EPIGENETICS

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

*David Monk[1], Deborah J. G. Mackay[2], Thomas Eggermann[3], Eamonn R. Maher[4] and Andrea Riccio[5]**

Abstract | Genomic imprinting, the monoallelic and parent-of-origin-dependent expression of a subset of genes, is required for normal development, and its disruption leads to human disease. Imprinting defects can involve isolated or multilocus epigenetic changes that may have no evident genetic cause, or imprinting disruption can be traced back to alterations of *cis*-acting elements or *trans*-acting factors that control the establishment, maintenance and erasure of germline epigenetic imprints. 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.

[1]*Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.*

[2]*Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton, UK.*

[3]*Institute of Human Genetics, Medical Faculty, RWTH Aachen University, Aachen, Germany.*

[4]*Department of Medical Genetics, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, UK.*

[5]*Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania 'Luigi Vanvitelli', Caserta; Institute of Genetics and Biophysics 'Adriano Buzzati–Traverso' CNR, Napoli, Italy.*

**e-mail: andrea.riccio@unicampania.it*

https://doi.org/10.1038/s41576-018-0092-0

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 parent-of-origin-dependent expression results from differential epigenetic marking, primarily from methylated cytosine at CpG dinucleotides of genes during gametogenesis in the male and female germ line. 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 among epialleles 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 (Supplementary 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 pathogenic gene sequence variants, copy number variants and uniparental disomy (UPD), or epigenetic changes that affect the regulation of imprinted loci (epimutations). The frequency of the four types of molecular alteration 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, imprinting errors originating in the germ line as primary or secondary epimutations are similarly permanently maintained in somatic tissues, resulting in disease phenotypes later in development. Primary or secondary epimutations and/or 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.

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. These studies are 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 the environment and the epigenomes of gametes and early embryos suggest mechanistic explanations for the sporadic occurrence of imprinting errors.

Box 1 | **Epigenetic mosaicism in imprinting disorders**

Several individuals with imprinting disorders, with or without multilocus imprinting disturbances (MLIDs), have somatic mosaicism, in which tissues contain cells with imprinting aberrations and cells with appropriate allelic methylation. Mosaicism is observed with all types of primary and secondary epimutation, with the exception of erasure and establishment errors (FIG. 4), indicating a more common post-zygotic aetiology[36]. In principle, the developmental period immediately before 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 phenomenon may account for the hypermethylation of imprinting centre 1 (IC1), seen in Beckwith–Wiedemann syndrome (BWS)[28,146], and imprinted gene–differentially methylated region (DMR) hypermethylation in Kagami–Ogata syndrome[147].

People with Silver–Russell syndrome (SRS) or BWS often present with body asymmetry, a feature credited to mosaicism, with recent mouse models for these two imprinting disorders identifying mosaicism in bilateral organs with asymmetric growth[101]. Mosaic *H19* hypomethylation is common in SRS, for which severity differs markedly between individuals[148]. Detailed studies in another imprinting disorder, Angelman syndrome, explored the timing of such an event. In a female patient with mosaic *SNURF* hypomethylation, X chromosome analysis showed that cells with the imprinting defect had either the paternally derived or maternally derived X chromosome inactivated, suggesting that the insult occurred before X inactivation and implantation[149]. In principle, somatic imprinting errors can occur at any time in dividing cells. Immediately following replication, the methylation pattern on the template DNA 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 hemi-methylated 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, in which one twin has the disorder (nearly always female and often with MLIDs) and the other is healthy, are over-represented among people with BWS[36,37,39,150] and SRS[151], suggesting that monozygotic twinning is connected to epigenetic disturbances in early development. The occasional presence of mild clinical features of BWS and intermediate methylation disturbance in the twin who is not affected[152] supports the hypothesis that imprinting centre epimutations precede and may possibly trigger the twinning process in the early embryo.

This Review focuses on imprints that effect essentially permanent and ubiquitous changes on gene expression potential at affected loci, as opposed to tissue-specific or transient changes (BOX 2). We begin with a brief overview of the genomic basis of imprinting and its control, before reviewing the life cycle of genomic imprinting and how disruption of the individual factors involved in the establishment, maintenance and erasure of imprints can result in disease. We discuss the heritability of imprinting defects and the role of environmental insults in imprinting disorders. Finally, we highlight areas requiring additional research that could complete our understanding of imprinting disorders, as well as the new technological advances that might correct imprinting errors. For details on the evolutionary importance of genomic imprinting[1,9], the methods of imprinting analysis[10], the physiological role of imprinted genes[6] or chromatin mechanisms in imprinting[11], the reader is referred to previous authoritative reviews.

The genomic basis of imprinting

The majority of imprinted genes are found in clusters, called imprinted domains, that enable coordinated regulation by shared regulatory elements such as long non-coding RNAs (lncRNAs) and differentially methylated regions (DMRs), that is, regions 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 a primary DMR (FIG. 1). Approximately 35 gDMRs associated with imprinted loci have been identified in the human genome[12] (Supplementary Table 2). 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), histone 3 lysine 9 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[4,11,13] (FIG. 1). 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[14] (FIG. 1). Whereas maternally methylated gDMRs are more numerous, intragenic and generally correspond to promoters, often of lncRNAs, gDMRs methylated on paternal chromosomes are intergenic and may function as insulators or enhancers[1,15] (Supplementary Table 2).

Of note, in multigenic imprinted domains, the imprinting centre often directs the expression of genes from both the chromosome on which it is methylated and the opposite parental chromosome; this situation arises from the regulatory interactions between imprinting centres and the gene products, both coding and non-coding, under their control (FIG. 2). 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[7] (FIG. 2; Supplementary Table 1).

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* (REF.[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 additional 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[20] (Supplementary Table 2), chromatin modifications, higher-order chromatin structures[22,23] (possibly resulting from CTCF–cohesin interactions; FIG. 2) and lncRNAs with silencing capacity for flanking imprinted genes in *cis*[1,24] (FIG. 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

Genomic imprinting
The epigenetic marking of a gene on the basis of parental origin that results in monoallelic expression.

Epialleles
Epigenetic profiles that are maintained in somatic tissues, resulting in interindividual variation.

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

Uniparental disomy
(UPD). A genetic defect characterized by the presence of two copies of a chromosome or part of the chromosome derived from only one parent.

Box 2 | **Transient imprinting**

A transient form of imprinting has been described in both mouse and human pre-implantation embryos[5,51,153], whereby 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 the non-coding RNA *Gpr1-As* mediates the accumulation of methylation at the *Zdbf2* differentially methylated region (DMR), whose stable maintenance in adult somatic tissues regulates *Zdbf2* allelic expression[153]. 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[26]. Further studies are needed to determine whether this form of DNA methylation imprinting is conserved in other species and what impact it has on gene expression and phenotypes at later developmental stages. It is possible that transient and DNA methylation-independent monoallelic expression controls the establishment of secondary germline DMRs and consequently leads to a more stable imprinted expression in somatic tissues.

their allele-specific expression may 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, tandem repeats in the *H19–IGF2* intergenic DMR concentrate methylation-restricted binding of ZFP57 and CTCF, which are critical for imprinting[28,29] (FIG. 1). In this case, recombination of the tandem repeats can result in recurrent imprinting defects[30]. By contrast, deletion of a large array of repeats of long-interspersed elements (LINE-1) in the *Dlk1–Dio3* imprinting domain in mouse embryonic stem cells did not disrupt imprinting or, seemingly, normal development of either maternal or paternal mutant mice[31], a finding that does not support a role for these repeats in imprinting.

Imprinted gene products intensify their fine-tuned regulation by cooperation in an imprinted gene network (IGN)[32,33]. For example, in mouse tissues, the transcription factor PLAGL1 (REF.[32]) and lncRNA *H19* (REF.[33]) have been shown to regulate, in a DNA-methylation-independent manner, the mRNA level of several members of an IGN controlling growth. As another example, 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 H3K9 histone methyltransferase G9A (also known as EHMT2) 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 might explain some of the observed overlap in the phenotypes of different imprinting disorders (Supplementary Table 1).

Multilocus imprinting disturbances. A subset of individuals with imprinting defects exhibit multilocus imprinting disturbances (MLIDs), that is, imprinting disruptions at multiple loci across the genome. MLIDs are confined to epimutation subgroups of imprinting disorders (Supplementary Table 1) and involve loci associated with known imprinting disorders and those not currently linked with specific phenotypes[36,37]. To date, most people with MLIDs have clinical features characteristic of one imprinting disorder, notably BWS, SRS or transient neonatal diabetes mellitus (TNDM), probably owing 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[37–39] (BOX 1).

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 are involved in these complex processes, and DNA binding sites can be targets of mutations that cause human imprinting disorders (Supplementary Table 3).

