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 allele[1]. 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 variants[2].

In humans, approximately 100 imprinted genes have been identified [3-5]. 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 risk[6]. 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

factors[8]. 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 analysis[10], the physiological role of imprinted genes[6] or the chromatin mechanisms in imprinting[11], 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 individuals[19-21]. 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 cis[24] (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 vicinity[20], and their allele-specific expression may possibly be controlled by epigenetic features other than DNA methylation[26].

Tandem repeats are a prominent feature of imprinting centres[27]. 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 defects[30]. 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 mice[31], 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 PLAGL1[32] and the *H19* lncRNA[33] 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 centre[34]. Furthermore, many imprinted gene clusters encode microRNAs (miRNAs) and small nucleolar RNAs (snoRNAs), which may be involved in the post-transcriptional control of imprinted genes[35]. 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 comparable[42-44].

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 imprints[47].

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 oocytes[52]; however, in humans, meiosis II oocytes and the first and second polar bodies have comparable methylation, including at imprinted maternally methylated gDMRs[49] (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 cells[55]. DNMT1 has an auxiliary role ensuring symmetric methylation of CpG sites in oocytes[56]. Transcription and underlying chromatin signature are important factors determining methylation acquisition[50]. Transcription in oocytes is required for methylation at numerous gDMRs[57], 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 DNMT3L[60-62]. Despite being a generic methylase in mouse oocytes, DNMT3L is not detectable by expression profiling in human oocytes between germinal vesicle phase and meiosis II[63], 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* methylation[64].

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 imprints[57]; 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) (REF[66] 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 REF[69]) 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 embryo[63]. 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 H3K9me2[73]. *DPPA3* is a maternal-effect gene: concepti of maternal null mice rarely progress beyond the two-cell stage, and their genomes are severely demethylated[74].

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 MLID[79].

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 blastulation[81]. 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 translation[82-84]. 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 defects[85].

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 aneuploidies[86]. 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 demethylation[87-89]. 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 hypermethylation[92]. 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 survival[87]. Human *ZFP57* acts zygotically, with recessive mutations associated with the imprinting disorders TNDM and a specific pattern of MLID[93], 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 humans[96], 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 BWS[30] and SRS[98], 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 species[101].

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 variants[99]). 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 penetrance[28] or apparent anticipation **[G]** with increased clinical severity over multiple generations[103]. 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 phenotype[20], 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 utero[105]. 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 IVF[112]. 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 fluids[113]. Furthermore, superovulation and embryo transfer induced developmental defects and imprinting centre epimutations in the placenta of mouse models[114].

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 culture[115]. More recently, impaired methylation of imprinting centres was reported in sperm of subfertile men[47]. 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 children[116] and animal models[117]. Maternal age and delayed ovulation or fertilization are associated with depletion of oocyte proteins and RNA stores and altered developmental fitness of embryos[118-120], 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 transfer[123], 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 states[124], 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 gDMRs[125].

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-adenosylhomocyteine (SAH) abundance correlate with aberrant imprinted methylation[126,127], which also revealed a link between low vitamin B12 levels and *H19* methylation maintenance[126] 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 ratios[97]. Furthermore, the *ZFP57* locus is a folate-sensitive region, and its genomic binding regions are metastable epialleles responsive to periconceptional 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 loss[130]. However, not all studies on isocaloric protein restriction during pregnancy have resulted in altered imprinted methylation in the newborn[131], 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 cells[132].

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 placenta[135] 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 obesity[138]. *Dnmt1* expression was found to be decreased in BPA-treated mouse spermatogonia[137], and BPA exposure during oocyte maturation altered other epigenetic marks, specifically the abundance of histone modifications, which was linked to induced oxidative stress[139]. Exposure-induced oxidative stress was shown to alter both TET enzyme expression and function, leading to altered 5-hmC levels at numerous imprinted loci[134], 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 alterations[140].

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 proposed[141-143]. 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 inhibitor[143] as well as antisense oligonucleotides[141] 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 model[142]. 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 cells[144], 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.

References

1 Barlow, D. P. & Bartolomei, M. S. Genomic imprinting in mammals. *Cold Spring Harb Perspect Biol* **6**, doi:10.1101/cshperspect.a018382 (2014).

2 Onuchic, V. *et al.* Allele-specific epigenome maps reveal sequence-dependent stochastic switching at regulatory loci. *Science* **361**, doi:10.1126/science.aar3146 (2018).

3 Baran, Y. *et al.* The landscape of genomic imprinting across diverse adult human tissues. *Genome Res* **25**, 927-936, doi:10.1101/gr.192278.115 (2015).

4 Court, F. *et al.* Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment. *Genome Res* **24**, 554-569, doi:10.1101/gr.164913.113 (2014).

Full description of allelic DMRs in humans and first report of tissue-specific ICs.

5 Smith, Z. D. *et al.* DNA methylation dynamics of the human preimplantation embryo. *Nature* **511**, 611-615, doi:10.1038/nature13581 (2014).

Describes methylation landscape in human gametes and pre-implantation embryos.

6 Peters, J. The role of genomic imprinting in biology and disease: an expanding view. *Nat Rev Genet* **15**, 517-530, doi:10.1038/nrg3766 (2014).

7 Eggermann, T. *et al.* Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clin Epigenetics* **7**, 123, doi:10.1186/s13148-015-0143-8 (2015).

8 Horsthemke, B. Mechanisms of imprint dysregulation. *Am J Med Genet C Semin Med Genet* **154C**, 321-328, doi:10.1002/ajmg.c.30269 (2010).

9 Haig, D. Colloquium papers: Transfers and transitions: parent-offspring conflict, genomic imprinting, and the evolution of human life history. *Proc Natl Acad Sci U S A* **107 Suppl 1**, 1731-1735, doi:10.1073/pnas.0904111106 (2010).

10 Eggermann, K. *et al.* EMQN best practice guidelines for the molecular genetic testing and reporting of chromosome 11p15 imprinting disorders: Silver-Russell and Beckwith-Wiedemann syndrome. *Eur J Hum Genet* **24**, 1377-1387, doi:10.1038/ejhg.2016.45 (2016).

11 Sanli, I. & Feil, R. Chromatin mechanisms in the developmental control of imprinted gene expression. *Int J Biochem Cell Biol* **67**, 139-147, doi:10.1016/j.biocel.2015.04.004 (2015).

12 Monk, D. *et al.* Recommendations for a nomenclature system for reporting methylation aberrations in imprinted domains. *Epigenetics* **13**, 117-121, doi:10.1080/15592294.2016.1264561 (2018).

13 Singh, P. *et al.* Chromosome-wide analysis of parental allele-specific chromatin and DNA methylation. *Mol Cell Biol* **31**, 1757-1770, doi:10.1128/MCB.00961-10 (2011).

14 Riso, V. *et al.* ZFP57 maintains the parent-of-origin-specific expression of the imprinted genes and differentially affects non-imprinted targets in mouse embryonic stem cells. *Nucleic Acids Res* **44**, 8165-8178, doi:10.1093/nar/gkw505 (2016).

15 Kota, S. K. *et al.* ICR noncoding RNA expression controls imprinting and DNA replication at the Dlk1-Dio3 domain. *Dev Cell* **31**, 19-33, doi:10.1016/j.devcel.2014.08.009 (2014).

16 Rougeulle, C., Glatt, H. & Lalande, M. The Angelman syndrome candidate gene, UBE3A/E6-AP, is imprinted in brain. *Nat Genet* **17**, 14-15, doi:10.1038/ng0997-14 (1997).

