult and not all studies have shown an association between ART and altered methylation, with some reports suggesting that there is no increase in mosaicism or methylation aberrations at imprinted

gDMRs^{121,122}. Other groups have reported perturbed imprinting in pre-implantation embryos suitable for transfer¹²³, suggesting that — similar to aneuploidy — epigenetic mosaicism in early embryos may be a normal occurrence. The rarity of ART-associated imprinting disorders suggests that they may result from a combination of multiple interacting factors, including specific aspects of ART protocols, infertility, genetic susceptibility and stochastic effects (Fig. 6). Moreover, epidemiological surveys often have ascertainment bias for liveborn offspring with clinically blatant phenotypes associated with imprinting disorders, while the frequency of clinical pregnancy, though well-known to be limited in ART, is not considered. Potentially, individuals with imprinting disorders represent the subset of IVF outcomes with the least pervasive disturbance and the most recognizable clinical features, and a more definitive study will require consideration of nonviable products of conception at all stages, including both epigenome and genome integrity.

[H2] Nutrition and metabolic disorders

Certain developmental windows are especially vulnerable to abnormal nutritional states¹²⁴, including pre- and early post-implantation development and lineage segregation when epigenetic modifications are re-established. Recent studies have indicated that maternal metabolic disorders can have lasting effects on offspring through many pathways, which are beginning to be characterized (Fig. 6). Maternal dietary and genetic obesity have been shown to reduce *Dppa3* expression in mouse oocytes; the resultant significant increase in 5hmC and concomitant reduction in 5mC in maternal pronuclei produces subsequent hypomethylation at several imprinted gDMRs¹²⁵.

Nutritional status may also affect epigenetic profiles at imprinted loci in a variety of ways. It is possible that the availability of free methyl donors, such as S-adenosylmethionine, a substrate for DNA and protein methylation, is limited, with evidence that methyl-deficient diets, folate levels and genetic variants in proteins involved in one-carbon metabolism all affect imprinted methylation patterns, at the 11p15.5 imprinted gene cluster^{97,126,127}. In these studies, the presence of missense amino acid substitutions in genes regulating the S-adenosyl methionine (SAM) or the inhibitory S-adenosylhomocysteine (SAH) abundance correlate with aberrant imprinted methylation^{126,127}, which also revealed a link between low vitamin B12 levels and *H19* methylation maintenance¹²⁶. Functional genetic variants of

DNMT1 in BWS patients were also observed in combination with SNV of folate metabolism pathway genes, suggesting that decreased DNMT1 enzymatic activity could be exaggerated by extreme SAM/SAH ratios⁹⁷. Furthermore, the *ZFP57* locus is a folate-sensitive region, and its genomic binding regions are metastable epialleles responsive to periconceptual conditions^{128,129}. In mouse, withdrawal of maternal dietary protein permanently altered imprinted expression of *Cdkn1c* in offspring, which was maintained into adulthood and occurred through a folate-dependent mechanism of DNA methylation loss¹³⁰. However, not all studies on isocaloric protein restriction during pregnancy have resulted in altered imprinted methylation in the newborn¹³¹, suggesting that any deregulation is likely a consequence of a general effect on global methylation. Recent evidence suggests that cells have important energy status sensors that protect the cells against metabolic stress by directly regulating epigenetic processes. The nicotinamide adenine dinucleotide (NAD)-dependent deacetylase, SIRT1 has been shown to protect methylation at imprinted loci by directly regulating acetylation of DNMT3L, at both the promoter and protein level in mouse embryonic stem cells¹³².

Endocrine disruptors

In addition to micronutrient availability, prenatal exposure to estrogenic endocrine-disrupting compounds (EDC), such as bisphenol A (BPA), results in deregulation of genomic methylation and hydroxymethylation^{133,134}, with imprinting and methylation anomalies being reported in both mouse placenta¹³⁵ and developing gametes^{136,137}. Endorsing the vulnerability of imprinted loci to EDCs, prenatal BPA exposure in humans has been associated with changes in methylation at the *MEST* locus and is linked with early childhood obesity¹³⁸. *Dnmt1* expression was found to be decreased in BPA-treated mouse spermatogonia¹³⁷, and BPA exposure during oocyte maturation altered other epigenetic marks, specifically the abundance of histone modifications, which was linked to induced oxidative stress¹³⁹. Exposure-induced oxidative stress was shown to alter both TET enzyme expression and function, leading to altered 5-hmC levels at numerous imprinted loci¹³⁴, which indicates that environmental toxicants also alter long-term imprinted gene regulation (Fig. 6). Indirect effects of the toxic compounds on DNA methylation could also be exerted as consequence of developmental and metabolic alterations¹⁴⁰.

In summary, combined genetic and environmental predispositions may erode the gametic and zygotic competence to reprogramme the epigenome, with consequences on imprint maintenance, and insights into these effects in humans may be gained by delineating the aetiology of apparently sporadic primary epimutations in individuals with imprinting disorders.

[H1] Conclusions and perspectives

The maintenance of differential DNA methylation of imprinting centres is fundamental for the survival of imprinting marks in the early embryo. Some of the key factors and genomic sequences involved in this process have been identified, but the causation and timing of their interactions require further clarification. This is particularly true for the SCMC proteins and possibly further oocyte-specific factors that affect DNA methylation maintenance in the early embryo, whose mechanisms of action and relationship with ZGA are still ill-defined. Importantly, further human-based studies are required, firstly to resolve key differences from mouse in the timing and mechanisms of epigenetic remodelling, and secondly to learn from rare cases of imprinting disorders by identifying genetic variants that predispose to imprinting centre epimutations.

It has also become evident that environmental changes can affect the epigenetic reprogramming of germ cells and early embryo, altering their developmental competence and causing imprinting disorders. The availability or activity of factors needed for imprint establishment or maintenance may be affected by exposure to chemical pollutants, under- or over-nourishment, or other emergent factors. Omic and functional analyses of early embryos and nonviable reproductive outcomes will clarify the relationship between epigenomic and genomic integrity, uncover the key processes involved, and enable the creation of model systems in which primary imprinting centre epimutations can be created and explored.