Imprinting centre methylation dynamics in germ cells. Of the ubiquitous gDMRs present in somatic tissues, all but two originate from the oocyte[5,12] (Supplementary Table 2). This disparity reflects fundamental differences in the mechanisms of methylation acquisition in the female and male germ lines and in the treatment of parent-of-origin-derived methylation in the zygote[21] (FIG. 3). In primordial germ cells (PGCs), the precursors of sperm and oocytes, germline specification requires remodelling of the epigenome as a prerequisite 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 demethylation 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 of 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 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 (FIG. 4). In the case of GOM at the PWS–Angelman syndrome (AS) imprinting centre (also known as the *SNURF*: transcription start site (TSS) DMR), grandmaternal methylation is not erased in paternal PGCs. As a result, the paternal allele retains this maternal imprint[46] (FIG. 4a). Similarly, the hypermethylation of imprinting centres in sperm from individuals with subfertility is consistent with incomplete erasure of imprints[47].

Remethylation and imprint acquisition occur asynchronously between the sexes, with de novo methylation in the male germ line occurring before birth and maintained through many cycles of mitotic division before

Epimutations
With reference to imprinting disorders, epigenetic changes that affect the regulation of imprinted loci. An epimutation is primary if there is no detectable genetic cause and secondary if it is associated with a genetic cause.

cis-acting elements
DNA sequences that regulate the expression of a gene that is present on the same chromosome.

trans-acting factors
Proteins that regulate the expression of a gene.

Epigenetic reprogramming
The erasure of pre-existing epigenetic marks that enable subsequent remodelling of chromatin.

Epigenome
The chromatin modifications influencing genome function but not involving the underlying DNA sequence that can be propagated through cell division.

Imprinting centre
Also known as imprinting control region. A functional definition of germline differentially methylated regions (gDMRs) that have been shown through either genetic targeting in mice or mutations in humans to regulate imprinted gene expression. Not all gDMRs have been shown to be imprinting centres.

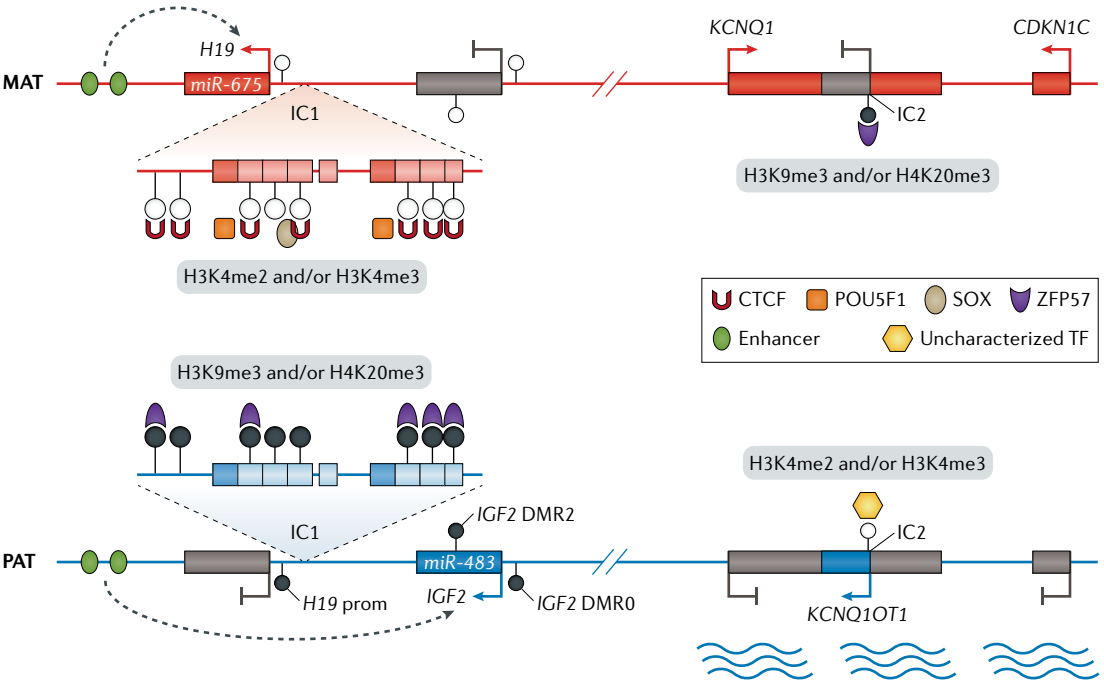


Fig. 1 | **Chromosome 11p15.5 as an example of an imprinted gene cluster.** Within the telomeric domain, enhancers (green ovals) direct transcription of the long non-coding RNA (lncRNA) *H19* and the intragenic microRNA (miRNA) *miR-675* on the maternal chromosome (MAT; red line) and that of the growth factor gene *IGF2* and the intragenic *miR-483* on the paternal chromosome (PAT; blue line). The imprinting centre of the telomeric domain (IC1; inset), also known as the *H19–IGF2* intergenic differentially methylated region (DMR), contains tandem repeats (light red and light 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 histone 3 lysine 9 dimethylation (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*:transcription start site (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) oblong rectangles, and inactive alleles are represented with grey oblong rectangles.

entry into meiosis, while 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 that the timings are similar to those of mice.

In mouse, 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 the underlying chromatin signature are important factors that determine methylation acquisition[50]. Transcription in oocytes is required for methylation at numerous gDMRs[57], which may render the chromatin more accessible to the de novo methylation machinery and/or might be associated with specific chromatin changes. The co-transcriptional histone H3K36me3 mark is deposited at intragenic CpG islands and is subsequently recognized by DNMT3A and DNMT3B[58,59]. Successive removal of dimethylation and trimethylation of histone H3K4 by KDM1A or KDM1B (known previously as AOF2 or LSD1 and AOF1 or LSD2, respectively) enables direct interaction between DNMT3L and the unmodified histone tail[60–62]. Despite being a generic methylase in mouse oocytes, DNMT3L is not detectable by expression profiling in human oocytes between the germinal vesicle phase and meiosis II[63], suggesting that it is not required for de novo methylation in the human female germ line. In male mouse germ cells, transcriptional readthrough 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. The establishment

Germline differentially methylated region
(gDMR). A region of differential DNA methylation between the parental alleles in somatic cells that originates from the gametes. gDMRs that survive embryonic reprogramming are generally associated with imprinted genes.

Loss of methylation
(LOM). With reference to imprinting disorders, loss of differential imprinting centre methylation detected in individuals that causes deregulation of imprinted genes in the domain controlled by the imprinting centre.

