# Human Imprinting Disorders: principles, practice, problems and progress.

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Abstract

Epigenetic regulation orchestrates gene expression with exquisite precision, over a huge dynamic range and across developmental space and time, permitting genomically-homogeneous humans to develop and adapt to their surroundings. Every generation, these epigenetic marks are re-set twice: in the germline, to enable differentiation of sperm and eggs, and at fertilisation, to create the totipotent zygote that then begins growth and differentiation into a new human. A small group of genes evades the second, zygotic wave of epigenetic reprogramming, and these genes retain an epigenetic ‘imprint’ of the parent from whom they were inherited.

Imprinted genes are (as a general rule) expressed from one parental allele only. Some imprinted genes are critical regulators of growth and development, and thus disruption of their normal monoallelic expression causes congenital imprinting disorders, with clinical features impacting growth, development, behaviour and metabolism.

Imprinting disorders as a group have characteristics that challenge diagnosis and management, including clinical and molecular heterogeneity, overlapping clinical features, somatic mosaicism, and multi-locus involvement. New insights into the biology and epigenomics of the early embryo offers new clues about the origin and importance of imprinting disorders.

## 1. Principles of imprinting.

We are genetic creatures. Essentially every somatic cell in our bodies contains essentially the same genome, and this genome contains all the genetic material required to encode and regulate every gene in the whole individual. But of course, not all cells in our bodies use this genome in the same way (Jaenisch and Bird, 2003). Different organs and tissues, at different developmental times, in different stages of health and disease, repair, regeneration and senescence – even in neighbouring cells in homogeneous tissues – express different numbers, types and levels of genes, both protein-coding and non-coding (Kundaje et al, 2015). These differences, from the gross level of tissues to the quantitative and stochastic variation between cells (Xue et al, 2013, Klein et al, 2015), underpin the growth, differentiation, adaptation and regeneration of the whole organism.

The origin of this extraordinary variation is not genetic, but epigenetic. Epigenetics, in its this sense, is modification of DNA that does not alter its sequence, but alters its expression, and thereby alters its phenotype (Jaenisch and Bird, 2003). Although epigenetic modifications do not change DNA sequence, they are transmitted faithfully from cells to their progeny, ensuring phenotypic continuity. Epigenetic signals are highly diverse and dynamic: the most-studied is DNA methylation on cytosine residues in CpG dinucleotide sequences, but it is only one epigenetic regulator, alongside RNA species, histones and other chromatin proteins, and their wide-ranging post-translational modifications (reviewed in Schubeler, 2015; He and Ecker, 2015; Su and Demu, 2015; Geisler and Coller, 2015; Li et al, 2016; Ali et al, 2016; Gonzalez-Sandoval and Gasser, 2016).

Epigenetic regulation orchestrates gene expression, with exquisite precision across a very high dynamic range, across developmental space and time. Nowhere is this more apparent than in reproduction. From a complex, highly-differentiated organism, the cells of one specific lineage, the germline, differentiate into two of the most differentiated and dimorphic cell types in humans: the egg and sperm; then after their fusion, the resultant zygote becomes totipotent, capable of populating all the lineages of the future organism; and thus it begins its development into a new, complex four-dimensional individual (reviewed in Lee et al, 2014; Dang-Nguyen and Torres-Padilla, 2015). These transformations are in large part epigenetic. Global epigenetic reprogramming occurs twice: once in the germline and once in the very early zygote (Seisenberger et al, 2012; Smallwood et al, 2011; Smith et al, 2012; Arand et al, 2015). Germline cells essentially ‘wipe the slate clean’ – they undergo almost complete loss of DNA methylation, before the eggs and sperm acquire their highly specialised and divergent epigenetic marks. In the cleavage-stage embryo there is a second wave of epigenetic reprogramming; both egg and sperm epigenetic marks are erased, with to a minimum of DNA methylation at around the blastula stage, before lineage-specific DNA methylation arises with the onset of differentiation (FIGURE 1A).

A small number of genomic regions evade the zygotic wave of epigenetic reprogramming. Their epigenetic marks are not demethylated during the first cell divisions of the cleavage-stage embryo; nor are they altered by any cell fate decisions during subsequent differentiation (FIGURE 1B). Thus, these regions retain the epigenetic marks of the eggs and sperm that constituted the zygote, an epigenetic ‘memory’ of their parent of origin. This phenomenon is termed genomic imprinting (Barlow and Bartolomei, 2014; Kelsey and Feil, 2013). Recently, epigenomic analysis of embryonic and extraembryonic tissues has revealed widespread transient and placental imprinting, but these are outside the scope of this review (Hanna et al, 2016, Sanchez-Delgado et al, 2016A, Duffié et al, 2014).

## 2. Putting principles into practice: imprinting and imprinting disorders

In humans over 40 genomic regions, distributed across the genome, show imprinting – that is, DNA methylation (and other epigenetic marking) on imprinting control regions. Comprehensive genomewide analyses suggest that all germline imprints of somatic tissues have now been identified (Court et al, 2013), but it remains possible that more remain to be found. All currently-known imprinting control regions (ICRs) include differentially methylated regions (DMRs). These ICRs restrict the expression of genes under their control, according to their parent of origin. Imprinting marks are permanent and ubiquitous: that is, they persist essentially throughout the lifecourse and in essentially all cells of the body.

Most ICRs are methylated on the maternal allele, and overlap with transcriptional start sites in the oocyte (Chotalia et al, 2009), whereas the known paternally-methylated ICRs are intergenic and form chromatin boundary elements.

Genes in imprinted loci include both protein-coding and non-coding genes. Some ICRs control the expression of only one or two genes, while other imprinted loci include numerous transcripts, both translated and untranslated. The ‘classical’ definition of an imprinted allele is the allele whose expression is downregulated epigenetically from the specified parent of origin. However, the complexity of some imprinted loci limits the value of such definitions; for example, the ICR on chromosome 14q32 is paternally methylated, but both maternally- and paternally-imprinted genes are under its control. Also, imprinted genes may be regulated by other DNA sequences besides ICRs, so their *expression* may not be ubiquitously imprinted: for example, the Angelman syndrome gene *UBE3A* shows imprinted expression only in the brain (Buiting, 2010); and *GNAS* is expressed from the maternal allele in only certain tissues such as kidney tubule and pituitary gland, and this accounts for the clinical features of PHP1b (Mantovani, 2016).

Despite these caveats, the asymmetrical regulation of imprinted genes makes them important in human development. At least at some critical developmental times and places, imprinted genes are expressed hemizygously (from one parental allele only). Some of these genes are regulators of growth and development, with tightly regulated expression, and altered expression of these genes has deleterious effects on growth and development. Additionally, imprinting effects are increasingly being recognised in population-level traits in growth and metabolism (Kong et al, 2009; Horikoshi et al, 2016; Benonisdottir et al, 2016). Many other imprinted genes are not currently associated with phenotypic traits.

In principle, either increased or decreased dosage of imprinted genes may be deleterious. And indeed, eight of the nine currently-defined primary imprinting disorders are ‘mirror’ disorders – pairs of disorders caused by opposing molecular alterations of specific imprinted loci, with broadly ‘opposing’ effects on development. Imprinting disorders may occur through three broad mechanisms:

-changes affecting the *expressed* allele of the gene in question. This includes coding mutations, and copy number changes (deletions or duplications). Such variations are genetic and therefore heritable, but whether a phenotype results depends on the parental allele on which the change is present.

-chromosomal errors. This includes large-scale reorganisation of genetic material, normally without overall change in copy number, including translocation, uniparental disomy (upd), and the more recently-described genomewide uniploidy. Translocations altering regulation of imprinted genes are a rare but recognised cause of imprinting disorders, and are in principle heritable, but whether a phenotype results depends on the parental allele on which the change is present. In most IDs, upd is a meiotic error, and is therefore not generally heritable, unless a predisposing parental chromosomal rearrangement is present. In Beckwith-Wiedemann and Silver-Russell syndromes, upd arises post-fertilisation, and almost always appears in mosaic and segmental form; it carries a low recurrence risk.