Cellular-physiological approaches are beginning to uncover key interactions of imprinted gene products, their effects on growth and metabolism, and their disturbance in imprinting disorders^{32-34,101}. Such approaches, and their extension into human pathophysiology, will shed further light on molecular mechanisms of disease, (epi)genotype–phenotype correlations, phenotypic modification by mosaicism and MLID, and potential therapies for some of the resultant endocrine and growth disturbances.

More fundamentally, there are possibilities for therapeutic correction of imprinting disorders based on the reversal of gene imprinting status. Three therapeutic approaches for the neurological disorders AS and PWS have been proposed¹⁴¹⁻¹⁴³. The AS/PWS locus contains a maternally methylated imprinting centre that directs the paternal expression of several genes, including a snoRNA cluster with a critical role in PWS and an antisense regulator (*UBE3A-ATS*) of the maternally-expressed E3 ubiquitin ligase *UBE3A*, the expression of which is lost in AS (Table 1). It was demonstrated that a topoisomerase inhibitor¹⁴³ as well as antisense oligonucleotides¹⁴¹ are able to downregulate *UBE3A-ATS* and reactivate the paternal *UBE3A* in mouse models of AS, whereas G9a inhibitors can unsilence the maternal snoRNAs in a PWS model¹⁴². Another exciting approach is the direct modification of epigenetic marks at imprinted genes using catalytically inactivated Cas9 (dCas9) fusion proteins. Although still in their infancy, dCas9–DNA methyltransferase fusions have been able to target methylation to IC1 in mouse cells¹⁴⁴, a promising technology if such alterations can be performed in an allelic fashion. Future experiments will demonstrate whether these approaches with small molecules may revert other epimutations in imprinting disorders and possibly be applied in other epigenetic-based human diseases.

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Box 1 | Epigenetic mosaicism in imprinting disorders.

Numerous patients with imprinting disorders, with or without MLID, have somatic mosaicism, in which tissues contains cells with imprinting aberrations as well as those with appropriate allelic methylation. Mosaicism is observed with all types of primary and secondary epimutations, with the exception of erasure and establishment errors (Fig. 4), indicating a more common post-zygotic aetiology³⁶. In principle, the developmental period immediately prior to implantation, when the embryonic epigenome is reprogrammed, is particularly vulnerable. During this time, failure to selectively protect imprints may result in hypomethylation in individual cells and their progeny. If the event occurs late in pre-implantation development, after lineage commitment, tissue-specific epimutations may result. Conversely, failure to preserve imprints from the substantial remethylation that occurs post-implantation may give rise to mosaic hypermethylation; this may account for the IC1 hypermethylation seen in BWS^{28,145}, and imprinted gene-DMR hypermethylation in Kagami-Ogata syndrome¹⁴⁶.

Patients with SRS or BWS often present with body asymmetry, a feature accredited to mosaicism, with recent mouse models for these two imprinting disorders identifying mosaicism in bilateral organs with asymmetric growth¹⁰⁰. Mosaic *H19* hypomethylation is common in SRS, for which severity differs markedly between patients¹⁴⁷. Detailed studies in another imprinting disorder, Angelman Syndrome, explored the timing of such an event. In a female patient with mosaic *SNURF* hypomethylation, X-chromosome analysis showed that cells with the imprinting defect had either the paternally-derived or maternally-derived X chromosome inactivated, suggesting that the insult occurred before X-inactivation and implantation¹⁴⁸. In principle, somatic imprinting errors may occur at any time in dividing cells. Immediately following replication, the methylation pattern on the template strand is recognized by the UHRF1-DNMT1 maintenance methyltransferase complex and copied onto the daughter strand. A failure to recognize or copy this pattern will result in a sustained hemimethylated profile that will segregate in subsequent cell divisions in a tissue-restricted manner.

A phenomenon related to epigenetic mosaicism is represented by discordant monozygotic twins. Discordant monozygotic twins, whereby one twin has the disorder (nearly always female and often with MLID) and the other is healthy, are over-represented in patients with BWS^{36,37,39,149} and SRS¹⁵⁰, suggesting that

monozygotic twinning is connected with epigenetic disturbances in early development. The occasional presence of mild clinical features of BWS and intermediate methylation disturbance in an unaffected twin¹⁵¹ supports the hypothesis that imprinting centre epimutations precede and possibly trigger the twinning process in the early embryo.

Box 2 | **Transient imprinting**

A transient form of imprinting has been described in both mouse and human pre-implantation embryos^{5,51,152} where DNA methylation is either lost on the maternal alleles or acquired by the paternal alleles post implantation. At the *Gpr1/Zdbf2* locus, transient monoallelic expression of *Gpr1-as1* mediates the accumulation of methylation at the *Zdbf2* DMR, whose stable maintenance in adult somatic tissues regulates *Zdbf2* allelic expression¹⁵². More recently, widespread transient imprinting derived from oocyte-specific methylation has been demonstrated in human placenta²¹. Epigenetic marks other than DNA methylation may also mediate transient imprinting, although this has not been reported in humans²⁶. In mouse morula, some loci that display maternal allele-specific histone 3 lysine 27 trimethylation (H3K27me3) marks are expressed from their paternal alleles. This form of imprinted expression is largely lost later in development in the embryonic cell lineage but is retained at a few loci in extra-embryonic tissues. Further studies are needed to determine if this form of DNA methylation imprinting is conserved in other species and what its impact is on gene expression and phenotypes at later developmental stages. It is possible that transient and DNA methylation-independent monoallelic expression control the establishment of secondary gDMRs and consequently lead to a more stable imprinted expression in somatic tissues.