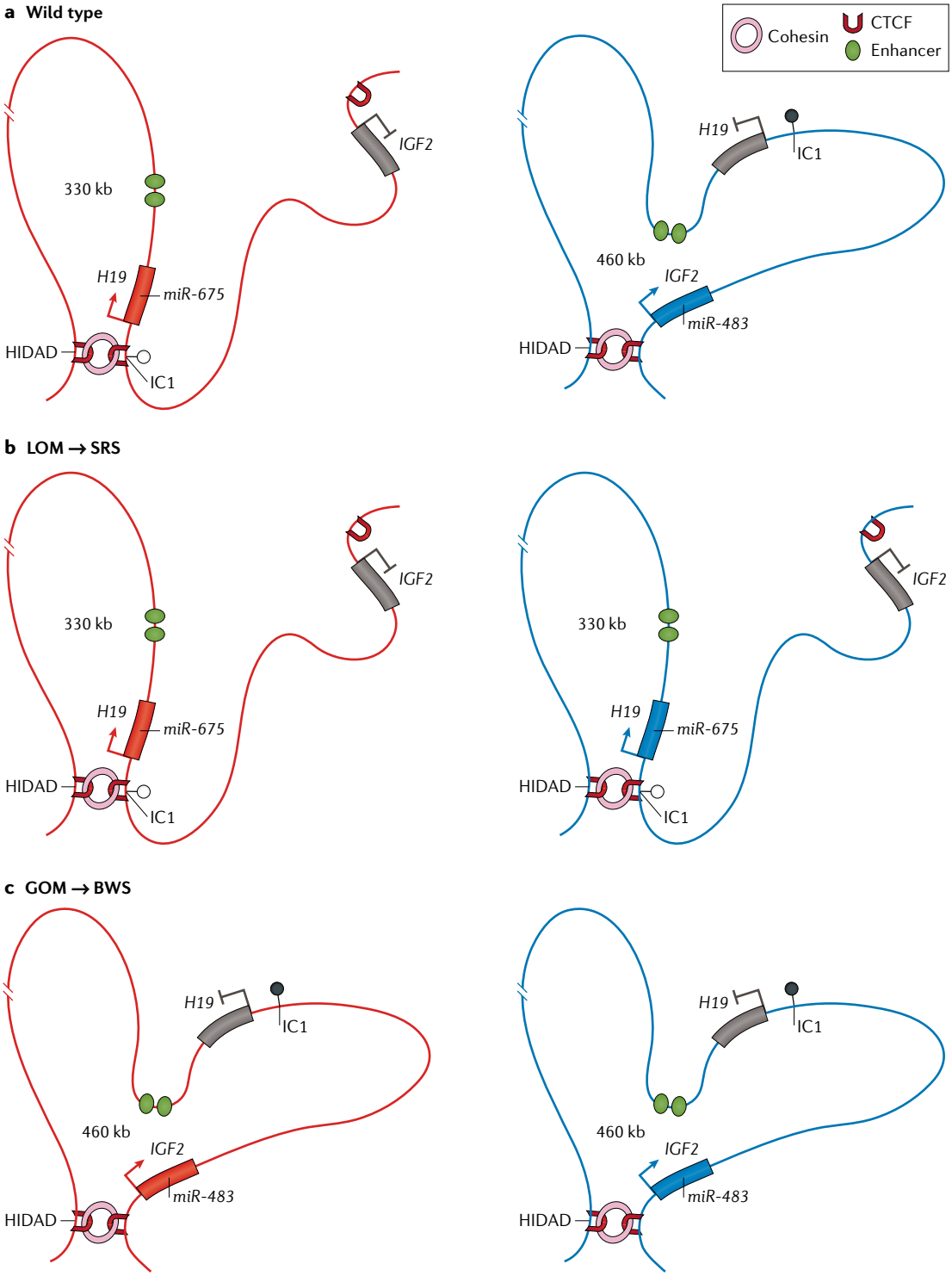


Fig. 2 | **The imprinted 11p15.5 region as an example of an epigenetic error in imprinting disorders. a** | Model of the 11p15.5 region represented as alternative chromatin loops on the maternal and paternal chromosomes. The model is based on the results of chromatin conformation capture studies in human cells[22,23]. Loop 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 imprinting centre 1 (IC1). On the paternal chromosome (blue line), the formation of this loop is prevented by methylation of IC1, and an alternative loop 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[156]. **b** | Chromosome interactions and gene expression changes predicted by the looping model as a consequence of IC1 loss of methylation (LOM) in Silver–Russell syndrome (SRS)[156]. *H19* is activated and *IGF2* is silenced on both parental chromosomes. **c** | Chromosome interactions and gene expression changes predicted by the looping model as a consequence of IC1 gain of methylation (GOM) in Beckwith–Wiedemann syndrome (BWS)[156]. *H19* is silenced and *IGF2* is activated on both parental chromosomes. Chromosome distances are not to scale.

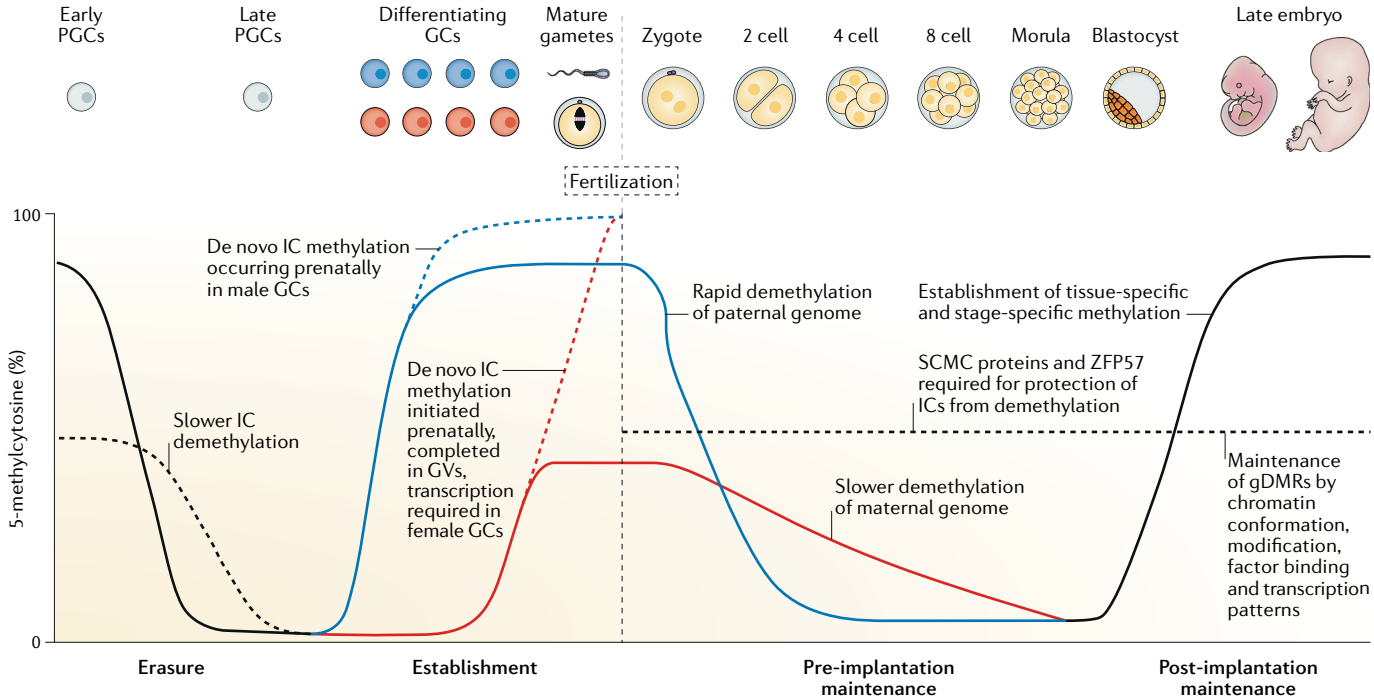


Fig. 3 | **The life cycle of imprints.** DNA methylation reprogramming during human development. Methylation of imprinting centres (ICs) (dashed black line) is erased more slowly than that of the rest of the genome (black line) in primordial germ cells (PGCs) and re-established with different kinetics in male (paternal ICs, dashed blue line; whole genome, blue line) and female (maternal ICs, 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 IC alleles (50% level) is maintained pre-implantation and post-implantation. Factors and events involved in each stage, 5-methylcytosine level and approximate timing of imprint erasure, establishment and pre-implantation and post-implantation maintenance are indicated. gDMRs, germline differentially methylated regions; GVs, germinal vesicles; SCMC, subcortical maternal complex.

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

Secondary DMRs
Also known as somatic differentially methylated regions (DMRs). Regions of differential DNA methylation between parental alleles that do not originate in the germ line. They are regulated in a hierarchical fashion by a nearby imprinting centre region.

Multilocus imprinting disturbances
(MLIDs). Methylation anomalies at imprinted differentially methylated regions in individuals with imprinting disorders in addition to those that are normally associated with disease.

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[65] (FIG. 4b). Genetic mutations that affect transcription through a gDMR have been identified in individuals with rare BWS with complete and isolated lack of methylation at imprinting centre 2 (IC2), the imprinting centre of the centromeric domain of the BWS–SRS locus (also known as *KCNQ1OT1*:TSS DMR)[66,67]. Disruption of germline transcription is probably also present in people with AS who have non-mosaic LOM of the *SNURF*:TSS DMR and deletions of the smallest region of deletion overlap for AS (AS-SRO)[68].

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[51,69], as part of the extensive epigenetic reprogramming that underpins zygotic genome activation (ZGA) and that is required first to acquire totipotency and subsequently to initiate differentiation (BOX 3; reviewed elsewhere[70]). The murine paternal genome is demethylated early in the first cell cycle, in part by TET3-induced oxidation of 5mC[5,49,69,71], whereas maternal demethylation occurs predominantly passively, by replicative dilution during cleavage-stage divisions, possibly through the restricted activity or localization of DNMT1 and its accessory factors[72,73] (FIG. 3). Studies in human systems are currently limited but indicate differences in the 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 of 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.

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 H3K9me2-marked chromatin[74]. *Dppa3* is a maternal-effect gene; concepti of maternal null mice rarely progress beyond the two-cell stage, and their genomes are severely demethylated[75].