17 Vu, T. H. & Hoffman, A. R. Imprinting of the Angelman syndrome gene, UBE3A, is restricted to brain. *Nat Genet* **17**, 12-13, doi:10.1038/ng0997-12 (1997).

18 Travers, M. E. *et al.* Insights into the molecular mechanism for type 2 diabetes susceptibility at the KCNQ1 locus from temporal changes in imprinting status in human islets. *Diabetes* **62**, 987-992, doi:10.2337/db12-0819 (2013).

19 Hanna, C. W. *et al.* Pervasive polymorphic imprinted methylation in the human placenta. *Genome Res* **26**, 756-767, doi:10.1101/gr.196139.115 (2016).

20 Zink, F. *et al.* Insights into imprinting from parent-of-origin phased methylomes and transcriptomes. *Nat Genet* **50**, 1542-1552, doi:10.1038/s41588-018-0232-7 (2018).

Description of partial phenotypes associated with parent-of-origin-specific genotypes that influence imprinted gene expression.

21 Sanchez-Delgado, M. *et al.* Human Oocyte-Derived Methylation Differences Persist in the Placenta Revealing Widespread Transient Imprinting. *PLoS Genet* **12**, e1006427, doi:10.1371/journal.pgen.1006427 (2016).

Demonstrates oocyte-derived methylation and polymorphic transient-DMRs.

22 Tan, L., Xing, D., Chang, C. H., Li, H. & Xie, X. S. Three-dimensional genome structures of single diploid human cells. *Science* **361**, 924-928, doi:10.1126/science.aat5641 (2018).

Identifies allelic contacts and novel CTCF-mediated loops at the H19-Igf2 locus by single cell Hi-C profiling.

23 Rao, S. S. *et al.* A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665-1680, doi:10.1016/j.cell.2014.11.021 (2014).

24 Kanduri, C. Long noncoding RNAs: Lessons from genomic imprinting. *Biochim Biophys Acta* **1859**, 102-111, doi:10.1016/j.bbagrm.2015.05.006 (2016).

25 Niemczyk, M. *et al.* Imprinted chromatin around DIRAS3 regulates alternative splicing of GNG12-AS1, a long noncoding RNA. *Am J Hum Genet* **93**, 224-235, doi:10.1016/j.ajhg.2013.06.010 (2013).

26 Inoue, A., Jiang, L., Lu, F., Suzuki, T. & Zhang, Y. Maternal H3K27me3 controls DNA methylation-independent imprinting. *Nature* **547**, 419-424, doi:10.1038/nature23262 (2017).

Demonstrates that maternal H3K27me3 controls DNA methylation-independent imprinting in early mouse embryos independent of DMRs.

27 Hutter, B., Helms, V. & Paulsen, M. Tandem repeats in the CpG islands of imprinted genes. *Genomics* **88**, 323-332, doi:10.1016/j.ygeno.2006.03.019 (2006).

28 Beygo, J. *et al.* The molecular function and clinical phenotype of partial deletions of the IGF2/H19 imprinting control region depends on the spatial arrangement of the remaining CTCF-binding sites. *Hum Mol Genet* **22**, 544-557, doi:10.1093/hmg/dds465 (2013).

29 Sparago, A., Cerrato, F. & Riccio, A. Is ZFP57 binding to H19/IGF2:IG-DMR affected in Silver-Russell syndrome? *Clin Epigenetics* **10**, 23, doi:10.1186/s13148-018-0454-7 (2018).

30 De Crescenzo, A. *et al.* A novel microdeletion in the IGF2/H19 imprinting centre region defines a recurrent mutation mechanism in familial Beckwith-Wiedemann syndrome. *Eur J Med Genet* **54**, e451-454, doi:10.1016/j.ejmg.2011.04.009 (2011).

31 Soares, M. L. *et al.* Targeted deletion of a 170-kb cluster of LINE-1 repeats and implications for regional control. *Genome Res*, doi:10.1101/gr.221366.117 (2018).

32 Varrault, A. *et al.* Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. *Dev Cell* **11**, 711-722, doi:10.1016/j.devcel.2006.09.003 (2006).

33 Gabory, A. *et al.* H19 acts as a trans regulator of the imprinted gene network controlling growth in mice. *Development* **136**, 3413-3421, doi:10.1242/dev.036061 (2009).
34 Stelzer, Y., Sagi, I., Yanuka, O., Eiges, R. & Benvenisty, N. The noncoding RNA IPW regulates the imprinted DLK1-DIO3 locus in an induced pluripotent stem cell model of Prader-Willi syndrome. *Nat Genet* **46**, 551-557, doi:10.1038/ng.2968 (2014).
35 Girardot, M., Cavaille, J. & Feil, R. Small regulatory RNAs controlled by genomic imprinting and their contribution to human disease. *Epigenetics* **7**, 1341-1348, doi:10.4161/epi.22884 (2012).
36 Court, F. *et al.* Genome-wide allelic methylation analysis reveals disease-specific susceptibility to multiple methylation defects in imprinting syndromes. *Hum Mutat* **34**, 595-602, doi:10.1002/humu.22276 (2013).
37 Docherty, L. E. *et al.* Mutations in NLRP5 are associated with reproductive wastage and multilocus imprinting disorders in humans. *Nat Commun* **6**, 8086, doi:10.1038/ncomms9086 (2015).
Identifies NLRP5 mutations in MLID.
38 Azzi, S. *et al.* A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome. *J Med Genet* **52**, 446-453, doi:10.1136/jmedgenet-2014-102979 (2015).
39 Begemann, M. *et al.* Maternal variants in NLRP and other maternal effect proteins are associated with multilocus imprinting disturbance in offspring. *J Med Genet* **55**, 497-504, doi:10.1136/jmedgenet-2017-105190 (2018).
Identifies deleterious variants in maternal effect genes associated with the SCMC in MLID patients.
40 Hackett, J. A. *et al.* Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* **339**, 448-452, doi:10.1126/science.1229277 (2013).
Demonstrates that erasure of CpG methylation in mouse PGCs occurs via conversion to 5-hydroxymethylcytosine (5hmC), driven by high levels of TET1 and TET2.
41 Seisenberger, S. *et al.* The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. *Mol Cell* **48**, 849-862, doi:10.1016/j.molcel.2012.11.001 (2012).
42 Guo, F. *et al.* The Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells. *Cell* **161**, 1437-1452, doi:10.1016/j.cell.2015.05.015 (2015).
First genome-wide description of the Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells.
43 Tang, W. W. *et al.* A Unique Gene Regulatory Network Resets the Human Germline Epigenome for Development. *Cell* **161**, 1453-1467, doi:10.1016/j.cell.2015.04.053 (2015).
44 Gkountela, S. *et al.* DNA Demethylation Dynamics in the Human Prenatal Germline. *Cell* **161**, 1425-1436, doi:10.1016/j.cell.2015.05.012 (2015).
45 Dawlaty, M. M. *et al.* Combined deficiency of Tet1 and Tet2 causes epigenetic abnormalities but is compatible with postnatal development. *Dev Cell* **24**, 310-323, doi:10.1016/j.devcel.2012.12.015 (2013).
46 Buiting, K. *et al.* Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. *Am J Hum Genet* **72**, 571-577, doi:10.1086/367926 (2003).
47 Poplinski, A., Tuttelmann, F., Kanber, D., Horsthemke, B. & Gromoll, J. Idiopathic male infertility is strongly associated with aberrant methylation of MEST and IGF2/H19 ICR1. *Int J Androl* **33**, 642-649, doi:10.1111/j.1365-2605.2009.01000.x (2010).
48 Hiura, H., Obata, Y., Komiyama, J., Shirai, M. & Kono, T. Oocyte growth-dependent progression of maternal imprinting in mice. *Genes Cells* **11**, 353-361, doi:10.1111/j.1365-2443.2006.00943.x (2006).
49 Guo, H. *et al.* The DNA methylation landscape of human early embryos. *Nature* **511**, 606-610, doi:10.1038/nature13544 (2014).
50 Gahurova, L. *et al.* Transcription and chromatin determinants of de novo DNA methylation timing in oocytes. *Epigenetics Chromatin* **10**, 25, doi:10.1186/s13072-017-0133-5 (2017).
51 Smallwood, S. A. *et al.* Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat Genet* **43**, 811-814, doi:10.1038/ng.864 (2011).
52 Yu, B. *et al.* Genome-wide, Single-Cell DNA Methylomics Reveals Increased Non-CpG Methylation during Human Oocyte Maturation. *Stem Cell Reports* **9**, 397-407, doi:10.1016/j.stemcr.2017.05.026 (2017).
53 Bourc'his, D., Xu, G. L., Lin, C. S., Bollman, B. & Bestor, T. H. Dnmt3L and the establishment of maternal genomic imprints. *Science* **294**, 2536-2539, doi:10.1126/science.1065848 (2001).
54 Kaneda, M. *et al.* Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* **429**, 900-903, doi:10.1038/nature02633 (2004).
55 Kato, Y. *et al.* Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. *Hum Mol Genet* **16**, 2272-2280, doi:10.1093/hmg/ddm179 (2007).