Box 3 | **Zygotic genome activation**

Epigenetic reprogramming and zygotic genome activation (ZGA) are intimately linked in the early embryo (see the figure; maternal (red line), early zygotic (blue line) and embryonic (black line) transcripts are shown). Approximate timing (days) of human cleavage-stage divisions and blastulation are shown. Upon maturation, the oocyte ceases transcription and translation, which do not resume in human embryo until around the 8-cell stage; therefore, the early embryo relies substantially on maternally

provided oocyte protein and RNA. From the time of fertilization, maternal RNA is progressively degraded. The paternal genome supports an early wave of transcription (see the figure; blue line) that is essential for major ZGA¹⁵³.

In the 1-cell embryo, the maternally-derived histones that replace sperm protamines do not at first establish the patterns of euchromatin and heterochromatin normally seen in somatic cells, and as a result, chromatin on the paternal genome remains atypically accessible. The paternal genome supports low-level transcription of sequences normally repressed within heterochromatin, such as retroviral repeats, and pioneer factors like DUX4¹⁵⁴. Pioneer factors support transcription of cleavage-stage transcription factors that trigger major ZGA. Upon ZGA the genome attains a more somatic organisation, blocking the return to the permissive, early pattern of transcription.

Fig. 1 | The imprinted 11p15.5 region as an example of epigenetic error in imprinting disorders. a | Model of the 11p15.5 region represented as alternative chromatin loops on the maternal and paternal chromosomes. The model is based on the results of chromatin conformation capture studies in human cells^{22,23}. Loops anchors occur at CTCF-cohesin binding sites. On the maternal chromosome (red line), a loop is formed between a distal region (HIDAD) located at 1.72 Mb and the unmethylated IC1. On the paternal chromosome (blue line), the formation of this loop is prevented by IC1 methylation and an alternative one is formed between HIDAD and the *IGF2* promoter. Alternative loops may facilitate differential activation of *H19* and *IGF2* by common enhancers on the maternal and paternal chromosomes, respectively¹⁵⁵. **b** | Chromosome interactions and gene expression changes predicted by the looping model as consequence of IC1 LOM in SRS¹⁵⁵. *H19* is activated and *IGF2* silenced on both parental chromosomes. **c** | Chromosome interactions and gene expression changes predicted by the looping model as consequence of IC1 GOM in BWS¹⁵⁵. *H19* is silenced and *IGF2* activated on both parental chromosomes. Chromosome distances are not in scale.

Fig. 2 | Chromosome 11p15.5 — an example of an imprinted gene cluster. Within the telomeric domain, enhancers (green ovals) direct transcription of the long non-coding RNA (lncRNA) *H19* and the intragenic microRNA (miRNA) miR-675 on the maternal chromosome (red line) and that of the growth factor gene *IGF2* and the

intragenic miR-483 on the paternal chromosome (blue line). The imprinting centre (IC) of the telomeric domain (IC1; inset panel), also known as *H19/IGF2* intergenic differentially methylated region (DMR), contains tandem repeats (light red and blue rectangles) and is bound by the transcription factors (TFs) CTCF, POU5F1 and SOX2, which maintain the unmethylated status of the maternal allele, whereas ZFP57 maintains the methylated status of the paternal allele. IC1 and IC2 are also characterized by different chromatin configurations on parental chromosomes, with repressive histone marks, such as H3K9me2, H3K9me3 and H4K20me3 on the methylated allele, and permissive histone marks, such as H3K4me2 and H3K4me3, on the unmethylated allele. Secondary DMRs (*H19* promoter (prom), *IGF2* DMR0 and *IGF2* DMR2) are paternally methylated. The imprinting centre of the centromeric domain (IC2), also known as *KCNQ1OT1:TSS*-DMR, is maternally methylated and directs maternal-specific expression of *KCNQ1* and the cell cycle regulator *CDKN1C*. On the paternal allele, a lncRNA intragenic to *KCNQ1* (*KCNQ1OT1*) is transcribed (wavy blue lines), suppressing in *cis* the expression of coding genes in the region. IC2 methylation and silencing of the *KCNQ1OT1* promoter are maintained through interaction with ZFP57 on the maternal chromosome, while as yet uncharacterized TFs sustain *KCNQ1OT1* transcription on the paternal allele. Active alleles are represented with red (maternal) and blue (paternal) oblongs, inactive alleles with grey oblongs.

Fig. 3 | **The life cycle of imprints.** DNA methylation reprogramming during human development. Methylation of imprinting centres (dashed black line) is erased more slowly than that of the rest of the genome (black line) in PGCs and re-established with different kinetics in male (paternal imprinting centres, dashed blue line; whole genome, blue line) and female (maternal imprinting centres, dashed red line; whole genome, red line) germ cells. After fertilization, the maternally and paternally derived genomes are widely demethylated, while differential methylation between maternal and paternal imprinting centre alleles (50% level) is maintained pre- and post-implantation. Factors and events involved in each stage, 5mC level and approximate timing of imprint erasure, establishment and pre/post-implantation maintenance are indicated. GVs, germinal vesicles.

Fig. 4 | **Mechanisms of imprinting errors in human diseases.** In each panel, normal mechanisms are on the left, defective mechanisms on the right. **a** | Defective IC methylation erasure in PGCs. **b** | Defective transcription (dashed red arrow) across imprinting centre and imprint establishment in oocyte. **c** | Defective oocyte factor (striked pink triangle) impacting imprint establishment in oocyte. **d** | Defective oocyte factor (striked pink diamond) impacting developmental competence and imprint maintenance in pre-implantation embryo. **e** | Defective zygotic factor (white asterisk) and imprint maintenance in pre-implantation embryo. **f** | Defective target site (indicated with x) for transcription factor protecting methylated imprinting centre and imprint maintenance in pre-implantation embryo. **g** | Defective target site for transcription factor recognizing unmethylated imprinting centre and imprint maintenance in post-implantation embryo. Methylated and unmethylated imprinting centres: black / white lollipops. Mosaic methylation is indicated by grey lollipops. MLID is indicated by LOM at multiple imprinting centres. Maternally methylated imprinting centres are in red, paternally methylated imprinting centres in blue. *Trans*-acting factors are as in Fig. 1.