Maternal-effect variants in NLRP proteins and associated factors have been implicated in pregnancy outcomes including hydatidiform mole and infertility, as well as monozygotic twinning, pregnancy loss and MLID[37,39,76,77] (FIG. 4c,d). Women with biallelic-inactivating *NLRP7*

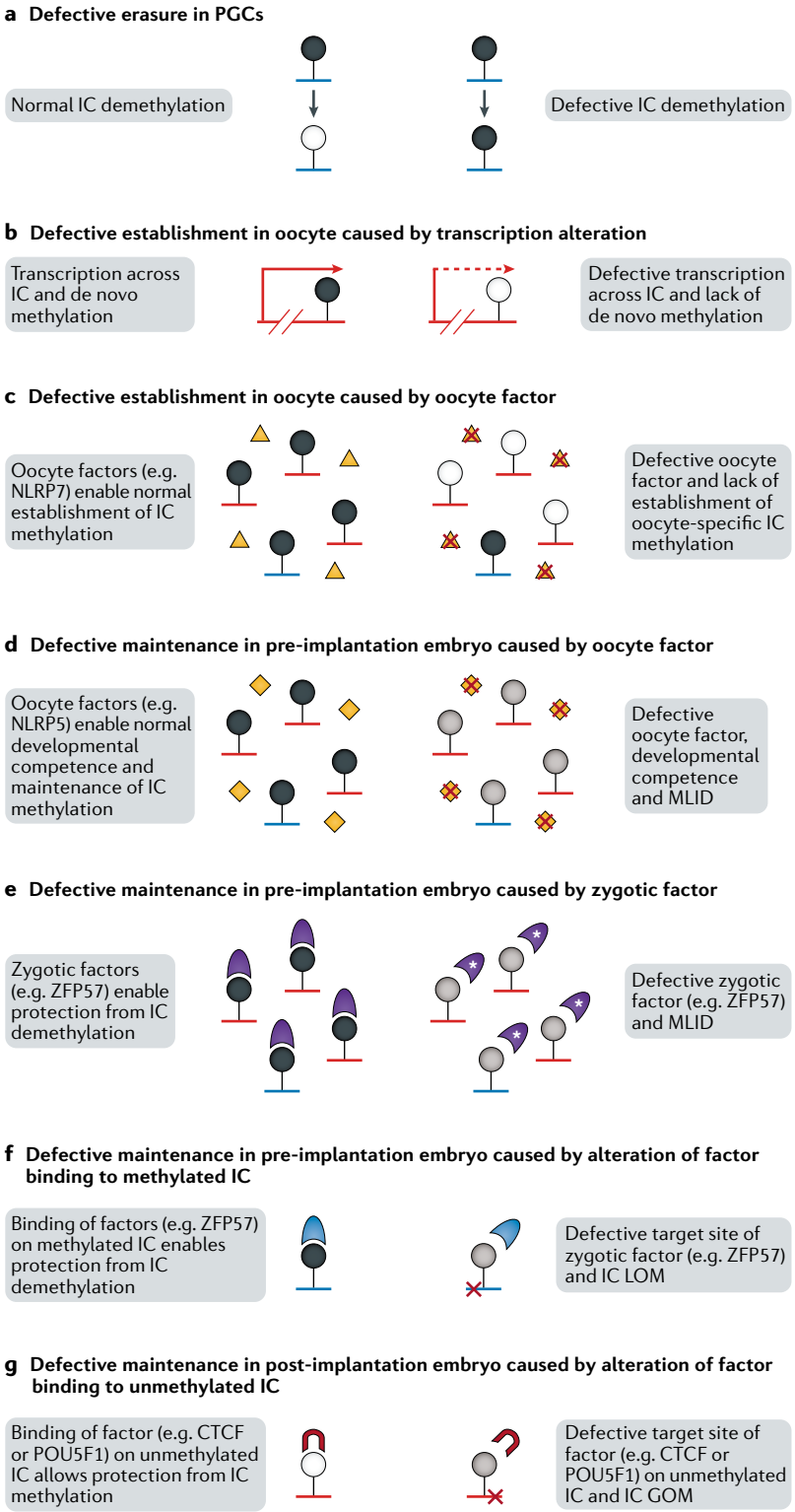


Fig. 4 | **Mechanisms of imprinting errors in human diseases.** In each panel, normal mechanisms are on the left, and defective mechanisms are on the right. **a** | Defective imprinting centre (IC) methylation erasure in primordial germ cells (PGCs). **b** | Defective transcription (dashed red arrow) across IC and imprint establishment in oocyte. **c** | Defective oocyte factor (striked pink triangle) affecting imprint establishment in oocyte. **d** | Defective oocyte factor (striked pink diamond) affecting 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 IC and imprint maintenance in pre-implantation embryo. **g** | Defective target site for transcription factor recognizing unmethylated IC and imprint maintenance in post-implantation embryo. Methylated and unmethylated ICs are represented by black lollipops and white lollipops, respectively. Mosaic methylation is indicated by grey lollipops. Multilocus imprinting disturbances (MLIDs) are indicated by loss of methylation (LOM) at multiple ICs. Maternally methylated ICs are in red, paternally methylated ICs in blue. *Trans*-acting factors are as in FIG. 1. GOM, gain of methylation.

mutations are affected by familial hydatidiform mole (FHM)[78], in which nonviable products of conception have normal biparental genomic constitution but complete loss of maternal imprinting marks. The high penetrance of FHM suggests that *NLRP7* is involved in oocyte-specific imprint establishment[79] (FIG. 4c), but hypomorphic maternal *NLRP7* variants have been associated with MLIDs[80].

In mouse, NLRP5 and its associated proteins are referred to as the subcortical maternal complex (SCMC)[81]. These proteins are highly expressed in the oocyte, but their mRNA and protein abundance decline to undetectable levels by blastulation[82]. Maternal ablation of SCMC gene function compromises embryo development, with frequent demise between the two-cell and blastula stage, and disruption of processes including maintenance of genome integrity, euploidy, mitochondrial function and gene transcription and translation[83–85]. A mouse model of maternal NLRP2 deficiency showed severe reproductive compromise, with embryo demise at all developmental stages and mosaic LOM and GOM at imprinted loci, indicating that abnormal subcellular localization of DNMT1 and/or SCMC members causes early embryonic loss and imprinting defects[86].

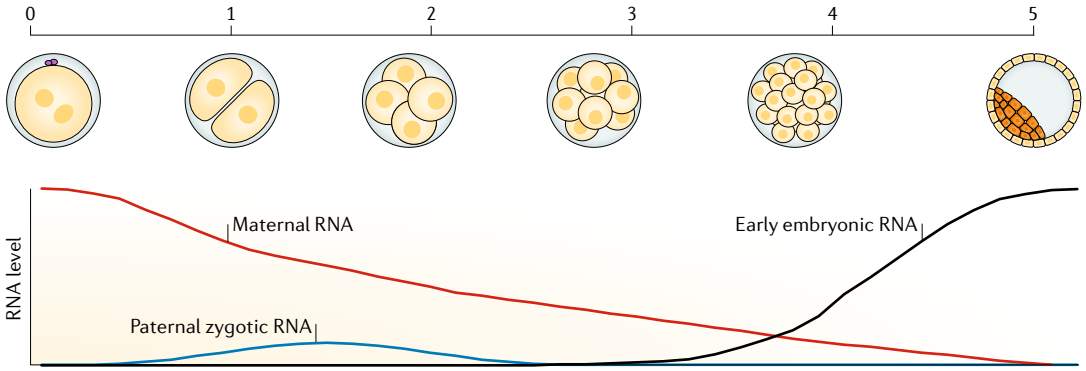
The effects of maternal SCMC variants suggest a link between DNA methylation, genome integrity and developmental competence in the early embryo. If the competence of an embryo is severely compromised, both ploidy and DNA methylation may be intolerably affected, leading to embryo demise. If errors in 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 process comes from reports of pre-implantation genetic diagnosis of embryos with maternal-effect *NLRP7* mutations in which all cleavage-stage embryos arrested and had various maternal aneuploidies[87]. Arguably, if an embryo had presented with a normal chromosome complement, it would have likely developed into a molar pregnancy or one with severe MLIDs owing 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.

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

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). The approximate timing (in days) of human cleavage-stage divisions and blastulation is shown. Upon maturation, the oocyte ceases transcription and translation, which do not resume in a human embryo until it reaches the eight-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[154].

In the one-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; 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 such as DUX4 (REF.[155]). Pioneer factors support transcription of cleavage-stage transcription factors that trigger major ZGA. Upon ZGA, the genome attains a more somatic organization, blocking the return to the permissive, early pattern of transcription.



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.

Blastocyst
The developmental stage of a 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 gives rise to the placenta.

Genome activation
The initiation of gene expression in the developing embryo. The initial burst of expression is termed zygotic genome activation 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.

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

Mothers with maternal-effect variants have children with variable disturbance of both paternally methylated and maternally methylated imprinting centres (FIG. 4d). Reproductive outcomes include apparent infertility, fetal loss, hydatidiform mole, live-born children with MLIDs who exhibit clinical symptoms and live-born children with MLIDs who exhibit no clinical phenotypes[37,39]; the only consistent feature of offspring of mothers with maternal-effect variants is MLID itself.