-epigenetic errors. This encompasses changes to the DNA methylation of DMRs controlling imprinted genes. While some have detectable underlying genetic causes, either in-cis or in-trans, it is generally believed that the majority of primary epimutations are purely epigenetic and stochastic in origin, and are not heritable.

## 3. Practical problems with imprinting disorders

Currently nine ‘classical’ imprinting disorders (IDs) are recognised: Classical imprinting disorders clinical syndromes caused by errors in the effective gene dosage of imprinted genes, with features affecting growth, development, metabolism and behaviour. In addition, a new growing class of disorders is being recognised, associated specifically with coding mutations of genes regulated by imprinting, such as MAGEL2 mutations causing Schaaf-Yang syndrome and MKRN3 mutations causing central precocious puberty type 2 (Schaaf et al, 2013; Abreu et al, 2013).

Individual imprinting disorders have been very well reviewed in published literature, so are summarised for reference in Table 1. Here I will focus on some of the characteristics that make IDs distinctive as a group of disorders. These include: heterogeneous and overlapping clinical features; heterogeneous molecular aetiology, including somatic mosaicism; and multi-locus imprinting disorder.

-heterogeneous and overlapping clinical features. Classical definitions of clinical IDs include groups of clinical features, very often featuring early growth, development and metabolism. Some of these features develop or disappear at different times in development, which can challenge clinical diagnosis. Not every child with an ID presents with sufficient cardinal features to secure a clinical diagnosis. Moreover, some clinical features – notably posnatal growth restriction – are shared by different IDs. The heterogeneity of presentation means that a child with certain features may warrant testing for more than one ID. For example, the cardinal features of Silver Russell syndrome (SRS) are pre- and postnatal growth restriction, relative macrocephaly, protruding forehead, asymmetry, and feeding difficulties (Wakeling et al, 2016). A clinical diagnosis of SRS requires four of these six features to be present, while molecular testing is warranted when three of six features are present. A minority of children with SRS do not have sufficient presenting features to trigger molecular testing. On the other hand, children with other IDs, notably Temple syndrome, but also upd20mat and even PWS, can be clinically diagnosed as SRS, particularly by clinicians less experienced with IDs (Poole et al, 2013; Kagami et al, 2015; Mulchandani et al, 2016). Moreover, a wide range of CNVs (affecting non-imprinted genes) is found in children clinically overlapping with SRS (reviewed in Wakeling et al, 2016). This is likely because numerous CNVs may have deleterious effects on pre- and postnatal growth. In summary, the heterogeneous and overlapping clinical features of IDs suggest a need for comprehensive approaches to maximise diagnosis.

-molecular heterogeneity. Children with IDs are molecularly as well as clinically heterogeneous. As mentioned above, IDs are caused by altered gene dosage of imprinted genes, which can arise through multiple causes: genetic changes including single nucleotide variants (SNV) or copy number variants (CNV), uniparental disomy (UPD), and epimutations (Eggermann et al, 2015). Careful phenotyping has shown that different molecular aetiologies can confer slightly different presenting features and management issues (eg le Fevre, 2017; Elli et al, 2016; Wakeling et al, 2016). Because of this, care must be taken with molecular diagnosis, (a) to include the range of epigenetic tests warranted in order to maximise diagnostic yield, and (b) to determine the aetiology accurately in order to support accurate counselling – particularly the different recurrence risks between genetic and other changes, and the different management risks, eg for neoplasm.

-mosaicism. Some molecular defects in IDs are mosaic, ie, not present in every cell of the body (reviewed in Sanchez-Delgado et al, 2016B). This strongly suggests that mosaic events arise after fertilisation, in very early development. Mosaicism is particularly associated with epimutations, but has been found rarely with genetic changes and with UPD. Mosaic presentation has been observed in most IDs, but most frequently in Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS), which involve the imprinted loci on chromosome 11p15.

Mosaicism represents a challenge for both clinical and molecular diagnosis, to the point where affected individuals may not be clinically recognisable, and thus may not be referred for appropriate molecular testing. For example, children with Angelman syndrome and mosaic imprinting disturbance generally have clinical features (including a degree of speech and balance) highly atypical of the ‘classical’ phenotype, and in some cases have been diagnosed because they were referred for molecular diagnosis of Prader-Willi syndrome. (le Fevre et al, 2017). Not all children with Beckwith-Wiedemann syndrome (BWS) show the classical BWS phenotype, probably because of (a) the heterogeneity of molecular causes and (b) the heterogeneity of somatic mosaicism. The recent BWS consensus meeting has recommended redefining BWS as a spectrum (Beckwith-Wiedemann spectrum or BWSp), to reflect this heterogeneity. It also recommends a low threshold of clinical suspicion to trigger molecular testing, to diagnose these atypical cases and instigate appropriate tumour surveillance **(BWSp 2017 – in preparation**).

Molecular mosaicism also can impede accurate diagnosis. The great majority of molecular testing uses blood-derived DNA; therefore, low-level mosaicism in blood can elude detection, even though the burden of mosaicism in other tissues may be high. This is a particular challenge in BWS and SRS where positive diagnosis guides appropriate management. In children with a negative diagnosis, where a clinical suspicion of disease persists, testing of other tissues can support diagnosis (eg Azzi et al, 2009, Russo et al, 2016). Conversely, in monozygotic twins affected by BWS (which occur at tenfold over the population rate, and are almost all discordant for disease) analysis of blood DNA may detect molecular anomalies in both twins, even though only one is clinically affected; this is because of the sharing of blood between twins in utero. In such twins, analysis of other tissues may be needed to *exclude* diagnosis in the unaffected twin. (Weksberg et al, 2002, Bliek et al, 2009)

Multi-locus involvement. Over the past ten years it has become apparent that some children with IDs show methylation disturbance at multiple imprinted loci across the genome. Multi-locus imprinting disorder (MLID) is seen in a significant fraction of ID cases with epimutations – in fact, the more sensitive and comprehensive the epigenomic analysis, the more prevalent MLID appears to be (Table 1). The frequency of MLID in different disorders reflects the contribution of epimutations to the overall disorder – for example, epimutations are rare in PWS and AS, so MLID is too, whereas the majority of BWS and SRS are epigenetic, so MLID is more prevalent (Table 1).

Loci affected by MLID include those associated with ‘classical’ IDs, and also numerous other ICRs not currently associated with disorders (eg Court et al, 2013; Docherty et al, 2014). While some patterns are discernible, in general MLID cases are highly heterogeneous, epigenetically and clinically. Since referral for molecular diagnosis normally follows clinical diagosis, the clinical heterogeneity of MLID cases can confound diagnosis. Most cases described to date have a ‘primary presentation’ of a single ID – ie, their clinical presentation broadly consistent with one ID, such as BWS or transient neonatal diabetes mellitus (Azzi et al, 2009; Mackay et al, 2006, Bliek et al, 2008). Others have clinical features that do not align with classical IDs, and have been diagnosed adventitiously (for example, Baple et al, 2010; Docherty et al, 2015). It seems increasingly likely that the ascertainment bias of classical clinical definitions is preventing recognition of MLID in some children.

MLID is generally mosaic, with different loci showing different degrees of methylation disturbance in a given tissue; moreover, when multiple tissues are available for study, patterns of imprinting disturbance vary (Azzi et al, 2014). These factors suggest that the mosaicism may be related to developmental lineage, and this in turn suggests that DNA methylation disturbance occurs post-fertilisation, in the first few cell divisions of the embryo.