Fig. 5 | **Modes of inheritance of phenotypes associated with imprinting errors.** **a** | Pedigree of sporadic SRS case in which IC1 epimutation is corrected in the germline of the proband. Black lollipop shows normal full methylation of paternal IC1 in blood of I:1 and in the sperm of II:1; grey lollipop, mosaic hypomethylation of paternal IC1 in blood of II:1; **b-c** | Pedigrees showing autosomal dominant inheritance with parent-of-origin-dependent penetrance (paternal, associated with PWS/AS imprinting centre mutation in b; maternal, associated with BWS/SRS IC1 mutation in c; **d** | Autosomal recessive (*ZFP57* mutation); **e** | Maternal effect (*NLRP5* mutations). Colored symbols indicate: SRS cases, purple; PWS cases, brown; BWS cases, blue; TNDM cases, green.

Fig. 6 | **Interaction between environmental and genetic factors and its impact on genomic imprinting.** The diagram summarizes the evidences obtained in humans and animal models of interaction of environmental factors and physio-pathological conditions (depicted in light blue) with gametic and zygotic factors (yellow) involved in *de novo* and maintenance methylation and impacting imprinting

establishment and maintenance (pink). Continuous lines indicate functional contribution; dashed lines, functional interference.

Table 1. Imprinting disorders and their main molecular defects and clinical features.

Imprinting disorder OMIM	Prevalence	Chromosome(s)	Molecular defect (frequency)	Main clinical features
Transient neonatal diabetes mellitus (TNDM) ^{93,156-158} 601410	1/300.000	Chr 6q24	- upd(6)pat (41%). - Paternal duplications (29%). - <i>PLAGL1:alt-TSS-DMR</i> LOM (30%) (MLID: 30% caused by pathogenic <i>ZFP57</i> variants ¹⁵⁹)	IUGR, transient diabetes mellitus, hyperglycaemia without ketoacidosis, macroglossia, abdominal wall defects
Silver-Russell syndrome (SRS) ¹⁶⁰ 180860	1/75.000- 1/100.000	Chr 7 Chr 11p15 Chr 12q14 Chr 8q12	- upd(7)mat (5–10% (MLID: rare ¹⁶¹)) - upd(11p15)mat (rare) - 11p15 CNVs (<1%) - <i>H19/IGF2:IG:DMR</i> LOM (30–60% (MLID: 7–10% ^{37,39,147})) - <i>CDKN1C</i> , <i>IGF2</i> , <i>HMGA2</i> , <i>PLAG1</i> point mutations (rare)	IUGR, PNGR, relative macrocephaly at birth, body asymmetry, prominent forehead, feeding difficulties
Birk-Barel syndrome ¹⁶² 612292	unknown	Chr 8q24.3	- <i>KCNK9</i> point mutations (100%)	Intellectual disability, hypotonia, dysmorphism
Beckwith-Wiedemann syndrome (BWS) ¹⁶³ 130650	1/15.000	Chr 11p15	- upd(11p15)pat (20%) - 11p15 CNVs (2–4%) - <i>H19/IGF2:IG:DMR</i> GOM ^a (5%) - <i>KCNQ1OT1:TSS-DMR</i> LOM (50% (MLID: 25% ^{37,39})) - <i>CDKN1C</i> point mutations (5% sporadic; 40–50% in families)	Macroglossia, exomphalos, lateralized overgrowth, Wilms tumour or nephroblastomatosis, hyperinsulinism, adrenal cortex cytomegaly, placental mesenchymal dysplasia, pancreatic adenomatosis
Kagami-Ogata syndrome (KOS14) ^{164,165} 608149	unknown	Chr 14q32	- upd(14)pat (65%) - 14q32 maternal deletion (20%) - <i>MEG3/DLK1:IG-DMR</i> GOM (15%)	IUGR, polyhydramnion, abdominal wall defects, bell-shaped thorax, coat-hanger ribs
Temple syndrome	unknown	Chr 14q32	- upd(14)mat (29%)	IUGR, PNGR,

(TS14) ¹⁶⁶⁻¹⁶⁸ 616222			-14q32 paternal deletion (10%) - <i>MEG3/DLK1</i> :IG-DMR LOM (61%)	neonatal hypotonia, feeding difficulties in infancy, truncal obesity, scoliosis, precocious puberty, small feet and hands
Prader–Willi syndrome (PWS) ¹⁶⁹⁻¹⁷¹ 176270	1/25.000 -1/10.000	Chr 15q11–q13	-15q11–q13 paternal deletion (75-80%) - Upd(15)mat (20-25%) - <i>SNURF</i> :TSS-DMR GOM ^a (~1% (MLID: rare ¹⁷²))	PNGR, Intellectual disability, neonatal hypotonia, hypogenitalism, hypopigmentation, obesity, hyperphagia
Angelman syndrome (AS) ^{173,174} 105830	1/20.000 -1/12.000	Chr 15q11–q13	- Maternal deletion (70-75%) - Upd(15)pat (3-7%) - <i>SNURF</i> :TSS-DMR LOM ^a (2-3% (MLID: rare ¹⁷²)) - <i>UBE3A</i> point mutations (10%)	Severe intellectual disability, microcephaly, no speech, unmotivated laughing, ataxia, seizures, scoliosis
Central precocious puberty 2 (CPPB2) ¹⁷⁵ 615356	Unknown	Chr 15q11.2	- <i>MKRN3</i> point mutations (100%)	Early activation of the hypothalamic–pituitary–gonadal axis resulting in gonadotropin-dependent precocious puberty
Schaaf–Yang syndrome (SYS) ¹⁷⁶ 615547	Unknown	Chr 15q11.2	- <i>MAGEL2</i> point mutations (100%)	Delayed psychomotor development, intellectual disability, hypotonia
Pseudohypoparathyroidism 1A (PHP1A) including PHP1C ¹⁷⁷ 103580 612462	Unknown	Chr 20q13:	- <i>GNAS</i> inactivating variants of the maternal allele (100%)	Resistance to PTH and other hormones, Albright hereditary osteodystrophy, moderately reduced birth weight, obesity, cognitive impairment (70% of patients)
Pseudohypoparathyroidism 1B (PHP1B) ¹⁷⁷ 603233	Unknown	Chr 20q13	-20q13 maternal deletion (8.5%) - <i>GNAS</i> DMRs LOM (42.5%; MLID: 12.5% ^{178,179}) - upd(20)pat (2.5%) -20q13 point mutations (46.5%)	Resistance to PTH and other hormones Albright hereditary osteodystrophy, subcutaneous ossifications, feeding behaviour anomalies,