Zygotic factors. The KRAB zinc-finger protein (ZFP) ZFP57 acts as the focus for a multiprotein complex that protects imprinting centres from both passive and active demethylation[88–90]. ZFP57 recognizes a hexameric motif enriched in all maternally and paternally methylated imprinting centres in mouse[91,92]. KRAB ZFPs make up a large, expanding family; their rapid evolution seems to keep pace with the endogenous retroviruses, whose expression they suppress through DNA hypermethylation[93]. 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 offspring survival[88]. Human ZFP57 acts zygotically, with recessive mutations associated with the imprinting disorder TNDM and a specific pattern of MLIDs[94]; however, this outcome may represent ascertainment bias of mutation patterns that are compatible with life (FIG. 4e). It is possible that additional KRAB ZFPs expressed in the oocyte, acting via a maternal effect, are involved in earlier imprinting centre maintenance in humans with a degree of redundancy among ZFPs in recruiting the KRAB repressor complex to specific gDMRs.

Human mutations have rarely been identified in other zygotic factors involved in methylation maintenance, presumably because complete ablation would be incompatible with life, as seen in mouse models (for example, *Trim28* (REF.[95]), *Uhrf1* (REF.[96]) and *Dnmt1* (REF.[72])). Nonetheless, haploinsufficiency of *TRIM28* has been associated with polyphenism, obesity and reduced expression of imprinted genes in mice and humans[97], and haploinsufficiencies of *DNMT1* and *UHRF1* have been described in association with BWS and MLIDs, respectively[39,98]. *Cis*-acting genetic variants have been identified in imprinting centre sequences, and study of these variants can help to 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[99] but, interestingly, their effects are seen only in maternal and paternal inheritance, respectively, and the methylation defects are generally mosaic. This observation suggests, once again, that imprinted states acquired in the early embryo are faithfully perpetuated in subsequent development. In individuals with SRS, no maternal transmissions have yet been reported, but it has been suggested that loss of ZFP57 binding sites may result in post-zygotic attrition of methylation[29] (FIG. 4f). In people with BWS, erroneous GOM of the maternal allele is thought to result from loss of SOX2 and POU5F1 binding or weakening of CTCF binding[28,100] (FIG. 4g). Murine models demonstrate that binding sequences of CTCF and ZFP57 are involved in embryonic maintenance of IC1 imprinting[91,101], although some differences may exist between human and mouse species[102].

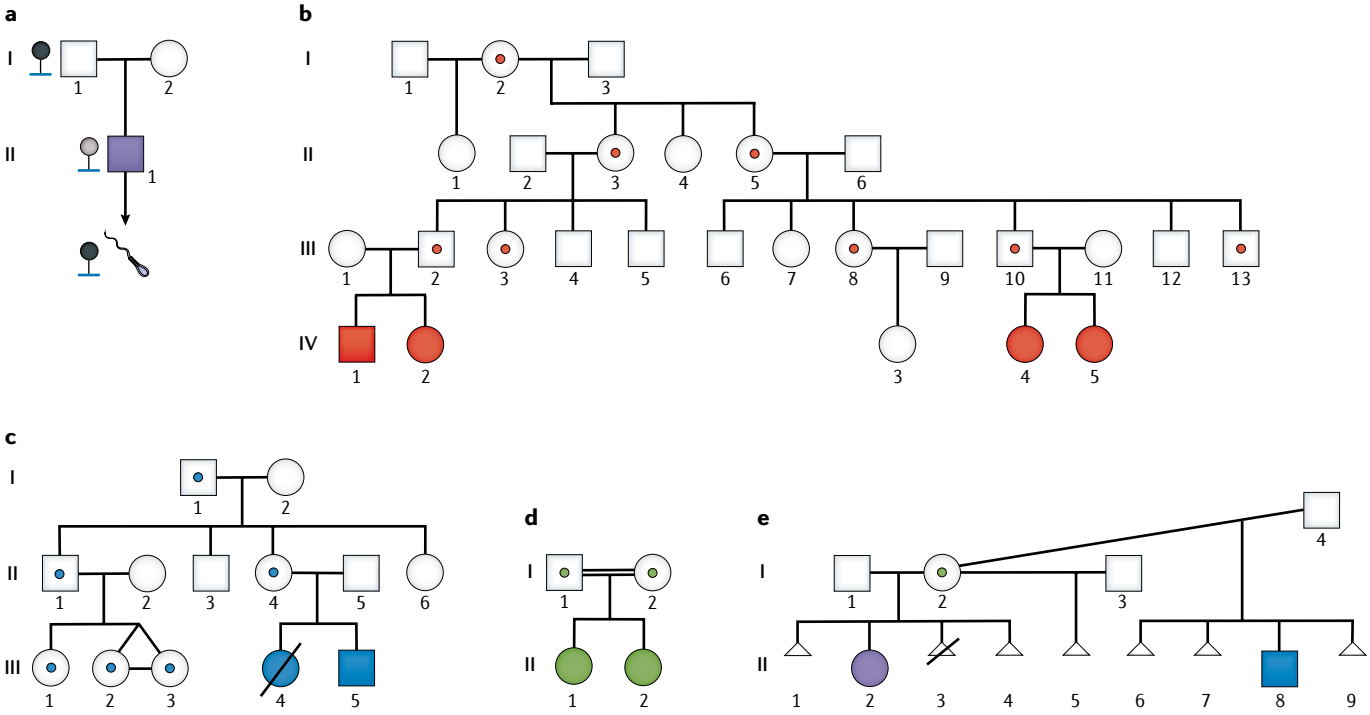


Fig. 5 | **Modes of inheritance of phenotypes associated with imprinting errors. a** | Pedigree of sporadic Silver–Russell syndrome (SRS) in which imprinting centre 1 (IC1) epimutation is corrected in the germ line of the proband. Black lollipop shows normal full methylation of paternal IC1 in blood of I:1 and in the sperm of II:1; grey lollipop, mosaic hypomethylation of paternal IC1 in blood of II:1. **b** | Pedigree showing autosomal dominant inheritance with parent-of-origin-dependent penetrance (paternal, associated with Prader–Willi syndrome (PWS)–Angelman syndrome (AS) imprinting centre mutation). **c** | Pedigree showing autosomal dominant inheritance with parent-of-origin-dependent penetrance (maternal, associated with Beckwith–Wiedemann syndrome (BWS) and/or SRS IC1 mutation). **d** | Autosomal recessive inheritance (*ZFP57* mutation). **e** | Maternal effect (*NLRP5* mutations). SRS cases, purple; PWS cases, red; BWS cases, blue; transient neonatal diabetes mellitus cases, green.

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

Hydatidiform mole
A benign gestational trophoblastic disease developing during pregnancy and resulting from 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.

Penetrance
The proportion of individuals in a population with a specific genotype who show an associated phenotypic trait. Incomplete penetrance occurs when not all individuals carrying a dominant deleterious genetic variant express the associated clinical phenotype.

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.

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 additional 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 the person's germ line[103] (FIG. 5a). 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 appear only upon maternal or paternal transmission (for example, familial PWS with paternally inherited imprinting centre mutations[46] (FIG. 5b) and familial BWS associated with maternally inherited IC1 microdeletions[30] (FIG. 5c) and POU5F1-binding site variants[100]). However, autosomal recessively inherited TNDM is caused by pathogenic variants in *ZFP57* (REF.[94]) (FIG. 5d), and maternal pathogenic variants in maternal-effect genes (*NLRP2*, *NLRP5*, *NLRP7*, *PADI6* and *OOEP*)[39] (FIG. 5e) are associated with MLIDs 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* FHMs), 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 with increased clinical severity over multiple generations[104]. These findings suggest that, whereas highly penetrant variants, such as those disrupting transcription factor binding, exhibit obvious, 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

Subcortical maternal complex
(SCMC). A large multiprotein complex comprising NLRP5, OOEP, TLE6, PADI6 and KHDC3L that localizes to the outermost regions of the cytoplasm in oocytes and is excluded from regions of cell-to-cell contact in cleavage embryos.

Endogenous retroviruses
Also known as retrotransposons. Repetitive genetic elements present in the genome that, similarly to retroviruses, use the activity of reverse transcriptase to move from one locus to another.

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

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

and phenotype[20], suggesting that imprinting is a more quantitative than categorical phenomenon.

Environmental influences on imprinting

In addition to genetic causes of imprinting centre epimutations, environmental factors can influence the imprinting process. In humans, evidence for this phenomenon derives from assisted reproductive technologies (ARTs)[105]. Other environmental influences on imprinting centres include nutritional status or exposure to chemical pollutants in utero[106]. 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 external stimuli.