## Progress in Imprinting Disorders: MLID and the biology of the early embryo

Imprinting disturbance across multiple loci suggests trans-acting or mechanistic problems with imprint maintenance. Moreover, the (rare) finding of MLID in siblings demonstrates that, at least in some cases, MLID has an underlying genetic cause. To date, two types of genetic mutation have been identified:

-The DNA-binding factor ZFP57 operates in concert with a multimeric complex to direct methylation to hemimethylated DNA sequences. In the early embryo, ZFP57 is required to maintain methylation that would otherwise be diluted through repeated DNA replication. ZFP57 recognises a hexameric motif that is present throughout the genome, including all ICRs (Li et al, 2008A; Quenneville et al, 2011; Strogantsev et al, 2015). In humans, recessive mutations of *ZFP57* are associated with a relatively specific pattern of MLID, with loss of imprinted methylation that is complete at the *PLAGL1* ICR and variable at *PEG3* and *GRB10*; clinically, affected children present with transient neonatal diabetes, though other clinical features may be present (Mackay et al, 2008; Boonen et al, 2010). To date, no other mutations in DNA-binding factors have been associated with failure of imprint maintenance in humans, perhaps because such mutations are incompatible with life.

-Maternal-effect mutations have been identified in some cases of MLID. The first such case was identified in a rare case of siblings with BWS and MLID, whose mother had homozygous inactivating mutations of *NLRP2* (Meyer et al, 2009). The mother of a child with MLID was found to have a heterozygous mutation of *NLRP7* (Caliebe et al, 2014). More recently, maternal mutations in *NLRP5* were found in five pedigrees affected by MLID (Docherty et al, 2015). It is striking that the epigenotypes of affected children are variable, and affect both paternally- and maternally-methylated ICRs. Affected pedigrees experienced a spectrum of reproductive outcomes, including infertility, miscarriage, hydatidiform mole, and liveborn children with MLID with variable phenotypes, and no clinical phenotypes (Docherty et al, 2015 & DJGM, unpublished data). The only consistent feature of probands is MLID itself.

The most extreme manifestation of MLID is caused by maternal-effect mutation of *NLRP7*, and is not compatible with life. Maternal inactivation of *NLRP7* causes hydatidiform mole, an adverse pregnancy outcome resembling overproliferation of placenta without formation of a foetus (Judson et al, 2002, Murdoch et al, 2006). Hydatidiform moles caused by maternal mutations have a normal biparental genomic constitution, but complete loss of maternal imprinting marks.

NLRP genes form a large family in mammals, some of which are critical for humoral immunity, and some (including *NLRP7*, *NLRP2* and *NLRP5*) expressed only in the oocyte and early embryo (Tian et al, 2009). NLRP5 and its associated proteins are referred to as the subcortical maternal complex (SCMC; Li et al, 2008B; Zhu et al, 2015; reviewed in Zhou and Dean, 2015). Human maternal-effect mutations of SCMC genes are associated with a range of adverse pregnancy outcomes including mole and infertility as well as miscarriage and MLID (Meyer et al, 2009; Parry et al, 2011; Caliebe et al, 2014; Docherty et al, 2015; Alazami et al, 2016; Xu et al, 2016). Their precise roles in the embryo are unknown. They are among the most highly expressed genes in the oocyte; but their mRNA and protein abundance decline to undetectable levels by blastulation (Virant-Klun et al, 2016). Pre- and post-ovulatory oocyte ageing are associated with increased rate of decline in transcript levels (Dankert et al, 2014). Loss of function models in mice show a range of adverse effects on the onset of development, including maintenance of genome integrity and ploidy, mitochondrial function, and gene transcription and translation (Tong et al, 2000; Li et al, 2008B; Yurttas et al, 2008; Zheng et al, 2009; Fernandes et al, 2012; Zhao et al, 2016; Mahadevan et al, 2017).

This is striking, because oocyte transcription in humans ceases upon maturation and onset of meiosis (Bouniol-Baly et al, 1999). After fertilisation, the first transcription from the zygote genome is non-canonical, and involves DUX-family transcription factors (de Iaco et al, 2017); embryonic transcription is fully activated only at the 8-cell stage (Braude et al, 1988). Transcription and translation are tightly and independently regulated in the zygote and very early embryo (Nothias et al, 1996; reviewed in Zhou and Dean, 2015). At the same time, the embryo undergoes comprehensive epigenetic remodelling, including passive demethylation of genomic DNA to a minimum level around the time of blastulation (Smith et al, 2012). Embryonic stem cell populations recapitulating this developmental stage likewise undergo passive DNA demethylation, and this is associated with decline in protein levels of DNMT1, the methyltransferase that protects DNA methylation from dilution due to DNA replication (Eckersley-Maslin et al, 2016, von Meyenn et al, 2016).

Taken together, these observations strongly suggest that epigenetic reprogramming and zygotic genome activation (ZGA) must occur on linked timescales to enable the embryo to grow and develop (Figure 2A, 2B). Maternal genetic variations, or environmental effects (including maternal age) – or both – may impede the embryo from co-ordinating these two programmes, and this in turn may lead to a ‘crisis’ in the embryo, with delayed ZGA and degradation of imprinted DNA methylation (Figure 2C). If the zygote is severely compromised, the crisis becomes a catastrophe and the embryo dies – depending on the nature of the compromise, between the 1-cell stage and the blastocyst. But if the embryo survives the crisis, it continues to develop. Ongoing differentiation erases the majority of early epigenetic errors. But imprinting marks are not overwritten, and therefore imprinting disturbance may persist as evidence of an embryonic crisis. Thus, ‘embryo crises’ are predicted to lead to a range of consequences, from embryo death and apparent maternal infertility, through nonviable outcomes such as hydatidiform mole or miscarriage, to imprinting disorders and MLID, and offspring with no imprinting disturbance, or epigenetic disturbance with no apparent phenotypic effect.

It follows that MLID, and arguably the majority of primary imprinting epimutations, are no more nor less than relics of developmental crises in early embryos. The contribution of maternal-effect mutation is hard to estimate, because (a) reproductive problems are not generally quantified or analysed in clinical practice, (b) maternal genomes are not routinely analysed with reference to offspring genetics, and (c) imprinting disturbance is not analysed in the normal or disease population. The role of environmental influences is also hard to estimate. It is well recognised that the frequencies of fertility problems, aneuploidies and upd rise dramatically with increasing maternal age, suggesting compromise of the oocyte under these conditions (Eichenlaub-Ritter, 2012). Moreover, it is recognised that assisted reproductive technology (ART) is associated with adverse outcomes, including growth disturbance (Qin et al, 2016; Luke et al, 2017) and increased frequency of classical imprinting disorders (Cox et al, 2002; Mussa et al, 2017). More comprehensive, large-scale analysis is needed to indicate whether children born after ART have an increased risk of MLID or of non-classical epigenetic disturbance.

## Summary and future directions

Imprinted genes are a small subset of human genes, whose epigenetic control is not re-set in the early embryo, and thus reflects their gamete of origin. Human imprinting disorders (IDs) are caused by inappropriate expression of imprinted genes; either through genetic changes which are heritable, depending on their parental origin, or through chromosomal or epigenetic changes (UPD and imprinting errors) which are normally not heritable. IDs affect growth, development, metabolism and behaviour. They were originally defined as clinical syndromes; but recent developments in molecular genetic and epigenetic diagnosis is bringing about a re-definition of many IDs, and suggesting they may be less rare and more heterogeneous than originally thought. Beyond ‘classical’ syndromes, imprinting effects may be more prevalent than currently recognised, through population-level effects on lifecourse disease, and effects on fetal development, particularly in relation to ART.

Use of epigenomic analysis has expanded the range of known imprinted genes. The same tools are now required to determine whether any are associated with phenotypes that are currently clinically unrecognised, including the fascinating – and presently unknown – possibilities of transient and placental imprinting effects on growth and development. At the same time, integrating studies of animal models and human disease will drive progress in the biology of our early development, as both genetic and epigenetic creatures.