56 Shirane, K. *et al.* Mouse oocyte methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. *PLoS Genet* **9**, e1003439, doi:10.1371/journal.pgen.1003439 (2013).
57 Chotalia, M. *et al.* Transcription is required for establishment of germline methylation marks at imprinted genes. *Genes Dev* **23**, 105-117, doi:10.1101/gad.495809 (2009).
58 Baubec, T. *et al.* Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* **520**, 243-247, doi:10.1038/nature14176 (2015).
Demonstrates the targeting of DNA methylation to transcribed gene bodies by SETD2-mediated methylation of H3K36me3 and a functional recruitment of DNMT3B.
59 Dhayalan, A. *et al.* The Dnmt3a PWWP domain reads histone 3 lysine 36 trimethylation and guides DNA methylation. *J Biol Chem* **285**, 26114-26120, doi:10.1074/jbc.M109.089433 (2010).
60 Ciccone, D. N. *et al.* KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* **461**, 415-418, doi:10.1038/nature08315 (2009).
61 Ooi, S. K. *et al.* DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **448**, 714-717, doi:10.1038/nature05987 (2007).
62 Stewart, K. R. *et al.* Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. *Genes Dev* **29**, 2449-2462, doi:10.1101/gad.271353.115 (2015).
63 Petrussa, L., Van de Velde, H. & De Rycke, M. Dynamic regulation of DNA methyltransferases in human oocytes and preimplantation embryos after assisted reproductive technologies. *Mol Hum Reprod* **20**, 861-874, doi:10.1093/molehr/gau049 (2014).
64 Henckel, A., Chebli, K., Kota, S. K., Arnaud, P. & Feil, R. Transcription and histone methylation changes correlate with imprint acquisition in male germ cells. *EMBO J* **31**, 606-615, doi:10.1038/emboj.2011.425 (2012).
65 Singh, V. B. *et al.* Blocked transcription through KvDMR1 results in absence of methylation and gene silencing resembling Beckwith-Wiedemann syndrome. *Development* **144**, 1820-1830, doi:10.1242/dev.145136 (2017).
66 Beygo, J. *et al.* A maternal deletion upstream of the imprint control region 2 in 11p15 causes loss of methylation and familial Beckwith-Wiedemann syndrome. *Eur J Hum Genet* **24**, 1280-1286, doi:10.1038/ejhg.2016.3 (2016).
67 Lewis, M. W. *et al.* Angelman syndrome imprinting center encodes a transcriptional promoter. *Proc Natl Acad Sci U S A* **112**, 6871-6875, doi:10.1073/pnas.1411261111 (2015).
68 Smith, Z. D. *et al.* A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* **484**, 339-344, doi:10.1038/nature10960 (2012).
69 Eckersley-Maslin, M. A., Alda-Catalinas, C. & Reik, W. Dynamics of the epigenetic landscape during the maternal-to-zygotic transition. *Nat Rev Mol Cell Biol* **19**, 436-450, doi:10.1038/s41580-018-0008-z (2018).
70 Shen, L. *et al.* Tet3 and DNA replication mediate demethylation of both the maternal and paternal genomes in mouse zygotes. *Cell Stem Cell* **15**, 459-471, doi:10.1016/j.stem.2014.09.002 (2014).
71 Hirasawa, R. *et al.* Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. *Genes Dev* **22**, 1607-1616, doi:10.1101/gad.1667008 (2008).
72 Maenohara, S. *et al.* Role of UHRF1 in de novo DNA methylation in oocytes and maintenance methylation in preimplantation embryos. *PLoS Genet* **13**, e1007042, doi:10.1371/journal.pgen.1007042 (2017).
73 Nakamura, T. *et al.* PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* **486**, 415-419, doi:10.1038/nature11093 (2012).
74 Nakamura, T. *et al.* PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nat Cell Biol* **9**, 64-71, doi:10.1038/ncb1519 (2007).
75 Meyer, E. *et al.* Germline mutation in NLRP2 (NALP2) in a familial imprinting disorder (Beckwith-Wiedemann Syndrome). *PLoS Genet* **5**, e1000423, doi:10.1371/journal.pgen.1000423 (2009).
76 Parry, D. A. *et al.* Mutations causing familial biparental hydatidiform mole implicate c6orf221 as a possible regulator of genomic imprinting in the human oocyte. *Am J Hum Genet* **89**, 451-458, doi:10.1016/j.ajhg.2011.08.002 (2011).
77 Murdoch, S. *et al.* Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. *Nat Genet* **38**, 300-302, doi:10.1038/ng1740 (2006).
78 Sanchez-Delgado, M. *et al.* Absence of Maternal Methylation in Biparental Hydatidiform Moles from Women with NLRP7 Maternal-Effect Mutations Reveals Widespread Placenta-Specific Imprinting. *PLoS Genet* **11**, e1005644, doi:10.1371/journal.pgen.1005644 (2015).
79 Soellner, L. *et al.* Maternal heterozygous NLRP7 variant results in recurrent reproductive failure and imprinting disturbances in the offspring. *Eur J Hum Genet* **25**, 924-929, doi:10.1038/ejhg.2017.94 (2017).
80 Zhu, K. *et al.* Identification of a human subcortical maternal complex. *Mol Hum Reprod* **21**, 320-329, doi:10.1093/molehr/gau116 (2015).