				abnormal growth patterns
Pseudopseudohypoparathyroidism (PPHP) ¹⁷⁷ 612463	Unknown	Chr 20q13	-GNAS inactivating variants of the paternal allele (100%)	Mild resistance to PTH and other hormones, subcutaneous ossifications, birth weight and length restrictions
Progressive osseous heteroplasia (POH) ¹⁷⁷ 166350	Unknown	Chr 20q13	-GNAS inactivating variants of the paternal allele (100%)	Ectopic ossifications
Mulchandani–Bhoj–Conlin syndrome (MBCS) ^{180,181} 617352	Unknown	Chr 20	-upd(20)mat (100%)	IUGR, PNGR, feeding difficulties

^a Imprinting defects can either be due to primary imprinting epimutations without DNA sequence alterations, or due to deletions in the imprinting center (IC) critical regions. GOM, gain of methylation; IUGR, intrauterine growth restriction; LOM, loss of methylation; PNGR, postnatal growth restriction; PTH, parathyroid hormone; UPD, uniparental disomy.

Table 2. Human germline and somatic DMRs with regulated imprinted genes.

HGVS approved DMR name ¹²	Previous names	Location (hg19/GRCh37)	Methylated allele	Germline or somatic	Known TF binding sites ^c	Cis-regulated genes
<i>PPIEL</i> :Ex1-DMR		1:40024626-40025540	M	Oocyte gDMR		<i>PPIEL</i>
<i>DIRAS3</i> :TSS-DMR	<i>NOEY2</i> , <i>ARH1</i>	1: 68513430-68517450	M	Oocyte gDMR		<i>DIRAS3</i> , <i>GNG12-AS1</i>
<i>DIRAS3</i> :Ex2-DMR		1:68512505-68513486	M	Oocyte gDMR	ZFP57	
<i>GPR1-AS</i> :TSS-DMR		2:207114583-207136544	M	Oocyte gDMR		<i>GPR1-AS</i> , <i>ZDBF2</i> , <i>ADAM23</i>
<i>ZDBF2/GPR1-AS</i> :IG-DMR		2:207114583-207136544	P	Sperm gDMR-secondary DMR	CTCF, RAD21	
<i>JAKMIP1</i> :Int2-DMR		chr4:6106594-6108185	M	Oocyte gDMR		

<i>NAP1L5</i> :TSS-DMR		4:89618184-89619237	M	Oocyte gDMR	ZFP57	<i>NAP1L5</i>
^a <i>VTRNA2-1</i> :DMR	<i>Nc886</i>	5:135414802-135416645	M	Oocyte gDMR	CTCF, RAD21	
^a <i>FAM50B</i> :TSS-DMR		6:3849082-3850359	M	Oocyte gDMR	CTCF, RAD21	<i>FAM50B</i> , <i>PXDC1</i>
<i>PLAGL1</i> :alt-TSS-DMR	<i>LOT1, ZAC1</i>	6:144328078-144329888	M	Oocyte gDMR	ZFP57	Multiple <i>PLAGL1</i> transcripts, <i>HYMAI</i>
<i>IGF2R</i> :Int2-DMR		6:160426558-160427561	M	Oocyte gDMR		
^a <i>WDR27</i> :Int13-DMR		6:170054504-170055618	M	Oocyte gDMR	CTCF	<i>WDR27</i>
<i>RPS2P32</i> :TSS-DMR		chr7:23530017-23530976	M	unclear		<i>RPS2P32</i>
<i>GRB10</i> :alt-TSS-DMR		7:50848726-50851312	M	Oocyte gDMR	CTCF, RAD21, ZFP57	<i>GRB10</i> , <i>DDC</i>
<i>PEG10</i> :TSS-DMR		7:94285537-94287960	M	Oocyte gDMR		<i>PEG10</i> , <i>SGCE</i> , <i>PPP1R9A</i> , <i>TFPI2</i> , <i>CALCR</i> , <i>DLX5</i>
<i>MEST</i> :alt-TSS-DMR	<i>PEG1</i>	7:130130122-130134388	M	Oocyte gDMR	CTCF, RAD21, ZFP57	<i>MEST</i> , <i>MESTIT1</i> , <i>COPG2IT1</i> , <i>CPA4</i> , <i>KLF14</i>
^a <i>SVOPL</i> :alt-TSS-DMR		7:138348118-138349069	M	Oocyte gDMR		<i>SVOPL</i>
<i>HTR5A</i> :TSS-DMR		7:154862719-154863382	M	Oocyte gDMR		<i>HTR5A</i>
^a <i>ERLIN2</i> :Int6-DMR		8:37604992-37606088	M	Oocyte gDMR		<i>ERLIN2</i>
<i>PEG13</i> :TSS-DMR	<i>TRAPPC9</i> intronic DMR	8:141108147-141111081	M	Oocyte gDMR	CTCF, RAD21, ZFP57	<i>PEG13</i> , <i>TRAPPC9</i> , <i>KCNK9</i>
^a <i>FANCC</i> :In61-DMR		9:98075400-98075744	M	Oocyte gDMR		
<i>PTCHD3</i> :TSS-DMR		chr10:27702514-27703363	M	Oocyte gDMR		<i>PTCHD3</i>
<i>INPP5F</i> :Int2-DMR	<i>INPP5FV2</i>	10:121578046-121578727	M	Oocyte gDMR		<i>INPP5FV2</i>
<i>H19/IGF2</i> :IG-DMR	<i>IC1, ICR1</i> , <i>H19</i> DMD	11:2018812-2024740	P	Sperm gDMR	CTCF, RAD21,	Multiple <i>IGF2</i> transcripts,