Assisted reproductive technologies. ARTs are usually performed for male and/or female infertility and include procedures such as ovarian hyperstimulation to obtain multiple oocytes for retrieval, in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) and embryo culture and transfer, which all 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[107–109] (FIG. 6). Epidemiological studies have provided further evidence for an increased risk of having children with BWS, AS or SRS when using ART[105,110]; however, the absolute risk is very small (for example, for BWS, the risk is as much as tenfold greater with ART than without ART, and it occurs in <0.1% of all children conceived with the help of ARTs)[110]. ARTs have also been associated with an increased frequency of MLIDs, although this finding has not been universal[111,112]. MLIDs and large offspring syndrome (a condition with similarities to BWS) have been observed in bovine fetuses conceived by IVF[113]. 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 were partially restored with the addition of natural reproductive fluids[114]. Furthermore, superovulation and embryo-transfer induced developmental defects and imprinting centre epimutations in the placenta of mouse models[115].

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[116]. Moreover, impaired methylation of imprinting centres was reported in sperm of subfertile men[47]. Furthermore, unrecognized ART-associated epigenetic alterations have been suggested to play a role in the increased risk of low birthweight and congenital anomalies that have been reported in ART-conceived children[117] and animal models[118]. Maternal age and delayed ovulation or fertilization are associated with depletion of oocyte proteins and RNA stores and altered developmental

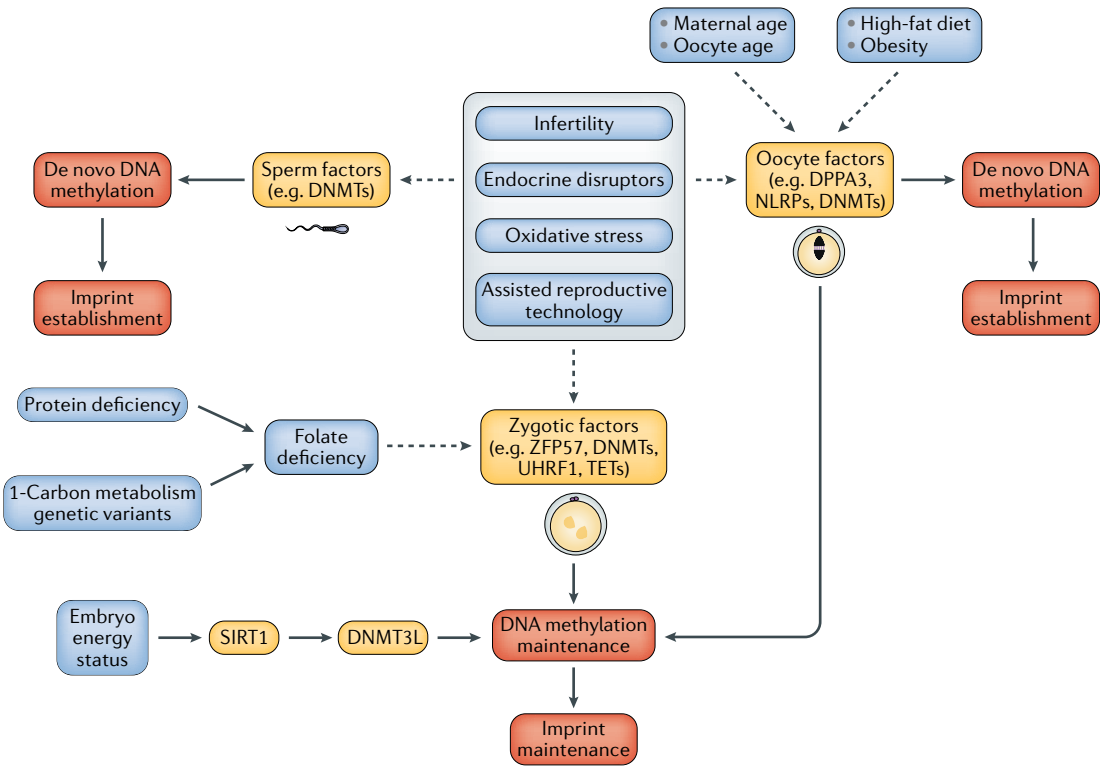


Fig. 6 | **Interaction between environmental and genetic factors and its impact on genomic imprinting.** The diagram summarizes the evidence obtained in humans and animal models of interaction of environmental factors and physiopathological conditions (depicted in light blue) with gametic and zygotic factors (yellow) involved in de novo and maintenance methylation and impacting imprinting establishment and maintenance (red). Continuous lines indicate functional contribution; dashed lines indicate functional interference.

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. ARTs cover a wide spectrum of treatments including the use of fertility drugs, intrauterine insemination and in vitro fertilization and/or intracytoplasmic sperm injection.

fitness of embryos[119–121], suggesting that maternal-effect genes can be critically vulnerable to these or other challenges that occur during ART procedures (FIG. 6).

Disentangling the effects of infertility and ART in the aetiology of 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[122,123]. Other groups have reported perturbed imprinting in pre-implantation embryos suitable for transfer[124], 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 live-born offspring with clinically blatant phenotypes associated with imprinting disorders, whereas the frequency of clinical pregnancy, though well known to be limited with ART, is not considered. Potentially, individuals with imprinting disorders represent the subset of IVF outcomes with the least pervasive disturbances and the most recognizable clinical features, and a more definitive study will require consideration of both the epigenome and genome integrity of nonviable products of conception at all stages.

Nutrition and metabolic disorders. Certain developmental windows are especially vulnerable to abnormal nutritional states[125], including pre-implantation 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). For example, 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 produce subsequent hypomethylation at several imprinted gDMRs[126].

Nutritional status can 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 (SAM), 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[98,127,128]. In these studies, the presence of missense amino acid substitutions in genes regulating the abundance of SAM or the inhibitory *S*-adenosylhomocysteine (SAH) correlated with aberrant imprinted methylation[127,128]; one of these studies also revealed a link between low vitamin B_{12} levels and *H19* methylation maintenance[127]. Functional genetic variants of DNMT1 in people with BWS were observed in combination with single-nucleotide variants of folate metabolism pathway genes[98], suggesting that decreased DNMT1 enzymatic activity could be exaggerated by extreme SAM:SAH ratios. Furthermore, the *ZFP57* locus is a folate-sensitive region, and its genomic binding regions are metastable epialleles responsive to periconceptional conditions[129,130]. 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[131]. However, not all studies on isocaloric protein restriction during pregnancy have shown altered imprinted methylation in the offspring[132], 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. For example, the NAD-dependent deacetylase SIRT1 has been shown to protect methylation at imprinted loci by directly regulating acetylation of DNMT3L, at both the promoter level and protein level in mouse embryonic stem cells[133].

Endocrine disruptors. In addition to micronutrient availability, prenatal exposure to oestrogenic endocrine-disrupting compounds, such as bisphenol A (BPA), results in deregulation of genomic methylation and hydroxymethylation[134,135], with imprinting and methylation anomalies being reported for both mouse placenta[136] and developing gametes[137,138]. Endorsing the vulnerability of imprinted loci to endocrine-disrupting compounds, prenatal BPA exposure in humans has been associated with changes in methylation at the *MEST* locus and is linked with early childhood obesity[139]. *Dnmt1* expression was found to be decreased in BPA-treated mouse spermatogonia[138], and BPA exposure during oocyte maturation altered other epigenetic marks, specifically the abundance of histone modifications, and this alteration was linked to induced oxidative stress[140]. Exposure-induced oxidative stress was shown to alter both TET enzyme expression and TET function, leading to altered 5hmC levels at numerous imprinted loci[135], 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 consequences of developmental and metabolic alterations[141].

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.

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 in recent years, but the causation and timing of their interactions require further clarification. This is particularly true for the SCMC proteins and possibly other oocyte-specific factors that affect DNA methylation maintenance in the early embryo, as their mechanisms of action and relationships with ZGA remain ill-defined.

Importantly, additional human-based studies are required, first to resolve key differences between human and mouse in the timing and mechanisms of epigenetic remodelling and, second, to identify genetic variants that predispose to imprinting centre epimutations on the basis of the study of individuals with rare imprinting disorders.