**References**

Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM, Gagliardi PC, Rodrigues T, Kochi C, Longui CA, Beckers D, de Zegher F, Montenegro LR, Mendonca BB, Carroll RS, Hirschhorn JN, Latronico AC, Kaiser UB. Central Precocious Puberty Caused by Mutations in the Imprinted Gene MKRN3. N Engl J Med 2013;368:2467-75. DOI: 10.1056/NEJMoa1302160

Alazami AM, Awad SM, Coskun S, Al-Hassan S, Hijazi H, Abdulwahab FM, Poizat C, Alkuraya FS. TLE6 mutation causes the earliest known human embryonic lethality. Genome Biol. 2015 Nov 5;16:240. doi: 10.1186/s13059-015-0792-0. PubMed PMID: 26537248; PubMed Central PMCID: PMC4634911.

Ali T, Renkawitz R, Bartkuhn M. Insulators and domains of gene expression. Curr Opin Genet Dev. 2016 Apr;37:17-26. doi: 10.1016/j.gde.2015.11.009. Epub 2016 Jan 20. Review. PMID: 26802288

Arand J, Wossidlo M, Lepikhov K, Peat JR, Reik W, Walter J. Selective impairment of methylation maintenance is the major cause of DNA methylation reprogramming in the early embryo. Epigenetics Chromatin. 2015 Jan 9;8(1):1. doi: 10.1186/1756-8935-8-1. eCollection 2015. PMID: 25621012

Azzi S, Rossignol S, Steunou V, Sas T, Thibaud N, Danton F, Le Jule M, Heinrichs C, Cabrol S, Gicquel C, Le Bouc Y, Netchine I. Multilocus methylation analysis in a large cohort of 11p15-related foetal growth disorders (Russell Silver and Beckwith Wiedemann syndromes) reveals simultaneous loss of methylation at paternal and maternal imprinted loci. Hum Mol Genet. 2009 Dec 15;18(24):4724-33. doi: 10.1093/hmg/ddp435. Epub 2009 Sep 14. PMID: 19755383

Azzi S, Blaise A, Steunou S, Harbison MD, Salem J, Brioude F, Rossignol S, Abi Habib W, Thibaud N, Das Neves C, Le Jule M, Brachet C, Heinrichs C, Le Bouc Y, Netchine I. Complex Tissue-Specific Epigenotypes in Russell–Silver Syndrome Associated with 11p15 ICR1 Hypomethylation. Hum Mut. 2014. 35:1211-1220.

Baple EL, Poole RL, Mansour S, Willoughby C, Temple IK, Docherty LE, Taylor R, Mackay DJG. An atypical case of hypomethylation at multiple imprinted loci. Eur J Hum Genet 19:360-362 2011

Barlow DP, Bartolomei MS. Genomic imprinting in mammals. Cold Spring Harb Perspect Biol. 2014 Feb 1;6(2). pii: a018382. doi: 10.1101/cshperspect.a018382.

Benonisdottir S, Oddsson A, Helgason A, Kristjansson RP, Sveinbjornsson G, Oskarsdottir A, Thorleifsson G, Davidsson OB, Arnadottir GA, Sulem G, Jensson BO, Holm H, Alexandersson KF, Tryggvadottir L, Walters GB, Gudjonsson SA, Ward LD, Sigurdsson JK, Iordache PD, Frigge ML, Rafnar T, Kong A, Masson G, Helgason H, Thorsteinsdottir U, Gudbjartsson DF, Sulem P, Stefansson K. Epigenetic and genetic components of height regulation. Nat Commun. 2016 Nov 16;7:13490. doi: 10.1038/ncomms13490. PMID: 27848971

Bliek J, Verde G, Callaway J, Maas S, De Crescenzo A, Sparago A, Cerrato F, Russo S, Ferraiuolo S, Rinaldi M, Fischetto R, Lalatta F, Giordano L, Ferrari P, Cubellis M, Larizza L, Temple IK, Mannens M, Mackay DJG, Riccio A. Hypomethylation at multiple maternally methylated imprinted regions including PLAGL1 and GNAS loci in Beckwith-Wiedemann syndrome. 2008 17:611-619

Bliek J, Alders M, Maas SM, Oostra RJ, Mackay DM, van der Lip K, Callaway JL, Brooks A, van 't Padje S, Westerveld A, Leschot NJ, Mannens MM. Lessons from BWS twins: complex maternal and paternal hypomethylation and a common source of haematopoietic stem cells. Eur J Hum Genet. 2009 17:1625-34.

Boonen SE, Mackay DJ, Hahnemann JM, Docherty L, Grønskov K, Lehmann A, Larsen LG, Haemers AP, Kockaerts Y, Dooms L, Vu DC, Ngoc CT, Nguyen PB, Kordonouri O, Sundberg F, Dayanikli P, Puthi V, Acerini C, Massoud AF, Tümer Z, Temple IK. Transient neonatal diabetes, ZFP57 and hypomethylation of multiple imprinted loci: a detailed follow-up. Diabetes Care. 2013 Mar;36(3):505-12 PMID: 23150280

Bouniol-Baly C, Hamraoui L, Guibert J, Beaujean N, Szöllösi MS, Debey P. Differential Transcriptional Activity Associated with Chromatin Configuration in Fully Grown Mouse Germinal Vesicle Oocytes. 1999. Biol Reprod 60, 580–587.

Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988 Mar 31;332(6163):459-61. PMID: 3352746

Buiting K. Prader–Willi Syndrome and Angelman Syndrome. Am J Med Genet. 2010. 154C:365–376.

Caliebe A, Richter J, Ammerpohl O, Kanber D, Beygo J, Bens S, Haake A, Jüttner E, Korn B, Mackay DJ, Martin-Subero JI, Nagel I, Sebire NJ, Seidmann L, Vater I, von Kaisenberg CS, Temple IK, Horsthemke B, Buiting K, Siebert R. A familial disorder of altered DNA-methylation. J Med Genet. 2014 Jun;51(6):407-12. doi: 10.1136/jmedgenet-2013-102149. PMID: 24721835

Chotalia M, Smallwood SA, Ruf N, Dawson C, Lucifero D, Frontera M, James K, Dean W, Kelsey G. Transcription is required for establishment of germline methylation marks at imprinted genes. Genes Dev. 2009 Jan 1;23(1):105-17. doi: 10.1101/gad.495809.

Choufani S, Shuman C, Weksberg R. Beckwith-Wiedemann syndrome. Am J Med Genet. 2010. 154C:343–354.

Court F, Martin-Trujillo A, Romanelli V, Garin I, Iglesias-Platas I, Salafsky I, Guitart M, Perez de Nanclares G, Lapunzina P, Monk D. Genome-wide allelic methylation analysis reveals disease-specific susceptibility to multiple methylation defects in imprinting syndromes. Hum Mutat. 2013 Apr;34(4):595-602

Cox GF, Bürger J, Lip V, Mau UA, Sperling K, Wu BL, Horsthemke B. Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet. 2002 Jul;71(1):162-4.

Dang-Nguyen TQ, Torres-Padilla ME. How cells build totipotency and pluripotency: nuclear, chromatin and transcriptional architecture. Curr Opin Cell Biol. 2015 Jun;34:9-15. doi: 10.1016/j.ceb.2015.04.006. Review. PubMed PMID: 25935759.

Dankert D, Demond H, Trapphoff T, Heiligentag M, Rademacher K, Eichenlaub-Ritter U, Horsthemke B, Grümmer R. Pre- and postovulatory aging of murine oocytes affect the transcript level and poly(A) tail length of maternal effect genes. PLoS One. 2014 Oct 1;9(10):e108907. doi: 10.1371/journal.pone.0108907. PubMed PMID: 25271735; PubMed Central PMCID: PMC4182777.