81 Virant-Klun, I., Leicht, S., Hughes, C. & Krijgsveld, J. Identification of Maturation-Specific Proteins by Single-Cell Proteomics of Human Oocytes. *Mol Cell Proteomics* **15**, 2616-2627, doi:10.1074/mcp.M115.056887 (2016).
82 Tong, Z. B. *et al.* Mater, a maternal effect gene required for early embryonic development in mice. *Nat Genet* **26**, 267-268, doi:10.1038/81547 (2000).
83 Yurttas, P. *et al.* Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. *Development* **135**, 2627-2636, doi:10.1242/dev.016329 (2008).
84 Zheng, P. & Dean, J. Role of Filia, a maternal effect gene, in maintaining euploidy during cleavage-stage mouse embryogenesis. *Proc Natl Acad Sci U S A* **106**, 7473-7478, doi:10.1073/pnas.0900519106 (2009).
85 Mahadevan, S. *et al.* Maternally expressed NLRP2 links the subcortical maternal complex (SCMC) to fertility, embryogenesis and epigenetic reprogramming. *Sci Rep* **7**, 44667, doi:10.1038/srep44667 (2017).
Generation of maternal-effect mouse model for Nlrp2 which revealed disrupted imprinted methylation consistent with human MLID.
86 Sills, E. S. *et al.* Pathogenic variant in NLRP7 (19q13.42) associated with recurrent gestational trophoblastic disease: Data from early embryo development observed during in vitro fertilization. *Clin Exp Reprod Med* **44**, 40-46, doi:10.5653/cerm.2017.44.1.40 (2017).
87 Li, X. *et al.* A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. *Dev Cell* **15**, 547-557, doi:10.1016/j.devcel.2008.08.014 (2008).
88 Quenneville, S. *et al.* In embryonic stem cells, ZFP57/KAP1 recognize a methylated hexanucleotide to affect chromatin and DNA methylation of imprinting control regions. *Mol Cell* **44**, 361-372, doi:10.1016/j.molcel.2011.08.032 (2011).
89 Coluccio, A. *et al.* Individual retrotransposon integrants are differentially controlled by KZFP/KAP1-dependent histone methylation, DNA methylation and TET-mediated hydroxymethylation in naive embryonic stem cells. *Epigenetics Chromatin* **11**, 7, doi:10.1186/s13072-018-0177-1 (2018).
90 Anvar, Z. *et al.* ZFP57 recognizes multiple and closely spaced sequence motif variants to maintain repressive epigenetic marks in mouse embryonic stem cells. *Nucleic Acids Res* **44**, 1118-1132, doi:10.1093/nar/gkv1059 (2016).
91 Strogantsev, R. *et al.* Allele-specific binding of ZFP57 in the epigenetic regulation of imprinted and non-imprinted monoallelic expression. *Genome Biol* **16**, 112, doi:10.1186/s13059-015-0672-7 (2015).
92 Imbeault, M., Helleboid, P. Y. & Trono, D. KRAB zinc-finger proteins contribute to the evolution of gene regulatory networks. *Nature* **543**, 550-554, doi:10.1038/nature21683 (2017).
Identifies human genome targets for 350 Krüppel-associated box (KRAB) domain-containing zinc-finger proteins
93 Mackay, D. J. *et al.* Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. *Nat Genet* **40**, 949-951, doi:10.1038/ng.187 (2008).
94 Messerschmidt, D. M. *et al.* Trim28 is required for epigenetic stability during mouse oocyte to embryo transition. *Science* **335**, 1499-1502, doi:10.1126/science.1216154 (2012).
95 Sharif, J. *et al.* The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450**, 908-912, doi:10.1038/nature06397 (2007).
96 Dalgaard, K. *et al.* Trim28 Haploinsufficiency Triggers Bi-stable Epigenetic Obesity. *Cell* **164**, 353-364, doi:10.1016/j.cell.2015.12.025 (2016).
97 Dagar, V. *et al.* Genetic variation affecting DNA methylation and the human imprinting disorder, Beckwith-Wiedemann syndrome. *Clin Epigenetics* **10**, 114, doi:10.1186/s13148-018-0546-4 (2018).
98 Abi Habib, W. *et al.* 11p15 ICR1 Partial Deletions Associated with IGF2/H19 DMR Hypomethylation and Silver-Russell Syndrome. *Hum Mutat* **38**, 105-111, doi:10.1002/humu.23131 (2017).
99 Demars, J. *et al.* Analysis of the IGF2/H19 imprinting control region uncovers new genetic defects, including mutations of OCT-binding sequences, in patients with 11p15 fetal growth disorders. *Hum Mol Genet* **19**, 803-814, doi:10.1093/hmg/ddp549 (2010).
100 Freschi, A. *et al.* Tissue-specific and mosaic imprinting defects underlie opposite congenital growth disorders in mice. *PLoS Genet* **14**, e1007243, doi:10.1371/journal.pgen.1007243 (2018).
101 Hur, S. K. *et al.* Humanized H19/Igf2 locus reveals diverged imprinting mechanism between mouse and human and reflects Silver-Russell syndrome phenotypes. *Proc Natl Acad Sci U S A* **113**, 10938-10943, doi:10.1073/pnas.1603066113 (2016).
102 Bruno, C. *et al.* Germline correction of an epimutation related to Silver-Russell syndrome. *Hum Mol Genet* **24**, 3314-3321, doi:10.1093/hmg/ddv079 (2015).
103 Berland, S. *et al.* Evidence for anticipation in Beckwith-Wiedemann syndrome. *Eur J Hum Genet* **21**, 1344-1348, doi:10.1038/ejhg.2013.71 (2013).
104 Cortessis, V. K. *et al.* Comprehensive meta-analysis reveals association between multiple imprinting disorders and conception by assisted reproductive technology. *J Assist Reprod Genet* **35**, 943-952, doi:10.1007/s10815-018-1173-x (2018).