It has become evident that environmental changes can affect the epigenetic reprogramming of germ cells and early embryos, altering their developmental competence and causing imprinting defects. 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 generated 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[32–34,102]. Such approaches, and their extension to human pathophysiology, will shed 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, therapeutic correction of imprinting disorders might be possible by reversal of the gene-imprinting status. Three therapeutic approaches for the neurological disorders AS and PWS have been proposed in preclinical studies[142–144]. 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 (Supplementary Table 1). In mouse models of AS, a topoisomerase inhibitor[142] and antisense oligonucleotides[143] were able to downregulate *Ube3a* ATS and reactivate expression of the paternal *UBE3A*. In a mouse model of PWS, G9A inhibitors were used to unsilence maternal snoRNAs[144]. 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–DNMT fusions have been able to target methylation to IC1 in mouse cells[145]; if such alterations can be performed in an allelic manner, this technology could prove promising. Future experiments will be needed to demonstrate whether approaches using small molecules can revert other epimutations in imprinting disorders and possibly be applied in other human diseases arising from disruption of the epigenome.

Published online 15 January 2019

1. Barlow, D. P. & Bartolomei, M. S. Genomic imprinting in mammals. *Cold Spring Harb. Perspect. Biol.* **6**, a018382 (2014).
2. Onuchic, V. et al. Allele-specific epigenome maps reveal sequence-dependent stochastic switching at regulatory loci. *Science* **361**, eaar3146 (2018).
3. Baran, Y. et al. The landscape of genomic imprinting across diverse adult human tissues. *Genome Res.* **25**, 927–936 (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 (2014).
A full description of allelic DMRs in humans and the first report of tissue-specific imprinting centres are presented.
5. Smith, Z. D. et al. DNA methylation dynamics of the human preimplantation embryo. *Nature* **511**, 611–615 (2014).
The methylation landscape in human gametes and pre-implantation embryos is described.
6. Peters, J. The role of genomic imprinting in biology and disease: an expanding view. *Nat. Rev. Genet.* **15**, 517–530 (2014).
7. Eggermann, T. et al. Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clin. Epigenet.* **7**, 123 (2015).
8. Horsthemke, B. Mechanisms of imprint dysregulation. *Am. J. Med. Genet.* **154C**, 321–328 (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. USA* **107** (Suppl. 1), 1731–1735 (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 (2016).
11. Sanli, I. & Feil, R. Chromatin mechanisms in the developmental control of imprinted gene expression. *Int. J. Biochem. Cell Biol.* **67**, 139–147 (2015).
12. Monk, D. et al. Recommendations for a nomenclature system for reporting methylation aberrations in imprinted domains. *Epigenetics* **13**, 117–121 (2018).
13. Singh, P. et al. Chromosome-wide analysis of parental allele-specific chromatin and DNA methylation. *Mol. Cell. Biol.* **31**, 1757–1770 (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 (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 (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 (1997).
17. Vu, T. H. & Hoffman, A. R. Imprinting of the Angelman syndrome gene, UBE3A, is restricted to brain. *Nat. Genet.* **17**, 12–13 (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 (2013).
19. Hanna, C. W. et al. Pervasive polymorphic imprinted methylation in the human placenta. *Genome Res.* **26**, 756–767 (2016).
20. Zink, F. et al. Insights into imprinting from parent-of-origin phased methylomes and transcriptomes. *Nat. Genet.* **50**, 1542–1552 (2018).
The partial phenotypes associated with parent-of-origin-specific genotypes that influence imprinted gene expression are described.
21. Sanchez-Delgado, M. et al. Human oocyte-derived methylation differences persist in the placenta revealing widespread transient imprinting. *PLOS Genet.* **12**, e1006427 (2016).
Oocyte-derived methylation and polymorphic transient DMRs are demonstrated.
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 (2018).
Allelic contacts and novel CTCF-mediated loops at the *H19–Igf2* locus are identified 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 (2014).
24. Kanduri, C. Long noncoding RNAs: lessons from genomic imprinting. *Biochim. Biophys. Acta* **1859**, 102–111 (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 (2013).
26. Inoue, A., Jiang, L., Lu, F., Suzuki, T. & Zhang, Y. Maternal H3K27me3 controls DNA methylation-independent imprinting. *Nature* **547**, 419–424 (2017).
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 (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 (2013).
29. Sparago, A., Cerrato, F. & Riccio, A. Is ZFP57 binding to H19/IGF2:IG-DMR affected in Silver-Russell syndrome? *Clin. Epigenet.* **10**, 23 (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–e454 (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.* https://doi.org/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 (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 (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 (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 (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 (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 (2015).
NLRP5 mutations in MLIDs are identified.