De Iaco A, Planet E, Coluccio A, Verp S, Duc J, Trono D. DUX-family transcription factors regulate zygotic genome activation in placental mammals. Nat Genet. 2017 Jun;49(6):941-945. doi: 10.1038/ng.3858. Epub 2017 May 1. PMID: 28459456

Docherty LE, Rezwan FI, Poole RL, Jagoe HT, Lake HA, Lockett G, Wilson DI, Holloway J, Temple IK, Mackay DJG, Genome-wide DNA methylation analysis of patients with imprinting disorders identifies differentially methylated regions associated with novel candidate imprinted genes. J Med Genet. 2014 Apr;51(4):229-38.

Docherty LE, Rezwan FI, Poole RL, Turner CL, Kivuva E, Maher ER, Smithson SF, Hamilton-Shield JP, Patalan M, Gizewska M, Peregud-Pogorzelski J, Beygo J, Buiting K, Horsthemke B, Soellner L, Begemann M, Eggermann T, Baple E, Mansour S, Temple IK, Mackay DJ. Mutations in NLRP5 are associated with reproductive wastage and multilocus imprinting disorders in humans. Nat Commun. 2015 Sep 1;6:8086. doi: 10.1038/ncomms9086. PMID: 26323243

Duffié R, Ajjan S, Greenberg MV, Zamudio N, Escamilla del Arenal M, Iranzo J, Okamoto I, Barbaux S, Fauque P, Bourc'his D. The Gpr1/Zdbf2 locus provides new paradigms for transient and dynamic genomic imprinting in mammals. Genes Dev. 2014 Mar 1;28(5):463-78. doi: 10.1101/gad.232058.113. PMID: 24589776

Eckersley-Maslin MA, Svensson V, Krueger C, Stubbs TM, Giehr P, Krueger F, Miragaia RJ, Kyriakopoulos C, Berrens RV, Milagre I, Walter J, Teichmann SA, Reik W. MERVL/Zscan4 Network Activation Results in Transient Genome-wide DNA Demethylation of mESCs. Cell Rep. 2016 Sep 27;17(1):179-92. doi: 10.1016/j.celrep.2016.08.087. PubMed PMID: 27681430; PubMed Central PMCID: PMC5055476.

Eggermann T. Russell-Silver syndrome. Am J Med Genet. 2010. 154C:355–364.

Eggermann T, Perez de Nanclares G, Maher ER, Temple IK, Tümer Z, Monk D, Mackay DJ, Grønskov K, Riccio A, Linglart A, Netchine I. Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. Clin Epigenetics. 2015 Nov 14;7:123. doi: 10.1186/s13148-015-0143-8. Review. PubMed PMID: 26583054; PubMed Central PMCID: PMC4650860.

Eichenlaub-Ritter U. Oocyte ageing and its cellular basis. Int J Dev Biol. 2012;56(10-12):841-52. doi: 10.1387/ijdb.120141ue. Review. PMID: 23417406

Elli FM, Linglart A, Garin I, de Sanctis L, Bordogna P, Grybek V, Pereda A, Giachero F, Verrua E, Hanna P, Mantovani G, Perez de Nanclares G. The Prevalence of GNAS Deficiency-Related Diseases in a Large Cohort of Patients Characterized by the EuroPHP Network. J Clin Endocrinol Metab 16; 101: 3657–3668.

Fernandes R, Tsuda C, Perumalsamy AL, Naranian T, Chong J, Acton BM, Tong ZB, Nelson LM, Jurisicova A. NLRP5 mediates mitochondrial function in mouse oocytes and embryos. Biol Reprod. 2012 May 3;86(5):138, 1-10. doi: 10.1095/biolreprod.111.093583. Print 2012 May. PMID: 22357545

Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol. 2013 Nov;14(11):699-712. doi: 10.1038/nrm3679. Epub 2013 Oct 9. Review. PMID: 24105322

Gonzalez-Sandoval A, Gasser SM. On TADs and LADs: Spatial Control Over Gene Expression. Trends Genet. 2016 Aug;32(8):485-95. doi: 10.1016/j.tig.2016.05.004. Epub 2016 Jun 13. Review. PMID: 27312344

Hanna CW, Peñaherrera MS, Saadeh H, Andrews S, McFadden DE, Kelsey G, Robinson WP. Pervasive polymorphic imprinted methylation in the human placenta. Genome Res. 2016 Jun;26(6):756-67. doi: 10.1101/gr.196139.115. Epub 2016 Jan 14. PMID: 26769960

He Y, Ecker JR. Non-CG Methylation in the Human Genome. Annu Rev Genomics Hum Genet. 2015;16:55-77. doi: 10.1146/annurev-genom-090413-025437. Epub 2015 Jun 4. Review. PMID: 26077819

Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN et al. Genome-wide associations for birth weight and correlations with adult disease. Nature. 2016 Oct 13;538(7624):248-252. doi: 10.1038/nature19806. Epub 2016 Sep 28.

PMID: 27680694.

Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet. 2014 Jun 2. pii: jmedgenet-2014-102396. doi: 10.1136/jmedgenet-2014-102396.

Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003 Mar;33 Suppl:245-54. Review. PMID: 12610534

Judson H, Hayward BE, Sheridan E, Bonthron DT. A global disorder of imprinting in the human female germ line. Nature. 2002. 416:539-542.

Kagami M, Mizuno S, Matsubara K, Nakabayashi K, Sano S, Fuke T, Fukami M, Ogata T. Epimutations of the IG‑DMR and the MEG3‑DMR at the 14q32.2 imprinted region in two patients with Silver–Russell syndrome-compatible phenotype. Eur. J. Hum. Genet. 23, 1062–1067 (2015).

Kagami M, Matsubara K, Nakabayashi K, Nakamura A, Sano S, Okamura K, Hata K, Fukami M, Ogata T. Genome-wide multilocus imprinting disturbance analysis in Temple syndrome and Kagami-Ogata syndrome. Genet Med. 2017 Apr;19(4):476-482. doi: 10.1038/gim.2016.123. Epub 2016 Sep 15. PMID: 27632690

Kelsey G, Feil R. New insights into establishment and maintenance of DNA methylation imprints in mammals. Philos Trans R Soc Lond B Biol Sci. 2013 Jan 5;368(1609):20110336. doi: 10.1098/rstb.2011.0336.

Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, Peshkin L, Weitz DA, Kirschner MW. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. Cell. 2015 May 21;161(5):1187-201. doi: 10.1016/j.cell.2015.04.044.

Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, Jonasdottir A, Sigurdsson A, Kristinsson KT, Jonasdottir A, Frigge ML, Gylfason A, Olason PI, Gudjonsson SA, Sverrisson S, Stacey SN, Sigurgeirsson B, Benediktsdottir KR, Sigurdsson H, Jonsson T, Benediktsson R, Olafsson JH, Johannsson OT, Hreidarsson AB, Sigurdsson G; DIAGRAM Consortium, Ferguson-Smith AC, Gudbjartsson DF, Thorsteinsdottir U, Stefansson K. Parental origin of sequence variants associated with complex diseases. Nature. 2009 Dec 17;462(7275):868-74. doi: 10.1038/nature08625. PMID: 20016592

Roadmap Epigenomics Consortium, Kundaje A et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015 Feb 19;518(7539):317-30. doi: 10.1038/nature14248. PMID: 25693563.

Lee MT, Bonneau AR, Giraldez AJ. Zygotic genome activation during the maternal-to-zygotic transition. Annu Rev Cell Dev Biol. 2014;30:581-613. doi: 10.1146/annurev-cellbio-100913-013027. Review. PubMed PMID: 25150012; PubMed Central PMCID: PMC4303375.