					ZFP57, POU5F1	<i>IGF2-AS</i> , <i>INS-IGF2</i> , <i>miR483</i> , <i>H19</i> , <i>HOTS</i> , <i>91H</i> , <i>miR675</i>
<i>IGF2:Ex9-DMR</i>	<i>IGF2-DMR2</i>	11:2153991- 2155112	P	Secondary DMR		
<i>IGF2:alt-TSS-DMR</i>	<i>IGF2-DMR0</i>	11:2168333- 2169768	P	Sperm gDMR		
<i>KCNQ10T1:TSS-DMR</i>	<i>IC2</i> , <i>ICR2</i> , <i>KvDMR1</i> , <i>KvLQT1</i> , <i>LIT1</i>	11:2719948- 2722529	M	Oocyte gDMR	ZFP57	<i>KCNQ10T1</i> , <i>CDKN1C</i> , <i>PHLDA2</i> , <i>SLC22A18</i> , <i>KCNQ1</i>
<i>RB1:Int-DMR</i>		13:48892341- 48895763	M	Oocyte gDMR	ZFP57	<i>LPAR6</i>
<i>LPAR6:TSS-DMR</i>		13:48984639- 48987689	M	Secondary DMR		
<i>DLK1:Int2-DMR</i>		14: 101193446- 101195447	M	Secondary DMR		
<i>MEG3/DLK1:IG-DMR</i>	<i>IG-DMR</i> , <i>GLT2-DLK1</i>	14:101275427- 101278058	P	Sperm gDMR		<i>MEG3</i> , <i>DLK1</i> , <i>MEG8</i> , <i>DIO3</i> , <i>RTL1</i> , <i>RTL-AS</i> , <i>MEG9</i> , <i>SNORD113</i> , <i>SNORD114</i> and miRNA cluster
<i>MEG3:TSS-DMR</i>	<i>GTL2</i>	14:101290524- 101293978	P	Secondary DMR	CTCF, ZFP57	
<i>MEG8:Int2-DMR</i>		14:101370410- 101371410	M	Secondary DMR	CTCF, RAD21	
<i>MKRN3:TSS-DMR</i>		15:23807086- 23812495	M	Oocyte gDMR- secondary DMR		
<i>MAGEL2:TSS-DMR</i>		15:23892425- 23894029	M	Secondary DMR		
<i>NDN:TSS-DMR</i>		15:23931451- 23932759	M	Secondary DMR		
^b <i>SNRPN:alt-TSS-DMR</i>		15:25068564- 25069481	M	Secondary DMR		
^b <i>SNRPN:Int1-DMR1</i>		15:25093008- 25193829	M	Secondary DMR	POU5F1	
^b <i>SNRPN:Int1-</i>		15:25123027-	M	Secondary		

DMR2		25123905		DMR		
<i>SNURF</i> :TSS-DMR		15:25200004-25201976	M	Oocyte gDMR	YY1	<i>MKRN3</i> , <i>MAGEL2</i> , <i>NDN</i> , <i>PWRN1</i> , <i>SNRPN</i> , <i>IPW</i> , <i>SNHG14</i> , <i>SNORD116</i> , <i>SNORD115</i> , <i>UBE3A</i>
<i>SNORD116</i> :DMR	<i>SNHG14</i> , <i>UBE3A-AS</i>	15: 25156289-25405834	P	Secondary DMR		
<i>IGF1R</i> :Int2-DMR	<i>IRIAN</i>	15:99408496-99409650	M	Oocyte gDMR		<i>IRIAN</i>
<i>ZNF597</i> : 3'-DMR		16:3481801-3482388	M	Oocyte gDMR		<i>ZNF597</i> , <i>NAA60</i>
<i>ZNF597</i> :TSS-DMR	<i>NAT15</i>	16:3492828-3494463	P	Secondary DMR		
<i>ZNF331</i> :alt-TSS-DMR1		19:54040510-54042212	M	Oocyte gDMR	CTCF	
<i>ZNF331</i> :alt-TSS-DMR2		19:54057086-54058425	M	Oocyte gDMR		
<i>PEG3</i> :TSS-DMR	<i>ZIM2</i> , <i>ZNF904</i>	19:57348493-57353271	M	Oocyte gDMR	ZFP57	<i>PEG3</i> , <i>ZIM2</i> , <i>MIMT1</i>
<i>MCTS2P</i> :TSS-DMR	<i>psMCT1</i> , <i>MCST2</i>	20:30134663-30135933	M	Oocyte gDMR	YY1	<i>MCST2</i> , <i>HM13</i>
<i>NNAT</i> :TSS-DMR	<i>PEG5</i>	20:36148604-36156528	M	Oocyte gDMR	ZFP57	<i>NNAT</i> , <i>BLACP</i>
<i>L3MBTL1</i> :alt-TSS-DMR	<i>ZC2HC3</i> , <i>KIAA0681</i>	20:42142365-42144040	M	Oocyte gDMR	CTCF, RAD21	<i>L3MBTL1</i> , <i>SGK2</i>
<i>GNAS</i> - <i>NESP</i> :TSS-DMR	<i>NESP55</i>	20:57414039-57418612	P	Secondary DMR	CTCF, RAD21	Multiple <i>GNAS</i> transcripts, <i>miR296</i> , <i>miR298</i>
<i>GNAS-AS1</i> :TSS-DMR	<i>NESP-AS</i>	20:57425649-57428033	M	Oocyte gDMR	CTCF, RAD21,	
<i>GNAS-XL</i> :TSS-DMR	Secretogranin VI	20:57428905-57431463	M	Oocyte gDMR	ZFP57	
<i>GNAS A/B</i> :TSS-DMR	Secretogranin VI	20:57463265-57465201	M	Oocyte gDMR		
<i>WRB</i> :alt-TSS-DMR		21:40757510-40758276	M	Oocyte gDMR		
^a <i>SNU13</i> :alt-TSS-DMR	<i>NHP2L1</i>	22:42077774-42078873	M	Oocyte gDMR		

^aThese imprinted DMRs show evidence for mosaicism in the general population with low frequency hypomethylation reported. ^bThe entire ~1MB interval between the *NDN* and *SNURF* DMRs (chr15:24112253-25108042) is preferentially methylated on the maternal allele. ^cTranscription factor binding sites taken from: YY1 ChIP-seq ENCODE datasets (March 2012); CTCF ChIP-seq ENCODE datasets (March 2012); ZFP57 ChIP-seq dataset Imbeault et al., 2017. Nature 543:550-554; POU5F1 ChIP-seq ENCODE datasets (March 2012). HGVS, Human Genome Variation Society; M, maternal; P, paternal.