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 (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 (2018).
Deleterious variants in maternal-effect genes associated with the SCMC in individuals with MLIDs are identified.
40. Hackett, J. A. et al. Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* **339**, 448–452 (2013).
Erasure of CpG methylation in mouse PGCs occurs via conversion to 5hmC that is 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 (2012).
42. Guo, F. et al. The transcriptome and DNA methylome landscapes of human primordial germ cells. *Cell* **161**, 1437–1452 (2015).
Paper presents first genome-wide descriptions of the transcriptome and the DNA methylome landscapes of human PGCs.
43. Tang, W. W. et al. A unique gene regulatory network resets the human germline epigenome for development. *Cell* **161**, 1453–1467 (2015).
44. Gkountela, S. et al. DNA demethylation dynamics in the human prenatal germline. *Cell* **161**, 1425–1436 (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 (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 (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 (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 (2006).
49. Guo, H. et al. The DNA methylation landscape of human early embryos. *Nature* **511**, 606–610 (2014).
50. Gahurova, L. et al. Transcription and chromatin determinants of de novo DNA methylation timing in oocytes. *Epigenetics Chromatin* **10**, 25 (2017).
51. Smallwood, S. A. et al. Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat. Genet.* **43**, 811–814 (2011).
52. Yu, B. et al. Genome-wide, single-cell DNA methylomics reveals increased Non-CpG methylation during human oocyte maturation. *Stem Cell Rep.* **9**, 397–407 (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 (2001).
54. Kaneda, M. et al. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* **429**, 900–903 (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 (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 (2013).
57. Chotalia, M. et al. Transcription is required for establishment of germline methylation marks at imprinted genes. *Genes Dev.* **23**, 105–117 (2009).
58. Baubec, T. et al. Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* **520**, 243–247 (2015).
Targeting of DNA methylation to transcribed gene bodies by SETD2-mediated methylation of H3K36me3 and a functional recruitment of DNMT3B is demonstrated.
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 (2010).
60. Ciccone, D. N. et al. KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* **461**, 415–418 (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 (2007).
62. Stewart, K. R. et al. Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. *Genes Dev.* **29**, 2449–2462 (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 (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 (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 (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 (2016).
67. Valente, F. M. et al. Transcription alterations of KCNQ1 associated with imprinted methylation defects in the Beckwith-Wiedemann locus. *Genet. Med.* https://doi.org/10.1038/s41436-018-0416-7 (2019).
68. Lewis, M. W. et al. Angelman syndrome imprinting center encodes a transcriptional promoter. *Proc. Natl Acad. Sci. USA* **112**, 6871–6875 (2015).
69. Smith, Z. D. et al. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* **484**, 339–344 (2012).
70. 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 (2018).
71. 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 (2014).
72. 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 (2008).
73. Maenohara, S. et al. Role of UHRF1 in de novo DNA methylation in oocytes and maintenance methylation in preimplantation embryos. *PLOS Genet.* **13**, e1007042 (2017).
74. Nakamura, T. et al. PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* **486**, 415–419 (2012).
75. Nakamura, T. et al. PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nat. Cell Biol.* **9**, 64–71 (2007).
76. Meyer, E. et al. Germline mutation in NLRP2 (NALP2) in a familial imprinting disorder (Beckwith-Wiedemann Syndrome). *PLOS Genet.* **5**, e1000423 (2009).
77. 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 (2011).
78. Murdoch, S. et al. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. *Nat. Genet.* **38**, 300–302 (2006).
79. 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 (2015).
80. 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 (2017).
81. Zhu, K. et al. Identification of a human subcortical maternal complex. *Mol. Hum. Reprod.* **21**, 320–329 (2015).
82. 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 (2016).
83. Tong, Z. B. et al. Mater, a maternal effect gene required for early embryonic development in mice. *Nat. Genet.* **26**, 267–268 (2000).
84. 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 (2008).
85. Zheng, P. & Dean, J. Role of Filia, a maternal effect gene, in maintaining euploidy during cleavage-stage mouse embryogenesis. *Proc. Natl Acad. Sci. USA* **106**, 7473–7478 (2009).
86. Mahadevan, S. et al. Maternally expressed NLRP2 links the subcortical maternal complex (SCMC) to fertility, embryogenesis and epigenetic reprogramming. *Sci. Rep.* **7**, 44667 (2017).
The generation of a maternal-effect mouse model for *Nlrp2* is presented, which reveals disrupted imprinted methylation consistent with human MLIDs.
87. 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 (2017).
88. Li, X. et al. A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. *Dev. Cell* **15**, 547–557 (2008).
89. 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 (2011).
90. 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 (2018).
91. 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 (2016).
92. Strogantsev, R. et al. Allele-specific binding of ZFP57 in the epigenetic regulation of imprinted and non-imprinted monoallelic expression. *Genome Biol.* **16**, 112 (2015).
93. Imbeault, M., Helleboid, P. Y. & Trono, D. KRAB zinc-finger proteins contribute to the evolution of gene regulatory networks. *Nature* **543**, 550–554 (2017).
Human genome targets for 350 KRAB-domain-containing ZFPs are identified.
94. 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 (2008).
95. Messerschmidt, D. M. et al. Trim28 is required for epigenetic stability during mouse oocyte to embryo transition. *Science* **335**, 1499–1502 (2012).
96. Sharif, J. et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450**, 908–912 (2007).
97. Dalgaard, K. et al. Trim28 haploinsufficiency triggers bi-stable epigenetic obesity. *Cell* **164**, 353–364 (2016).
98. Dagar, V. et al. Genetic variation affecting DNA methylation and the human imprinting disorder, Beckwith-Wiedemann syndrome. *Clin. Epigenetics* **10**, 114 (2018).
99. Abi Habib, W. et al. 11p15 ICR1 partial deletions associated with IGF2/H19 DMR hypomethylation and Silver-Russell syndrome. *Hum. Mutat.* **38**, 105–111 (2017).
100. 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 (2010).
101. Freschi, A. et al. Tissue-specific and mosaic imprinting defects underlie opposite congenital growth disorders in mice. *PLOS Genet.* **14**, e1007243 (2018).
102. 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. USA* **113**, 10938–10943 (2016).
103. Bruno, C. et al. Germline correction of an epimutation related to Silver-Russell syndrome. *Hum. Mol. Genet.* **24**, 3314–3321 (2015).
104. Berland, S. et al. Evidence for anticipation in Beckwith-Wiedemann syndrome. *Eur. J. Hum. Genet.* **21**, 1344–1348 (2013).
105. 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 (2018).
This meta-analysis demonstrates the association between imprinting disorders and ARTs.
106. Taniguchi, K., Kawai, T. & Hata, K. Placental development and nutritional environment. *Adv. Exp. Med. Biol.* **1012**, 63–73 (2018).
107. Cox, G. F. et al. Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am. J. Hum. Genet.* **71**, 162–164 (2002).
108. 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 (2003).
109. Maher, E. R. et al. Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). *J. Med. Genet.* **40**, 62–64 (2003).
110. Mussa, A. et al. Assisted reproductive techniques and risk of Beckwith-Wiedemann syndrome. *Pediatrics* **140**, e20164311 (2017).
111. Tee, L. et al. Epimutation profiling in Beckwith-Wiedemann syndrome: relationship with assisted reproductive technology. *Clin. Epigenet.* **5**, 23 (2013).
112. Tenorio, J. et al. Clinical and molecular analyses of Beckwith-Wiedemann syndrome: comparison between spontaneous conception and assisted reproduction techniques. *Am. J. Med. Genet.* **170A**, 2740–2749 (2016).
113. Chen, Z. et al. Characterization of global loss of imprinting in fetal overgrowth syndrome induced by assisted reproduction. *Proc. Natl Acad. Sci. USA* **112**, 4618–4623 (2015).
114. Canovas, S. et al. DNA methylation and gene expression changes derived from assisted reproductive technologies can be decreased by reproductive fluids. *eLife* **6**, e23670 (2017).
115. 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 (2015).
116. Ludwig, M. et al. Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples. *J. Med. Genet.* **42**, 289–291 (2005).
117. 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 (2014).
118. 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 (2017).
119. 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 (2014).
120. 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 (2016).
121. 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 (2016).
122. 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 (2013).
123. 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 (2018).
124. White, C. R. et al. High frequency of imprinted methylation errors in human preimplantation embryos. *Sci. Rep.* **5**, 17311 (2015).
125. Conradt, E. et al. Incorporating epigenetic mechanisms to advance fetal programming theories. *Dev. Psychopathol.* **30**, 807–824 (2018).
126. Han, L. et al. Embryonic defects induced by maternal obesity in mice derive from Stella insufficiency in oocytes. *Nat. Genet.* **50**, 432–442 (2018).
This study demonstrates that a maternal environmental insult may affect genomic integrity, epigenetic marking and developmental competence at the cleavage stage of offspring.
127. 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 (2017).
128. Motzek, A. et al. Abnormal hypermethylation at imprinting control regions in patients with S-adenosylhomocysteine hydrolase (AHCY) Deficiency. *PLOS ONE* **11**, e0151261 (2016).
129. 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 (2014).
130. 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 (2018).
This analysis of metastable epialleles in a rural Gambian population demonstrates epigenetic sensitivity to season of birth in early embryonic development.
131. 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 (2017).
A mouse model demonstrates that maternal dietary change promotes transient alteration in DNA methylation and enduring misexpression of an imprinted gene.
132. 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 (2012).
133. Heo, J. et al. Sirt1 regulates DNA methylation and differentiation potential of embryonic stem cells by antagonizing Dnmt3l. *Cell Rep.* **18**, 1930–1945 (2017).
134. Kim, J. H. et al. Perinatal bisphenol A exposure promotes dose-dependent alterations of the mouse methylome. *BMC Genomics* **15**, 30 (2014).
135. 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 (2018).
136. Susiarjo, M., Sasson, I., Mesaros, C. & Bartolomei, M. S. Bisphenol a exposure disrupts genomic imprinting in the mouse. *PLOS Genet.* **9**, e1003401 (2013).
137. 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 (2013).
138. 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 (2018).
139. Junge, K. M. et al. MEST mediates the impact of prenatal bisphenol A exposure on long-term body weight development. *Clin. Epigenetics* **10**, 58 (2018).
140. 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 (2017).
141. Pathak, R. & Feil, R. Environmental effects on chromatin repression at imprinted genes and endogenous retroviruses. *Curr. Opin. Chem. Biol.* **45**, 139–147 (2018).
142. Huang, H. S. et al. Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons. *Nature* **481**, 185–189 (2011).
143. Meng, L. et al. Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. *Nature* **518**, 409–412 (2015).
144. 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 (2017).
This proof-of-principle mouse study identifies small molecules that selectively reduce H3K9me2 at a PWS imprinting centre and highlights a potential therapy for PWS.
145. Lei, Y. et al. Targeted DNA methylation in vivo using an engineered dCas9-MQ1 fusion protein. *Nat. Commun.* **8**, 16026 (2017).
146. 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 (2008).
147. 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 (2008).
148. Azzi, S. et al. Complex tissue-specific epigenotypes in Russell-Silver Syndrome associated with 11p15 ICR1 hypomethylation. *Hum. Mutat.* **35**, 1211–1220 (2014).
149. Nazlican, H. et al. Somatic mosaicism in patients with Angelman syndrome and an imprinting defect. *Hum. Mol. Genet.* **13**, 2547–2555 (2004).
150. Weksberg, R. et al. Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Hum. Mol. Genet.* **11**, 1317–1325 (2002).
151. Riess, A. et al. First report on concordant monozygotic twins with Silver-Russell syndrome and ICR1 hypomethylation. *Eur. J. Med. Genet.* **59**, 1–4 (2016).
152. 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 (2009).
153. Greenberg, M. V. et al. Transient transcription in the early embryo sets an epigenetic state that programs postnatal growth. *Nat. Genet.* **49**, 110–118 (2017).
This study demonstrates that transient transcription in pre-implantation embryo sets an epigenetic state that programmes postnatal growth.
154. 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 (2010).
155. De Iaco, A. et al. DUX-family transcription factors regulate zygotic genome activation in placental mammals. *Nat. Genet.* **49**, 941–945 (2017).
156. 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 (2011).

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 owing to the concise nature of this Review. Individual authors thank the following funders for research support: Ministry of Education, Universities and Research, Research Projects of National Interest (MIUR PRIN) 2015 (JHLY35), Telethon-Italia (GGP15131 and AIRC IG18671) (A.R.); Deutsche Forschungsgemeinschaft (DFG) (EG110/15-1) (T.E.); Spanish Ministry of Economy and Competitiveness (MINECO; BFU2014-53093-R and BFU2017-85571-R) co-funded with the European Union Regional Development Fund (FEDER) (D.M.). E.R.M. acknowledges support from National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre and an NIHR Senior Investigator Award. The University of Cambridge has received salary support in respect of E.R.M. from the National Health Service (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.

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
The authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Reviewer information
Nature Reviews Genetics thanks Y.-H. Jiang and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Supplementary information
Supplementary information is available for this paper at https://doi.org/10.1038/s41576-018-0092-0.