Le Fevre A, Beygo J, Silveira C, Kamien B, Clayton-Smith J, Colley A, Buiting K, Dudding-Byth T. Atypical Angelman Syndrome Due to a Mosaic Imprinting Defect: Case Reports and Review of the Literature. 2017. Am J Med Genet Part A 173A:753–757.

Li X, Ito M, Zhou F, Youngson N, Zuo X, Leder P, Ferguson-Smith AC. A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. Dev Cell. **2008A** Oct;15(4):547-57. doi: 10.1016/j.devcel.2008.08.014. PubMed PMID: 18854139; PubMed Central PMCID: PMC2593089.

Li L, Baibakov B, Dean J. A subcortical maternal complex essential for preimplantation mouse embryogenesis. Dev Cell. **2008B** Sep;15(3):416-25. doi: 10.1016/j.devcel.2008.07.010. PubMed PMID: 18804437; PubMed Central PMCID: PMC2597058.

Li W, Notani D, Rosenfeld MG. Enhancers as non-coding RNA transcription units: recent insights and future perspectives. Nat Rev Genet. 2016 Apr;17(4):207-23. doi: 10.1038/nrg.2016.4. Epub 2016 Mar 7. Review. PMID: 26948815

Luke B, Brown MB, Wantman E, Stern JE, Toner JP, Coddington CC 3rd. Increased risk of large-for-gestational age birthweight in singleton siblings conceived with in vitro fertilization in frozen versus fresh cycles. J Assist Reprod Genet. 2017 Feb;34(2):191-200. doi: 10.1007/s10815-016-0850-x. Epub 2016 Dec 1. PMID: 27909843

Mackay DJG, Boonen SE, Clayton-Smith J, Goodship J, Hahnemann JMD, Kant SG, Njølstad PR, Robin NH, Robinson DO, Siebert R, Shield JPH, White HE, Temple IK. A maternal hypomethylation syndrome presenting as transient neonatal diabetes mellitus. 2006. Hum Gen 120:262-269.

Mackay DJ, Callaway JL, Marks SM, White HE, Acerini CL, Boonen SE, Dayanikli P, Firth HV, Goodship JA, Haemers AP, Hahnemann JM, Kordonouri O, Masoud AF, Oestergaard E, Storr J, Ellard S, Hattersley AT, Robinson DO, Temple IK. Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. Nat Genet. 2008 Aug;40(8):949-51. doi: 10.1038/ng.187. PubMed PMID: 18622393.

Mackay DJ, Temple IK. Transient neonatal diabetes mellitus type 1. Am J Med Genet C Semin Med Genet. 2010 Aug 15;154C(3):335-42. doi: 10.1002/ajmg.c.30272. Review. PMID: 20803656

Mahadevan S, Sathappan V, Utama B, Lorenzo I, Kaskar K, Van den Veyver IB. Maternally expressed NLRP2 links the subcortical maternal complex (SCMC) to fertility, embryogenesis and epigenetic reprogramming. Sci Rep. 2017 Mar 20;7:44667. doi: 10.1038/srep44667. PMID: 28317850

Mantovani G, Spada A, Elli FM. Pseudohypoparathyroidism and Gsα–cAMP-linked disorders: current view and open issues. Nat Rev Endo. 2016. 12:347-356 doi:10.1038/nrendo.2016.52.

Meyer E, Lim D, Pasha S, Tee LJ, Rahman F, Yates JR, Woods CG, Reik W, Maher ER. Germline mutation in NLRP2 (NALP2) in a familial imprinting disorder (Beckwith-Wiedemann Syndrome). PLoS Genet. 2009 Mar;5(3):e1000423. doi: 10.1371/journal.pgen.1000423.PMID: 19300480

Mulchandani S, Bhoj EJ, Luo M, Powell-Hamilton N, Jenny K, Gripp KW, Elbracht M, Eggermann T, Turner CL, Temple IK, Mackay DJ, Dubbs H, Stevenson DA, Slattery L, Zackai EH, Spinner NB, Krantz ID, Conlin LK. Maternal uniparental disomy of chromosome 20: a novel imprinting disorder of growth failure. Genet Med. 2015 Aug 6. doi: 10.1038/gim.2015.103.

Murdoch S, Djuric U, Mazhar B, Seoud M, Khan R, Kuick R, Bagga R, Kircheisen R, Ao A, Ratti B, Hanash S, Rouleau GA, Slim R. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. Nat Genet. 2006 Mar;38(3):300-2. PubMed PMID: 16462743.

Mussa A, Molinatto C, Cerrato F, Palumbo O, Carella M, Baldassarre G, Carli D, Peris C, Riccio A, Ferrero GB. Assisted Reproductive Techniques and Risk of Beckwith-Wiedemann Syndrome. Pediatrics. 2017 Jul;140(1). pii: e20164311. doi: 10.1542/peds.2016-4311. Epub 2017 Jun 20. PMID: 28634246

Nothias JY, Miranda M, DePamphilis ML. Uncoupling of transcription and translation during zygotic gene activation in the mouse. EMBO J. 1996 Oct 15;15(20):5715-25. PubMed PMID: 8896464; PubMed Central PMCID: PMC452315.

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

Parry DA, Logan CV, Hayward BE, Shires M, Landolsi H, Diggle C, Carr I, Rittore C, Touitou I, Philibert L, Fisher RA, Fallahian M, Huntriss JD, Picton HM, Malik S, Taylor GR, Johnson CA, Bonthron DT, Sheridan EG. Mutations causing familial biparental hydatidiform mole implicate c6orf221 as a possible regulator of genomic imprinting in the human oocyte. Am J Hum Genet. 2011 Sep 9;89(3):451-8. doi: 10.1016/j.ajhg.2011.08.002. PubMed PMID: 21885028; PubMed Central PMCID: PMC3169823.

Poole RL, Docherty LE, Al Sayegh A, Caliebe A, Turner C, Baple E, Wakeling E, Harrison L, Lehmann A, Temple IK, Mackay DJ; On behalf of the International Clinical Imprinting Consortium. Targeted methylation testing of a patient cohort broadens the epigenetic and clinical description of imprinting disorders. Am J Med Genet A. 2013 Aug 2. doi: 10.1002/ajmg.a.36049.

Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. Fertil Steril. 2016 Jan;105(1):73-85.e1-6. doi: 10.1016/j.fertnstert.2015.09.007. Epub 2015 Oct 9. Review. PMID: 26453266

Quenneville S, Verde G, Corsinotti A, Kapopoulou A, Jakobsson J, Offner S, Baglivo I, Pedone PV, Grimaldi G, Riccio A, Trono D. In embryonic stem cells, ZFP57/KAP1 recognize a methylated hexanucleotide to affect chromatin and DNA methylation of imprinting control regions. Mol Cell. 2011 Nov 4;44(3):361-72. doi: 10.1016/j.molcel.2011.08.032. PubMed PMID: 22055183; PubMed Central PMCID:PMC3210328.

Russo S, Calzari L, Mussa A, Mainini E, Cassina M, Di Candia S, Clementi M, Guzzetti S, Tabano S, Miozzo M, Sirchia S, Finelli P, Prontera P, Maitz S, Sorge G, Calcagno A, Maghnie M, Divizia MT, Melis D, Manfredini E, Ferrero GB, Pecile V, Larizza L. A multi-method approach to the molecular diagnosis of overt and borderline 11p15.5 defects underlying Silver-Russell and Beckwith-Wiedemann syndromes. Clin Epigenetics. 2016 Mar 1;8:23. doi: 10.1186/s13148-016-0183-8. eCollection 2016. Erratum in: Clin Epigenetics. 2016;8:40. PMID: 26933465

Sanchez-Delgado M, Court F, Vidal E, Medrano J, Monteagudo-Sánchez A, Martin-Trujillo A, Tayama C, Iglesias-Platas I, Kondova I, Bontrop R, Poo-Llanillo ME, Marques-Bonet T, Nakabayashi K, Simón C, Monk D. Human Oocyte-Derived Methylation Differences Persist in the Placenta Revealing Widespread Transient Imprinting. PLoS Genet. **2016A** Nov 11;12(11):e1006427. doi: 10.1371/journal.pgen.1006427. eCollection 2016 Nov. PMID: 27835649

Sanchez-Delgado M, Riccio A, Eggermann T, Maher ER, Lapunzina P, Mackay D, Monk D. Causes and Consequences of Multi-Locus Imprinting Disturbances in Humans. Trends Genet. **2016B** Jul;32(7):444-55. doi: 10.1016/j.tig.2016.05.001. Review. PubMed PMID: 27235113.