Meta-analysis demonstrating association between imprinting disorders and ART
105 Taniguchi, K., Kawai, T. & Hata, K. Placental Development and Nutritional Environment. *Adv Exp Med Biol* **1012**, 63-73, doi:10.1007/978-981-10-5526-3_7 (2018).
106 Cox, G. F. *et al.* Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet* **71**, 162-164, doi:10.1086/341096 (2002).
107 DeBaun, M. R., Niemitz, E. L. & Feinberg, A. P. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* **72**, 156-160, doi:10.1086/346031 (2003).
108 Maher, E. R. *et al.* Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). *J Med Genet* **40**, 62-64 (2003).
109 Mussa, A. *et al.* Assisted Reproductive Techniques and Risk of Beckwith-Wiedemann Syndrome. *Pediatrics* **140**, doi:10.1542/peds.2016-4311 (2017).
110 Tee, L. *et al.* Epimutation profiling in Beckwith-Wiedemann syndrome: relationship with assisted reproductive technology. *Clin Epigenetics* **5**, 23, doi:10.1186/1868-7083-5-23 (2013).
111 Tenorio, J. *et al.* Clinical and molecular analyses of Beckwith-Wiedemann syndrome: Comparison between spontaneous conception and assisted reproduction techniques. *Am J Med Genet A* **170**, 2740-2749, doi:10.1002/ajmg.a.37852 (2016).
112 Chen, Z. *et al.* Characterization of global loss of imprinting in fetal overgrowth syndrome induced by assisted reproduction. *Proc Natl Acad Sci U S A* **112**, 4618-4623, doi:10.1073/pnas.1422088112 (2015).
113 Canovas, S. *et al.* DNA methylation and gene expression changes derived from assisted reproductive technologies can be decreased by reproductive fluids. *Elife* **6**, doi:10.7554/eLife.23670 (2017).
114 de Waal, E. *et al.* The cumulative effect of assisted reproduction procedures on placental development and epigenetic perturbations in a mouse model. *Hum Mol Genet* **24**, 6975-6985, doi:10.1093/hmg/ddv400 (2015).
115 Ludwig, M. *et al.* Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples. *J Med Genet* **42**, 289-291, doi:10.1136/jmg.2004.026930 (2005).
116 Hyrapetian, M., Loucaides, E. M. & Sutcliffe, A. G. Health and disease in children born after assistive reproductive therapies (ART. *J Reprod Immunol* **106**, 21-26, doi:10.1016/j.jri.2014.08.001 (2014).
117 Weinerman, R., Ord, T., Bartolomei, M. S., Coutifaris, C. & Mainigi, M. The superovulated environment, independent of embryo vitrification, results in low birthweight in a mouse model. *Biol Reprod* **97**, 133-142, doi:10.1093/biolre/iox067 (2017).
118 Dankert, D. *et al.* Pre- and postovulatory aging of murine oocytes affect the transcript level and poly(A) tail length of maternal effect genes. *PLoS One* **9**, e108907, doi:10.1371/journal.pone.0108907 (2014).
119 Demond, H. *et al.* Preovulatory Aging In Vivo and In Vitro Affects Maturation Rates, Abundance of Selected Proteins, Histone Methylation Pattern and Spindle Integrity in Murine Oocytes. *PLoS One* **11**, e0162722, doi:10.1371/journal.pone.0162722 (2016).
120 Lu, Y. Q., He, X. C. & Zheng, P. Decrease in expression of maternal effect gene Mater is associated with maternal ageing in mice. *Mol Hum Reprod* **22**, 252-260, doi:10.1093/molehr/gaw001 (2016).
121 Camprubi, C. *et al.* Stability of genomic imprinting and gestational-age dynamic methylation in complicated pregnancies conceived following assisted reproductive technologies. *Biol Reprod* **89**, 50, doi:10.1095/biolreprod.113.108456 (2013).
122 Ji, M. *et al.* ART manipulation after controlled ovarian stimulation may not increase the risk of abnormal expression and DNA methylation at some CpG sites of H19,IGF2 and SNRPN in foetuses: a pilot study. *Reprod Biol Endocrinol* **16**, 63, doi:10.1186/s12958-018-0344-z (2018).
123 White, C. R. *et al.* High Frequency of Imprinted Methylation Errors in Human Preimplantation Embryos. *Sci Rep* **5**, 17311, doi:10.1038/srep17311 (2015).
124 Conradt, E. *et al.* Incorporating epigenetic mechanisms to advance fetal programming theories. *Dev Psychopathol* **30**, 807-824, doi:10.1017/S0954579418000469 (2018).
125 Han, L. *et al.* Embryonic defects induced by maternal obesity in mice derive from Stella insufficiency in oocytes. *Nat Genet* **50**, 432-442, doi:10.1038/s41588-018-0055-6 (2018).
Demonstrates that a maternal environmental insult may affect genomic integrity, epigenetic marking and developmental competence in cleavage stage of offspring.
126 Tserga, A., Binder, A. M. & Michels, K. B. Impact of folic acid intake during pregnancy on genomic imprinting of IGF2/H19 and 1-carbon metabolism. *FASEB J* **31**, 5149-5158, doi:10.1096/fj.201601214RR (2017).
127 Motzek, A. *et al.* Abnormal Hypermethylation at Imprinting Control Regions in Patients with S-Adenosylhomocysteine Hydrolase (AHCY) Deficiency. *PLoS One* **11**, e0151261, doi:10.1371/journal.pone.0151261 (2016).

128 Amarasekera, M. *et al.* Genome-wide DNA methylation profiling identifies a folate-sensitive region of differential methylation upstream of ZFP57-imprinting regulator in humans. *FASEB J* **28**, 4068-4076, doi:10.1096/fj.13-249029 (2014).
129 Kessler, N. J., Waterland, R. A., Prentice, A. M. & Silver, M. J. Establishment of environmentally sensitive DNA methylation states in the very early human embryo. *Sci Adv* **4**, eaat2624, doi:10.1126/sciadv.aat2624 (2018).
Analysis of metastable epialleles in a rural Gambian population demonstrates epigenetic sensitivity to season of birth in early embryonic development.
130 Van de Pette, M. *et al.* Visualizing Changes in Cdkn1c Expression Links Early-Life Adversity to Imprint Mis-regulation in Adults. *Cell Rep* **18**, 1090-1099, doi:10.1016/j.celrep.2017.01.010 (2017).
Demonstrates in a mouse model that maternal dietary change promotes transient alteration in DNA methylation and enduring mis-expression of an imprinted gene.
131 Ivanova, E., Chen, J. H., Segonds-Pichon, A., Ozanne, S. E. & Kelsey, G. DNA methylation at differentially methylated regions of imprinted genes is resistant to developmental programming by maternal nutrition. *Epigenetics* **7**, 1200-1210, doi:10.4161/epi.22141 (2012).
132 Heo, J. *et al.* Sirt1 Regulates DNA Methylation and Differentiation Potential of Embryonic Stem Cells by Antagonizing Dnmt3l. *Cell Rep* **18**, 1930-1945, doi:10.1016/j.celrep.2017.01.074 (2017).
133 Kim, J. H. *et al.* Perinatal bisphenol A exposure promotes dose-dependent alterations of the mouse methylome. *BMC Genomics* **15**, 30, doi:10.1186/1471-2164-15-30 (2014).
134 Kochmanski, J. J. *et al.* Longitudinal Effects of Developmental Bisphenol A Exposure on Epigenome-Wide DNA Hydroxymethylation at Imprinted Loci in Mouse Blood. *Environ Health Perspect* **126**, 077006, doi:10.1289/EHP3441 (2018).
135 Susiarjo, M., Sasson, I., Mesaros, C. & Bartolomei, M. S. Bisphenol a exposure disrupts genomic imprinting in the mouse. *PLoS Genet* **9**, e1003401, doi:10.1371/journal.pgen.1003401 (2013).
136 Trapphoff, T., Heiligentag, M., El Hajj, N., Haaf, T. & Eichenlaub-Ritter, U. Chronic exposure to a low concentration of bisphenol A during follicle culture affects the epigenetic status of germinal vesicles and metaphase II oocytes. *Fertil Steril* **100**, 1758-1767 e1751, doi:10.1016/j.fertnstert.2013.08.021 (2013).
137 Li, Y., Duan, F., Zhou, X., Pan, H. & Li, R. Differential responses of GC1 spermatogonia cells to high and low doses of bisphenol A. *Mol Med Rep* **18**, 3034-3040, doi:10.3892/mmr.2018.9256 (2018).
138 Junge, K. M. *et al.* MEST mediates the impact of prenatal bisphenol A exposure on long-term body weight development. *Clin Epigenetics* **10**, 58, doi:10.1186/s13148-018-0478-z (2018).
139 Ding, Z. M. *et al.* Bisphenol AF negatively affects oocyte maturation of mouse in vitro through increasing oxidative stress and DNA damage. *Chem Biol Interact* **278**, 222-229, doi:10.1016/j.cbi.2017.10.030 (2017).
140 Pathak, R. & Feil, R. Environmental effects on chromatin repression at imprinted genes and endogenous retroviruses. *Curr Opin Chem Biol* **45**, 139-147, doi:10.1016/j.cbpa.2018.04.015 (2018).
141 Meng, L. *et al.* Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. *Nature* **518**, 409-412, doi:10.1038/nature13975 (2015).
142 Kim, Y. *et al.* Targeting the histone methyltransferase G9a activates imprinted genes and improves survival of a mouse model of Prader-Willi syndrome. *Nat Med* **23**, 213-222, doi:10.1038/nm.4257 (2017).
Proof-of-principal mouse study identifying small molecules that selectively reduced H3K9me2 at PWS-IC and highlighting a potential therapy for PWS.
143 Huang, H. S. *et al.* Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons. *Nature* **481**, 185-189, doi:10.1038/nature10726 (2011).
144 Lei, Y. *et al.* Targeted DNA methylation in vivo using an engineered dCas9-MQ1 fusion protein. *Nat Commun* **8**, 16026, doi:10.1038/ncomms16026 (2017).
145 Cerrato, F. *et al.* Different mechanisms cause imprinting defects at the IGF2/H19 locus in Beckwith-Wiedemann syndrome and Wilms' tumour. *Hum Mol Genet* **17**, 1427-1435, doi:10.1093/hmg/ddn031 (2008).
146 Kagami, M. *et al.* Deletions and epimutations affecting the human 14q32.2 imprinted region in individuals with paternal and maternal upd(14)-like phenotypes. *Nat Genet* **40**, 237-242, doi:10.1038/ng.2007.56 (2008).
147 Azzi, S. *et al.* Complex tissue-specific epigenotypes in Russell-Silver Syndrome associated with 11p15 ICR1 hypomethylation. *Hum Mutat* **35**, 1211-1220, doi:10.1002/humu.22623 (2014).
148 Nazlican, H. *et al.* Somatic mosaicism in patients with Angelman syndrome and an imprinting defect. *Hum Mol Genet* **13**, 2547-2555, doi:10.1093/hmg/ddh296 (2004).
149 Weksberg, R. *et al.* Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Hum Mol Genet* **11**, 1317-1325 (2002).
150 Riess, A. *et al.* First report on concordant monozygotic twins with Silver-Russell syndrome and ICR1 hypomethylation. *Eur J Med Genet* **59**, 1-4, doi:10.1016/j.ejmg.2015.12.003 (2016).