Table 3. Factors with potential roles in imprinting control.

Factor	Function	Mouse knockout phenotype	Associated imprinting disorders
AID and APOBEC1	Possible role in active demethylation in PGCs	Modest global hypermethylation in deficient PGCs ¹⁸²	not reported
TET1 and TET2	Implicated in demethylation as they have specific activity for converting 5mC to 5hmC	DKO mice have reduced 5hmC and increased 5mC levels at some imprinted loci (<i>Mest</i> , <i>Peg3</i> , <i>Igf2r</i> , <i>H19</i>) ⁴⁵	not reported
DNMT3A/B	<i>De novo</i> DNA methyltransferase	No methylation at gDMRs ¹⁸³	not reported
DNMT3L	Associate factor for DNMT3	No methylation at gDMRs ⁵³	No pathogenic variants identified in SRS ¹⁸⁴
KDM1B	Oocyte-specific H3K4 demethylase	LOM at multiple gDMRs (<i>Mest</i> , <i>Grb10</i> , <i>Zac1</i> , <i>Impact</i> , <i>U2af1-rs1</i> , <i>Peg10</i> , <i>Nnat</i> , <i>KvDMR1</i> , <i>Igf2r</i> , <i>Gnas</i>) ^{60,62}	not reported
KDM1A	H3K4 demethylase	LOM at transient <i>Cdh15</i> gDMRs ⁶²	not reported
NLRP7	NACHT, LRR and PYD domains-containing protein 7 potentially a SCMC interacting proteins	No mouse orthologue	Biparental hydatidiform moles with complete lack of maternal imprints ^{77,78} ; MLID in rare hypomorphs ^{79,185,186}
DNMT1 (both oocyte and somatic isoforms)	Maintenance DNA methyltransferase	Widespread LOM during embryonic cleavage stages ^{187,188}	BWS with IC2 LOM ⁹⁷
DNMT3A/B	<i>De novo</i> DNA methyltransferase	No methylation at gDMRs ^{54,183}	not reported
ZFP57	KAP1 recruiting zinc-finger protein	Widespread LOM during embryonic cleavage stages ⁸⁷	TNDM patients with MLID ⁹³
TRIM28/KAP1	KRAB1-associated protein repressor	Partial LOM during embryonic cleavage stages (<i>H19</i> , <i>Snrpn</i>) ⁹⁴	No pathogenic variants identified in MLID ³⁶

	complex		
UHRF1	Guides DNMT1 to hemimethylated DNA during replication	Partial LOM during embryonic cleavage stages (<i>H19</i> , <i>KvDMR1</i> , <i>Ig-DMR</i>) ⁹⁵	Single case of MLID ³⁹
Histone H1	Linker histones	Triple KOs result in Partial LOM (<i>H19</i> , <i>Ig-DMR</i>)	not reported
MBD3	Methyl-CpG binding protein	Partial LOM at <i>H19</i> ¹⁸⁹	No pathogenic variants identified in SRS ¹⁸⁴
MTA2	Metastasis tumour antigen 2, member of NuRD complex	Partial LOM at <i>H19</i> and <i>Peg3</i> ¹⁹⁰	not reported
RBBP1/Arid4a RBBP1L1/Arid4b	Retinoblastoma-binding proteins	LOM at <i>Snrpn</i> in double KOs ¹⁹¹	not reported
DPPA3/STELLA	Binds to methylated and H3K9 decorated gDMRs	Partial LOM at several gDMRs (<i>Snrpn</i> , <i>Mest</i> , <i>Peg3</i> , <i>Nnat</i> , <i>Peg10</i> , <i>H19</i> , <i>Rasgrf1</i>) ⁷⁴	No pathogenic variants identified in MLID ¹⁴⁷
NLRP2	NACHT, LRR and PYD domains-containing protein 2 potentially a SCMC member	Aberrant gDMRs (GOM and LOM) in embryos that survive to mid-gestation ⁸⁵	MLID ⁷⁵
KHDC3L/ C6orf221/FILIA	KH Domain Containing 3 Like, Subcortical Maternal Complex Member	Imprinting not assessed ¹⁸⁴	Biparental hydatidiform moles. LOM at several DMRs (<i>KvDMR1</i> , <i>SNRPN</i> , <i>MEST</i> , <i>PEG3</i> , <i>GNAS XL</i> , <i>GNAS A/B</i>) ^{76,192} No pathogenic variants identified in MLID ^{36,186}
NLRP5/MATER	Member of SCMC	Imprinting not assessed ¹⁸²	MLID ³⁷
OOEP	Member of SCMC	Imprinting not assessed ¹⁹³	Single case of MLID ³⁹
PADI6	Member of SCMC	Imprinting not assessed ¹⁸³	MLID ³⁹
ZAR1	Oocyte-specific zinc finger protein	Imprinting not assessed ¹⁹⁴	Single case of MLID ³⁹
VEZF1	Zinc finger transcription factor DB1	Partial LOM at <i>H19</i> and <i>Igf2r</i> gDMRs ¹⁹⁵	No pathogenic identified in MLID ¹⁴⁷
SMCHD1	Structural Maintenance of Chromosome Flexible Hinger Domain-Containing protein	LOM of secondary DMRs in mouse 7qB5 domain ¹⁹⁶	not reported
YY1	Transcriptional repressor protein	Aberrant gDMRs (GOM and LOM) ¹⁹⁷	not reported
ZBTB33	Zinc finger an BTB	Imprinting not assessed ¹⁹⁸	shRNA-targeting resulting in