Schaaf CP, Gonzalez-Garay ML, Xia F, et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. Nat Genet 2013;45:1405–1408.

Schübeler D. Function and information content of DNA methylation. Nature. 2015 Jan 15;517(7534):321-6. doi: 10.1038/nature14192. Review. PMID: 25592537

Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, Popp C, Thienpont B, Dean W, Reik W. The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. Mol Cell. 2012 Dec 28;48(6):849-62. doi: 10.1016/j.molcel.2012.11.001. Epub 2012 Dec 6. PMID: 23219530

Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G. Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet. 2011 Jun 26;43(8):811-4. doi: 10.1038/ng.864. PubMed PMID: 21706000; PubMed Central PMCID: PMC3146050.

Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, Meissner A. A unique regulatory phase of DNA methylation in the early mammalian embryo. Nature. 2012 Mar 28;484(7394):339-44. doi: 10.1038/nature10960. PubMed PMID: 22456710; PubMed Central PMCID: PMC3331945.

Strogantsev R, Krueger F, Yamazawa K, Shi H, Gould P, Goldman-Roberts M, McEwen K, Sun B, Pedersen R, Ferguson-Smith AC. Allele-specific binding of ZFP57 in the epigenetic regulation of imprinted and non-imprinted monoallelic expression. Genome Biol. 2015 May 30;16:112. doi: 10.1186/s13059-015-0672-7. PubMed PMID: 26025256; PubMed Central PMCID: PMC4491874.

Su Z, Denu JM. Reading the Combinatorial Histone Language. ACS Chem Biol. 2016 Mar 18;11(3):564-74. doi: 10.1021/acschembio.5b00864. Epub 2015 Dec 21. Review. PMID: 26675328

Tian X, Pascal G, Monget P. Evolution and functional divergence of NLRP genes in mammalian reproductive systems. BMC Evol Biol. 2009 Aug 14;9:202. doi: 10.1186/1471-2148-9-202. PubMed PMID: 19682372; PubMed Central PMCID: PMC2735741.

Tong ZB, Gold L, Pfeifer KE, Dorward H, Lee E, Bondy CA, Dean J, Nelson LM. Mater, a maternal effect gene required for early embryonic development in mice. Nat Genet. 2000 Nov;26(3):267-8. PubMed PMID: 11062459.

Virant-Klun I, Leicht S, Hughes C, Krijgsveld J. Identification of Maturation-Specific Proteins by Single-Cell Proteomics of Human Oocytes. Mol Cell Proteomics. 2016 Aug;15(8):2616-27. doi: 10.1074/mcp.M115.056887. PubMed PMID: 27215607; PubMed Central PMCID: PMC4974340.

von Meyenn F, Iurlaro M, Habibi E, Liu NQ, Salehzadeh-Yazdi A, Santos F, Petrini E, Milagre I, Yu M, Xie Z, Kroeze LI, Nesterova TB, Jansen JH, Xie H, He C, Reik W, Stunnenberg HG. Impairment of DNA Methylation Maintenance Is the Main Cause of Global Demethylation in Naive Embryonic Stem Cells. Mol Cell. 2016 Jun 16;62(6):983. doi: 10.1016/j.molcel.2016.06.005.

Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, Canton AP, Chrzanowska KH, Davies JH, Dias RP, Dubern B, Elbracht M, Giabicani E, Grimberg A, Grønskov K, Hokken-Koelega AC, Jorge AA, Kagami M, Linglart A, Maghnie M, Mohnike K, Monk D, Moore GE, Murray PG, Ogata T, Petit IO, Russo S, Said E, Toumba M, Tümer Z, Binder G, Eggermann T, Harbison MD, Temple IK, Mackay DJ, Netchine I. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol. 2016 Sep 2. doi: 10.1038/nrendo.2016.138

Weksberg R, Shuman C, Caluseriu O Smith AC, Fei Y, Nishikawa J, Stockley TL, Best L, Chitayat D, Olney A, Ives E, Schneider A, Bestor TH, Li M, Sadowski P, Squire D. Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith –Wiedemann syndrome. Hum Mol Genet 2002; 15:1317– 1325.

Xu Y, Shi Y, Fu J, Yu M, Feng R, Sang Q, Liang B, Chen B, Qu R, Li B, Yan Z, Mao X, Kuang Y, Jin L, He L, Sun X, Wang L. Mutations in PADI6 Cause Female Infertility Characterized by Early Embryonic Arrest. Am J Hum Genet. 2016 Sep 1;99(3):744-52. doi: 10.1016/j.ajhg.2016.06.024. PubMed PMID: 27545678; PubMed Central PMCID: PMC5010645.

Yurttas P, Vitale AM, Fitzhenry RJ, Cohen-Gould L, Wu W, Gossen JA, Coonrod SA. Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. Development. 2008 Aug;135(15):2627-36. doi: 10.1242/dev.016329. Epub 2008 Jul 3. PMID: 18599511

Zhao B, Zhang WD, Duan YL, Lu YQ, Cun YX, Li CH, Guo K, Nie WH, Li L, Zhang R, Zheng P. Filia Is an ESC-Specific Regulator of DNA Damage Response and Safeguards Genomic Stability. Cell Stem Cell. 2015 Jun 4;16(6):684-98. doi: 10.1016/j.stem.2015.03.017. Epub 2015 Apr 30. PMID: 25936915

Zheng P, Dean J. Role of Filia, a maternal effect gene, in maintaining euploidy during cleavage-stage mouse embryogenesis. Proc Natl Acad Sci U S A. 2009 May 5;106(18):7473-8. doi: 10.1073/pnas.0900519106. Epub 2009 Apr 17. PMID: 19376971

Zhou LQ, Dean J. Reprogramming the genome to totipotency in mouse embryos. Trends Cell Biol. 2015 Feb;25(2):82-91. doi: 10.1016/j.tcb.2014.09.006. Review. PubMed PMID: 25448353; PubMed Central PMCID: PMC4312727.

Zhu K, Yan L, Zhang X, Lu X, Wang T, Yan J, Liu X, Qiao J, Li L. Identification of a human subcortical maternal complex. Mol Hum Reprod. 2015 Apr;21(4):320-9. doi: 10.1093/molehr/gau116. PubMed PMID: 25542835.

Figure Legends

**Figure 1: Epigenetic reprogramming during reproduction.**

**A: Somatic epigenetic marks.** In cell populations destined to become germ cells, epigenetic marks (including DNA methylation) are removed to reach an epigenetic ‘clean slate’. The developing eggs and sperm then establish divergent epigenetic marks, reflecting their very different developmental programmes. At fertilisation, the sperm genome enters the egg. The newly-formed zygote undergoes epigenetic reprogramming during zygotic cleavage stages: paternal DNA methylation is removed principally by active demethylation in the one-cell embryo, while successive cell divisions dilute maternal DNA methylation. Reaching a minimum in late cleavage stages, DNA methylation then evolves through subsequent differentiation to re-establish normal somatic patterns.