151 Bliek, J. *et al.* Lessons from BWS twins: complex maternal and paternal hypomethylation and a common source of haematopoietic stem cells. *Eur J Hum Genet* **17**, 1625-1634, doi:10.1038/ejhg.2009.77 (2009).

152 Greenberg, M. V. *et al.* Transient transcription in the early embryo sets an epigenetic state that programs postnatal growth. *Nat Genet* **49**, 110-118, doi:10.1038/ng.3718 (2017).

Demonstrates that transient transcription in pre-implantation embryo sets an epigenetic state that programs postnatal growth.

153 Probst, A. V. *et al.* A strand-specific burst in transcription of pericentric satellites is required for chromocenter formation and early mouse development. *Dev Cell* **19**, 625-638, doi:10.1016/j.devcel.2010.09.002 (2010).

154 De Iaco, A. *et al.* DUX-family transcription factors regulate zygotic genome activation in placental mammals. *Nat Genet* **49**, 941-945, doi:10.1038/ng.3858 (2017).

155 Nativio, R. *et al.* Disruption of genomic neighbourhood at the imprinted IGF2-H19 locus in Beckwith-Wiedemann syndrome and Silver-Russell syndrome. *Hum Mol Genet* **20**, 1363-1374, doi:10.1093/hmg/ddr018 (2011).

156 Temple, I. K., Mackay, D. J. G. & Docherty, L. E. in *GeneReviews((R))* (eds M. P. Adam *et al.*) (1993).

157 Mackay, D. J. & Temple, I. K. Transient neonatal diabetes mellitus type 1. *Am J Med Genet C Semin Med Genet* **154C**, 335-342, doi:10.1002/ajmg.c.30272 (2010).

158 Mackay, D., Bens, S., Perez de Nanclares, G., Siebert, R. & Temple, I. K. Clinical utility gene card for: Transient Neonatal Diabetes Mellitus, 6q24-related. *Eur J Hum Genet* **22**, doi:10.1038/ejhg.2014.27 (2014).

159 Mackay, D. J. *et al.* A maternal hypomethylation syndrome presenting as transient neonatal diabetes mellitus. *Hum Genet* **120**, 262-269, doi:10.1007/s00439-006-0205-2 (2006).

160 Wakeling, E. L. *et al.* Diagnosis and management of Silver-Russell syndrome: first international consensus statement. *Nat Rev Endocrinol* **13**, 105-124, doi:10.1038/nrendo.2016.138 (2017).

161 Begemann, M., Spengler, S., Kordass, U., Schroder, C. & Eggermann, T. Segmental maternal uniparental disomy 7q associated with DLK1/GTL2 (14q32) hypomethylation. *Am J Med Genet A* **158A**, 423-428, doi:10.1002/ajmg.a.34412 (2012).

162 Barel, O. *et al.* Maternally inherited Birk Barel mental retardation dysmorphism syndrome caused by a mutation in the genomically imprinted potassium channel KCNK9. *Am J Hum Genet* **83**, 193-199, doi:10.1016/j.ajhg.2008.07.010 (2008).

163 Brioude, F. *et al.* Expert consensus document: Clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. *Nat Rev Endocrinol* **14**, 229-249, doi:10.1038/nrendo.2017.166 (2018).

164 Ogata, T. & Kagami, M. Kagami-Ogata syndrome: a clinically recognizable upd(14)pat and related disorder affecting the chromosome 14q32.2 imprinted region. *J Hum Genet* **61**, 87-94, doi:10.1038/jhg.2015.113 (2016).

165 Kagami, M. *et al.* Comprehensive clinical studies in 34 patients with molecularly defined UPD(14)pat and related conditions (Kagami-Ogata syndrome). *Eur J Hum Genet* **23**, 1488-1498, doi:10.1038/ejhg.2015.13 (2015).

166 Geoffron, S. *et al.* Chromosome 14q32.2 Imprinted Region Disruption as an Alternative Molecular Diagnosis of Silver-Russell Syndrome. *J Clin Endocrinol Metab* **103**, 2436-2446, doi:10.1210/jc.2017-02152 (2018).

167 Kagami, M. *et al.* Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients. *Genet Med* **19**, 1356-1366, doi:10.1038/gim.2017.53 (2017).

168 Ioannides, Y., Lokulo-Sodipe, K., Mackay, D. J., Davies, J. H. & Temple, I. K. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. *J Med Genet* **51**, 495-501, doi:10.1136/jmedgenet-2014-102396 (2014).

169 Driscoll, D. J., Miller, J. L., Schwartz, S. & Cassidy, S. B. in *GeneReviews((R))* (eds M. P. Adam *et al.*) (1993).

170 Deal, C. L. *et al.* GrowthHormone Research Society workshop summary: consensus guidelines for recombinant human growth hormone therapy in Prader-Willi syndrome. *J Clin Endocrinol Metab* **98**, E1072-1087, doi:10.1210/jc.2012-3888 (2013).

171 Buiting, K. Prader-Willi syndrome and Angelman syndrome. *Am J Med Genet C Semin Med Genet* **154C**, 365-376, doi:10.1002/ajmg.c.30273 (2010).

172 Baple, E. L. *et al.* An atypical case of hypomethylation at multiple imprinted loci. *Eur J Hum Genet* **19**, 360-362, doi:10.1038/ejhg.2010.218 (2011).

173 Dagli, A. I., Mueller, J. & Williams, C. A. in *GeneReviews((R))* (eds M. P. Adam *et al.*) (1993).

174 Bird, L. M. Angelman syndrome: review of clinical and molecular aspects. *Appl Clin Genet* **7**, 93-104, doi:10.2147/TACG.S57386 (2014).

175 Abreu, A. P., Macedo, D. B., Brito, V. N., Kaiser, U. B. & Latronico, A. C. A new pathway in the control of the initiation of puberty: the MKRN3 gene. *J Mol Endocrinol* **54**, R131-139, doi:10.1530/JME-14-0315 (2015).

176 Fountain, M. D. & Schaaf, C. P. Prader-Willi Syndrome and Schaaf-Yang Syndrome: Neurodevelopmental Diseases Intersecting at the MAGEL2 Gene. *Diseases* **4**, doi:10.3390/diseases4010002 (2016).

177 Mantovani, G. *et al.* Diagnosis and management of pseudohypoparathyroidism and related disorders: first international Consensus Statement. *Nat Rev Endocrinol* **14**, 476-500, doi:10.1038/s41574-018-0042-0 (2018).