	Domain Containing transcription factor		partial LOM at H19 ¹⁹⁹
CTCF	Zinc finger protein involved in chromatin organisation	GOM at <i>H19</i> ²⁰⁰	Microdeletions of CTCF binding sites result in IC1 GOM in BWS ^{28,201,202}
ZFP42/REX1	Zinc finger protein	GOM at Peg3 and GNAS ²⁰³	not reported
POU5F1	Pioneer pluripotency transcription factor	Imprinting not assessed ²⁰⁴	Binding site pathogenic variants leads to IC1 GOM in BWS ^{99,205,206}
SOX2	Pioneer pluripotency transcription factor	Imprinting not assessed ²⁰⁷	Binding site pathogenic variants leads to IC1 GOM in BWS ^{99,103,205,206}

PGCs, primordial germ cells.

Glossary

Genomic imprinting

The epigenetic marking of a gene on the basis of parental origin, which results in monoallelic expression.

Anticipation

A phenomenon whereby the symptoms of a genetic disorder become apparent at an earlier age or with greater severity in succeeding generations.

Assisted reproductive technologies

(ART). Techniques used to achieve pregnancy during the treatment of infertility. ART covers a wide spectrum of treatments including the use of fertility drugs, intrauterine insemination and in vitro *fertilization*/intracytoplasmic sperm injection.

Blastocyst

Developmental stage of mammalian embryo just before implantation consisting of an inner cell mass which will form the embryo, and a cavity with an outer layer called trophoblast, which will give rise to the placenta.

***cis*-acting element**

DNA sequence regulating the expression of a gene that is present on the same chromosome.

Copy number variation

(CNV). Type of structural variation of a chromosome consisting in duplication or deletion of DNA sequence.

Endogenous retrovirus

Repetitive genetic element present in the genome that, similarly to retroviruses, uses the activity of reverse transcriptase to move from one locus to another (also known as retrotransposons).

Epiallele

Epigenetic profile which is maintained in somatic tissues resulting in interindividual variation.

Epigenome

Chromatin modifications influencing genome function and not involving the underlying DNA sequence that can be propagated through cell division.

Epigenetic reprogramming

The erasure of pre-existing epigenetic marks that allow for subsequent remodelling of chromatin.

Epimutation

When referred to imprinting disorders, epigenetic change that affects the regulation of imprinted loci. The epimutation is primary if there is no detectable genetic cause, secondary if it is associated with a genetic cause.

Imprinting disorders

Diseases associated with disruption of imprinted gene expression that can be caused by genetic or epigenetic defects.

Incomplete penetrance

A situation in which not all individuals carrying a dominant deleterious genetic variant express the associated clinical phenotype.

Gain of methylation

(GOM). When referred to imprinting disorders, gain of methylation on the unmethylated allele of imprinting centre. It is detected in patients and causes deregulation of the imprinted genes in the domain controlled by the imprinting centre.

Genome activation

The initiation of gene expression in the developing embryo. The initial burst of expression is termed zygotic genome activation (ZGA) and is regulated by pioneer transcription factors during the oocyte-to-embryo transition. Initiation of expression in cleavage embryos is referred to as embryonic genome activation.

Germline differentially methylated region

(gDMR): Regions of differential DNA methylation between parental alleles in somatic cells that originate from the gametes. gDMRs that survive embryonic reprogramming are generally associated with imprinted genes.

Haploinsufficiency

A situation in which half of the normal level of a gene product, usually consequence of a loss-of-function mutation, is not sufficient for the normal function.

Hydatidiform mole

Benign gestational trophoblastic disease developing during pregnancy and resulting from an abnormal fertilization. It is characterized by trophoblastic proliferation and little or no embryonic tissue. It is commonly sporadic and contains only sperm DNA. Occasionally, it can be biparental, recurrent and familial following an autosomal recessive mode of inheritance.

Imprinting centre

A function definition for gDMRs that have been shown to regulate imprinted genes expression through either genetic targeting in mouse or through mutations in patients. Also known as imprinting control region (ICR). Not all gDMRs have been shown to be imprinting centre regions.

Loss of methylation

(LOM). When referred to imprinting disorders, loss of differential imprinting centre methylation detected in patients and causing deregulation of the imprinted genes in the domain controlled by the imprinting centre.

Multi-locus imprinting disturbance

(MLID). Methylation anomalies at imprinted DMRs in patients with imprinting disorders in addition to those that are normally associated with the disease.

Maternal effect gene

A gene coding for an oocyte-derived *transcript or protein* that is required for the early development of the embryo.

Penetrance

Proportion of individuals in a population with a specific genotype who show an associated phenotypic trait.

Primordial germ cells

(PGCs). Stem cell-like cells found in the gonadal ridge of developing embryos that develop into gametes following sex-specific epigenetic reprogramming and meiosis.

Pronucleus

The haploid nucleus from a male or female gamete before the genetic material fuse at syngamy.

Protamines

Basic proteins that largely replace histones in the nucleus of mature sperm for more condensed DNA packaging.

Secondary differentially methylated region

A region of differential DNA methylation between parental alleles that does not originate from the germline. They are often referred to as somatic DMRs and are regulated in a hierarchical fashion by a nearby imprinting centre region.

Subcortical maternal complex

(SCMC). A large multi-protein complex comprising of NLRP5, OOEP, TLE6, PADI6 and KHDC3L that localises to the outermost regions of the cytoplasm in oocytes and excluded from regions of cell-to-cell contact in cleavage embryos.

trans-acting factor

Protein regulating the expression of a gene.

Uniparental disomy

(UPD). Genetic defect characterized by the presence of two copies of a chromosome or part of it derived from only one parent.

Zygote

A fertilized ovum before the first cell division which represents the earliest stage of embryonic development. The zygote's genome is a combination of the DNA in each gamete.