178 Maupetit-Mehouas, S. *et al.* Simultaneous hyper- and hypomethylation at imprinted loci in a subset of patients with GNAS epimutations underlies a complex and different mechanism of multilocus methylation defect in pseudohypoparathyroidism type 1b. *Hum Mutat* **34**, 1172-1180, doi:10.1002/humu.22352 (2013).

179 Perez-Nanclares, G. *et al.* Detection of hypomethylation syndrome among patients with epigenetic alterations at the GNAS locus. *J Clin Endocrinol Metab* **97**, E1060-1067, doi:10.1210/jc.2012-1081 (2012).

180 Mulchandani, S. *et al.* Maternal uniparental disomy of chromosome 20: a novel imprinting disorder of growth failure. *Genet Med* **18**, 309-315, doi:10.1038/gim.2015.103 (2016).

181 Kawashima, S. *et al.* Maternal Uniparental Disomy for Chromosome 20: Physical and Endocrinological Characteristics of Five Patients. *J Clin Endocrinol Metab* **103**, 2083-2088, doi:10.1210/jc.2017-02780 (2018).

182 Popp, C. *et al.* Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. *Nature* **463**, 1101-1105, doi:10.1038/nature08829 (2010).

183 Chen, T., Ueda, Y., Dodge, J. E., Wang, Z. & Li, E. Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. *Mol Cell Biol* **23**, 5594-5605 (2003).

184 Begemann, M. *et al.* Silver-Russell patients showing a broad range of ICR1 and ICR2 hypomethylation in different tissues. *Clin Genet* **80**, 83-88, doi:10.1111/j.1399-0004.2010.01514.x (2011).

185 Akoury, E. *et al.* Live births in women with recurrent hydatidiform mole and two NLRP7 mutations. *Reprod Biomed Online* **31**, 120-124, doi:10.1016/j.rbmo.2015.03.011 (2015).

186 Caliebe, A. *et al.* A familial disorder of altered DNA-methylation. *J Med Genet* **51**, 407-412, doi:10.1136/jmedgenet-2013-102149 (2014).

187 Li, E., Beard, C. & Jaenisch, R. Role for DNA methylation in genomic imprinting. *Nature* **366**, 362-365, doi:10.1038/366362a0 (1993).

188 Howell, C. Y. *et al.* Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. *Cell* **104**, 829-838 (2001).

189 Reese, K. J., Lin, S., Verona, R. I., Schultz, R. M. & Bartolomei, M. S. Maintenance of paternal methylation and repression of the imprinted H19 gene requires MBD3. *PLoS Genet* **3**, e137, doi:10.1371/journal.pgen.0030137 (2007).

190 Ma, P., Lin, S., Bartolomei, M. S. & Schultz, R. M. Metastasis tumor antigen 2 (MTA2) is involved in proper imprinted expression of H19 and Peg3 during mouse preimplantation development. *Biol Reprod* **83**, 1027-1035, doi:10.1095/biolreprod.110.086397 (2010).

191 Wu, M. Y., Tsai, T. F. & Beaudet, A. L. Deficiency of Rbbp1/Arid4a and Rbbp1l1/Arid4b alters epigenetic modifications and suppresses an imprinting defect in the PWS/AS domain. *Genes Dev* **20**, 2859-2870, doi:10.1101/gad.1452206 (2006).

192 Judson, H., Hayward, B. E., Sheridan, E. & Bonthron, D. T. A global disorder of imprinting in the human female germ line. *Nature* **416**, 539-542, doi:10.1038/416539a (2002).

193 Li, L., Baibakov, B. & Dean, J. A subcortical maternal complex essential for preimplantation mouse embryogenesis. *Dev Cell* **15**, 416-425, doi:10.1016/j.devcel.2008.07.010 (2008).

194 Wu, X. *et al.* Zygote arrest 1 (Zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nat Genet* **33**, 187-191, doi:10.1038/ng1079 (2003).

195 Gowher, H., Stuhlmann, H. & Felsenfeld, G. Vezf1 regulates genomic DNA methylation through its effects on expression of DNA methyltransferase Dnmt3b. *Genes Dev* **22**, 2075-2084, doi:10.1101/gad.1658408 (2008).

196 Mould, A. W. *et al.* Smchd1 regulates a subset of autosomal genes subject to monoallelic expression in addition to being critical for X inactivation. *Epigenetics Chromatin* **6**, 19, doi:10.1186/1756-8935-6-19 (2013).

197 Kim, J. D., Hinz, A. K., Choo, J. H., Stubbs, L. & Kim, J. YY1 as a controlling factor for the Peg3 and Gnas imprinted domains. *Genomics* **89**, 262-269, doi:10.1016/j.ygeno.2006.09.009 (2007).

198 Prokhortchouk, A. *et al.* Kaiso-deficient mice show resistance to intestinal cancer. *Mol Cell Biol* **26**, 199-208, doi:10.1128/MCB.26.1.199-208.2006 (2006).

199 Bohne, F. *et al.* Kaiso mediates human ICR1 methylation maintenance and H19 transcriptional fine regulation. *Clin Epigenetics* **8**, 47, doi:10.1186/s13148-016-0215-4 (2016).

200 Engel, N., West, A. G., Felsenfeld, G. & Bartolomei, M. S. Antagonism between DNA hypermethylation and enhancer-blocking activity at the H19 DMD is uncovered by CpG mutations. *Nat Genet* **36**, 883-888, doi:10.1038/ng1399 (2004).
201 Sparago, A. *et al.* Microdeletions in the human H19 DMR result in loss of IGF2 imprinting and Beckwith-Wiedemann syndrome. *Nat Genet* **36**, 958-960, doi:10.1038/ng1410 (2004).
202 Sparago, A. *et al.* Mechanisms causing imprinting defects in familial Beckwith-Wiedemann syndrome with Wilms' tumour. *Hum Mol Genet* **16**, 254-264, doi:10.1093/hmg/ddl448 (2007).
203 Kim, J. D. *et al.* Rex1/Zfp42 as an epigenetic regulator for genomic imprinting. *Hum Mol Genet* **20**, 1353-1362, doi:10.1093/hmg/ddr017 (2011).
204 Nichols, J. *et al.* Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95**, 379-391 (1998).
205 Hori, N. *et al.* A dyad oct-binding sequence functions as a maintenance sequence for the unmethylated state within the H19/Igf2-imprinted control region. *J Biol Chem* **277**, 27960-27967, doi:10.1074/jbc.M202280200 (2002).
206 Abi Habib, W. *et al.* Extensive investigation of the IGF2/H19 imprinting control region reveals novel OCT4/SOX2 binding site defects associated with specific methylation patterns in Beckwith-Wiedemann syndrome. *Hum Mol Genet* **23**, 5763-5773, doi:10.1093/hmg/ddu290 (2014).
207 Avilion, A. A. *et al.* Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev* **17**, 126-140, doi:10.1101/gad.224503 (2003).

Acknowledgements

The authors thank F. Cerrato, M. V. Cubellis and A. Sparago for careful reading of the manuscript. The authors apologize to all authors of studies that could not be cited due to the concise nature of this Review. Individual authors would like to thank the following funders for research support: MIUR PRIN 2015 JHLY35, Telethon-Italia GGP15131 and AIRC IG18671 (A. R.); the Deutsche Forschungsgemeinschaft (DFG), EG110/15-1 (T. E.); Spanish Ministry of Economy and Competitiveness (MINECO; BFU2014-53093-R and BFU2017-85571-R) to D. M., co-funded with the European Union Regional Development Fund (FEDER). E. R. M. acknowledges support from NIHR Cambridge Biomedical Research Centre and a NIHR Senior Investigator Award. The University of Cambridge has received salary support in respect of E. R. M. from the NHS in the East of England through the Clinical Academic Reserve. The views expressed are those of the authors and not necessarily those of the NIHR, NHS or Department of Health.

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 aetiology[36]. 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 syndrome[146].

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 growth[100]. Mosaic *H19* hypomethylation is common in SRS, for which severity differs markedly between patients[147]. Detailed studies in another imprinting disorder, Angelman Sydrome, 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 implantation[148]. 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 SRS[150]. 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 twin[151] 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 expression[152]. More recently, widespread transient imprinting derived from oocyte-specific methylation has been demonstrated in human placenta[21]. Epigenetic marks other than DNA methylation may also mediate transient imprinting, although this has not been reported in humans[26]. 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 ZGA[153].

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 DUX4[154]. 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, respectively[155]. **b** | Chromosome interactions and gene expression changes predicted by the looping model as consequence of IC1 LOM in SRS[155]. *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 BWS[155]. *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%). - *PLAGL1:alt-TSS-DMR* LOM (30%) (MLID: 30% caused by pathogenic *ZFP57* 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%) - *H19/*IGF2:IG:DMR LOM (30-60% (MLID: 7–10% [37,39,147])) - *CDKN1C, IGF2, HMGA2, PLAG1* point mutations (rare)	IUGR, PNGR, relative macrocephaly at birth, body asymmetry, prominent forehead, feeding difficulties
Birk–Barel syndrome[162] 612292	unknown	Chr 8q24.3	- *KCNK9* 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%) - *H19/*IGF2:IG:DMR GOM[a] (5%) - *KCNQ1OT1*:TSS-DMR LOM (50% (MLID: 25%[37,39])) - *CDKN1C* 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%) - *MEG3/DLK1*:IG-DMR 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%) - *MEG3/DLK1*: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%) - *SNURF*: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%) - *SNURF*:TSS-DMR LOM[a] (2-3% (MLID: rare[172])) - *UBE3A* 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	- *MKRN3* 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	- *MAGEL2* point mutations (100%)	Delayed psychomotor development, intellectual disability, hypotonia
Pseudohypo-parathyroidism 1A (PHP1A) including PHP1C[177] 103580 612462	Unknown	Chr 20q13:	- *GNAS* 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)
Pseudohypo-parathyroidism 1B (PHP1B)[177] 603233	Unknown	Chr 20q13	- 20q13 maternal deletion (8.5%) - *GNAS* 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
Pseudopseudohypo-parathyroidism (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**
PPIEL:Ex1-DMR		1:40024626-40025540	M	Oocyte gDMR		*PPIEL*
DIRAS3:TSS-DMR	*NOEY2, ARH1*	1: 68513430-68517450	M	Oocyte gDMR		*DIRAS3, GNG12-AS1*
DIRAS3:Ex2-DMR		1:68512505-68513486	M	Oocyte gDMR	ZFP57	
GPR1-AS:TSS-DMR		2:207114583-207136544	M	Oocyte gDMR		*GPR1-AS, ZDBF2, ADAM23*
ZDBF2/GPR1-AS:IG-DMR		2:207114583-207136544	P	Sperm gDMR-secondary DMR	CTCF, RAD21	
JAKMIP1:Int2-DMR		chr4:6106594-6108185	M	Oocyte gDMR		

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

					ZFP57, POU5F1	*IGF2-AS, INS-IGF2,* miR483, *H19, HOTS, 91H,* miR675
IGF2:Ex9-DMR	*IGF2*-DMR2	11:2153991-2155112	P	Secondary DMR		
IGF2:alt-TSS-DMR	*IGF2*-DMR0	11:2168333-2169768	P	Sperm gDMR		
KCNQ1OT1:TSS-DMR	*IC2, ICR2, KvDMR1, KvLQT1, LIT1*	11:2719948-2722529	M	Oocyte gDMR	ZFP57	*KCNQ1OT1, CDKN1C, PHLDA2, SLC22A18, KCNQ1*
RB1:Int-DMR		13:48892341-48895763	M	Oocyte gDMR	ZFP57	*LPAR6*
LPAR6:TSS-DMR		13:48984639-48987689	M	Secondary DMR		
DLK1:Int2-DMR		14: 101193446-101195447	M	Secondary DMR		
MEG3/DLK1:IG-DMR	IG-DMR, *GLT2-DLK1*	14:101275427-101278058	P	Sperm gDMR		*MEG3, DLK1, MEG8, DIO3, RTL1, RTL-AS, MEG9, SNORD113, SNORD114* and miRNA cluster
MEG3:TSS-DMR	*GTL2*	14:101290524-101293978	P	Secondary DMR	CTCF, ZFP57	
MEG8:Int2-DMR		14:101370410-101371410	M	Secondary DMR	CTCF, RAD21	
MKRN3:TSS-DMR		15:23807086-23812495	M	Oocyte gDMR-secondary DMR		
MAGEL2:TSS-DMR		15:23892425-23894029	M	Secondary DMR		
NDN:TSS-DMR		15:23931451-23932759	M	Secondary DMR		
[b]*SNRPN*:alt-TSS-DMR		15:25068564-25069481	M	Secondary DMR		
[b]*SNRPN*:Int1-DMR1		15:25093008-25193829	M	Secondary DMR	POU5F1	
[b]*SNRPN*:Int1-		15:25123027-	M	Secondary		

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

[a]These imprinted DMRs show evidence for mosaicism in the general population with low frequency hypomethylation reported. [b]The 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 (*Mest, Peg3, Igf2r, H19*)[45]	not reported
DNMT3A/B	*De novo* 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 (Mest, *Grb10, Zac1, Impact, U2af1-rs1, Peg10, Nnat, KvDMR1, Igf2r, Gnas*)[60,62]	not reported
KDM1A	H3K4 demethylase	LOM at transient *Cdh15* 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	*De novo* 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 (*H19, Snrpn*)[94]	No pathogenic variants identified in MLID[36]

	complex		
UHRF1	Guides DNMT1 to hemimethylated DNA during replication	Partial LOM during embryonic cleavage stages (*H19*, KvDMR1, Ig-DMR)[95]	Single case of MLID[39]
Histone H1	Linker histones	Triple KOs result in Partial LOM (*H19*, Ig-DMR)	not reported
MBD3	Methyl-CpG binding protein	Partial LOM at *H19*[189]	No pathogenic variants identified in SRS[184]
MTA2	Metastasis tumour antigen 2, member of NuRD complex	Partial LOM at *H19* and *Peg3*[190]	not reported
RBBP1/Arid4a RBBP1L1/Arid4b	Retinoblastoma-binding proteins	LOM at *Snrpn* in double KOs[191]	not reported
DPPA3/STELLA	Binds to methylated and H3K9 decorated gDMRs	Partial LOM at several gDMRs (*Snrpn, Mest, Peg3, Nnat, Peg10, H19, Rasgrf1*)[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[84]	Biparental hydatidiform moles. LOM at several DMRs (KvDMR1, SNRPN, MEST, PEG3, GNAS XL, GNAS A/B)[76,192] No pathogenic variants identified in MLID[36,186]
NLRP5/MATER	Member of SCMC	Imprinting not assessed[82]	MLID[37]
OOEP	Member of SCMC	Imprinting not assessed[193]	Single case of MLID[39]
PADI6	Member of SCMC	Imprinting not assessed[83]	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 *H19* and *Igf2r* 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 *H19*[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.