**B: Imprinted epigenetic marks.** Germline epigenetic reprogramming proceeds as for all other epigenetic marks. However, after fertilisation, imprints evade zygotic reprogramming: they are not removed in the zygote, and not overwritten by subsequent developmental marks. Thus they retain an epigenetic ‘memory’ of their parent of origin.

**Figure 2: Hypothesis: epigenetic reprogramming, zygotic genome activation and embyro crisis.**

**A:** The five cartoons represent the growth of the embryo from a fertilised one-cell zygote, through the 2-cell and 8-cell embryo, and the morula, to the blastocyst, where different cell lineages of trophectoderm and inner cell mass are well established.

**B**: The schematic represents the changes in transcription (black lines) and DNA methylation (as a proxy for epigenetic reprogramming, green lines) in a healthy embryo. Most DNA methylation (solid green line) is progressively lost in cleavage stages, reaching a minimum around the time of blastulation, but then re-established in tissue-specific patterns. Imprinted DNA methylation (dashed green line) is unaffected. After a transient burst of non-canonical transcription mediated by pioneer factors (dashed black line) the zygote genome is activated from the 8-cell stage.

**C**: The schematic hypothesises an embryo crisis resulting from a genetic or environmental lesion. In this hypothetical crisis, onset of transcription is delayed. As a result, maternal factors required to maintain imprinting become insufficient, and imprinting is progressively degraded. If this crisis is prolonged, epigenetic dysregulation or delayed zygotic genome activation may lead to embryo death. If the embryo survives the crisis, it may have mosaic loss of imprinted DNA methylation, with or without other developmental dysregulation.

Table 1: Imprinting disorders

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Disorder | chromosome(s) | prevalence | OMIM | % genetic error(SNV / CNV)  | % chromosomal error (UPD) | % imprinting error (% MLID) | references |
| Angelman Syndrome | 15q11.2 | 1:15000 | #105830 | 70% CNV (del15mat)15% SNV (*UBE3A*) | <5% (upd15pat) | <5% (rare) | Buiting, 2010 |
| Prader-Willi syndrome | 15q11.2 | 1:15000 | #176270 | 70% CNV (del15pat) | <30% (upd15mat) | <1% (nk) | Buiting, 2010 |
| Beckwith-Wiedemann syndrome | 11p15.5 | 1:10500 | #130650 | 5% SNV (*CDKN1C*)<5% CNV and SNV of H19/IGF2 IG-DMR | 20%  | 10% H19/IGF2 IG-DMR hypermethylation (rare)60% KCNQ1OT1 TSS-DMR hypomethylation (30%) | Choufani et al, 2010 |
| Silver-Russell syndrome | 11p15.5, chr7 | 1:50000? | #180860 | <1% | 10% (upd7mat)<1% (upd11mat) | 40% (15-38%)  | Eggermann 2010, wakeling et al, 2016 |
| Pseudohypoparathyroidism type 1b | 20q13.3 | ? | #603233 | 27% CNV (delSTX16mat)3% SNV (GNAS) | 5% (upd20mat) | 61% (rare) | Mantovani et al, 2016; Elli et al, 2016 |
| Transient neonatal diabetes mellitus type 1 | 6q24 | 1:300000 | #601410 | 40% CNV (dup6pat) | 40% (upd6pat) | 20% (50%) | Mackay and Temple, 2010 |
| Kagami-Ogata syndrome | 14q32 | ? | #608140 | 15% CNV (del14mat) | 65% (upd14pat) | 20% (nk) | Ogata et al, 2016; Kagami et al, 2017 |
| Temple syndrome | 14q32 | ? | #616222 | 10% CNV (del14pat) | 78% (upd14mat) | 12% (rare) | Ioannides et al, 2014; Kagami et al, 2017 |
| Mulchandani-Bhoj-Conlin syndrome | chr20 | ? | #617352 | nk | 100% (upd20mat) | nk (nk) | Mulchandani et al, 2016 |
| Schaaf-Yang syndrome | chr15 | ? | #615547 | 100% inactivation of *MAGEL2* (SNV / CNV) | - | - | Fountain et al, 2017 |
| Central precocious puberty 2 (CPPB2) | chr15 | ? | #615436 | 100% inactivation of *MKRN3* (SNV) | - | - | Abreu et al, 2013 |

nk: not known; SNV: single nucleotide variant; CNV: copy number variant; upd: uniparental disomy. References here are reviews of individual imprinting disorders. References specifically concerning the frequency of multi-locus imprinting disorder in each IDs may be found in Sanchez-Delgado et al 2016; only reports of MLID post-dating this review are cited in this table.

OMIM: Online Mendelian Inheritance in Man (<http://omim.org)>.

# Human Imprinting Disorders: principles, practice, problems and progress.

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Abstract

Epigenetic regulation orchestrates gene expression with exquisite precision, over a huge dynamic range and across developmental space and time, permitting genomically-homogeneous humans to develop and adapt to their surroundings. Every generation, these epigenetic marks are re-set twice: in the germline, to enable differentiation of sperm and eggs, and at fertilisation, to create the totipotent zygote that then begins growth and differentiation into a new human. A small group of genes evades the second, zygotic wave of epigenetic reprogramming, and these genes retain an epigenetic ‘imprint’ of the parent from whom they were inherited.

Imprinted genes are (as a general rule) expressed from one parental allele only. Some imprinted genes are critical regulators of growth and development, and thus disruption of their normal monoallelic expression causes congenital imprinting disorders, with clinical features impacting growth, development, behaviour and metabolism.

Imprinting disorders as a group have characteristics that challenge diagnosis and management, including clinical and molecular heterogeneity, overlapping clinical features, somatic mosaicism, and multi-locus involvement. New insights into the biology and epigenomics of the early embryo offers new clues about the origin and importance of imprinting disorders.

## 1. Principles of imprinting.

We are genetic creatures. Essentially every somatic cell in our bodies contains essentially the same genome, and this genome contains all the genetic material required to encode and regulate every gene in the whole individual. But of course, not all cells in our bodies use this genome in the same way (Jaenisch and Bird, 2003). Different organs and tissues, at different developmental times, in different stages of health and disease, repair, regeneration and senescence – even in neighbouring cells in homogeneous tissues – express different numbers, types and levels of genes, both protein-coding and non-coding (Kundaje et al, 2015). These differences, from the gross level of tissues to the quantitative and stochastic variation between cells (Xue et al, 2013, Klein et al, 2015), underpin the growth, differentiation, adaptation and regeneration of the whole organism.

The origin of this extraordinary variation is not genetic, but epigenetic. Epigenetics, in its this sense, is modification of DNA that does not alter its sequence, but alters its expression, and thereby alters its phenotype (Jaenisch and Bird, 2003). Although epigenetic modifications do not change DNA sequence, they are transmitted faithfully from cells to their progeny, ensuring phenotypic continuity. Epigenetic signals are highly diverse and dynamic: the most-studied is DNA methylation on cytosine residues in CpG dinucleotide sequences, but it is only one epigenetic regulator, alongside RNA species, histones and other chromatin proteins, and their wide-ranging post-translational modifications (reviewed in Schubeler, 2015; He and Ecker, 2015; Su and Demu, 2015; Geisler and Coller, 2015; Li et al, 2016; Ali et al, 2016; Gonzalez-Sandoval and Gasser, 2016).

Epigenetic regulation orchestrates gene expression, with exquisite precision across a very high dynamic range, across developmental space and time. Nowhere is this more apparent than in reproduction. From a complex, highly-differentiated organism, the cells of one specific lineage, the germline, differentiate into two of the most differentiated and dimorphic cell types in humans: the egg and sperm; then after their fusion, the resultant zygote becomes totipotent, capable of populating all the lineages of the future organism; and thus it begins its development into a new, complex four-dimensional individual (reviewed in Lee et al, 2014; Dang-Nguyen and Torres-Padilla, 2015). These transformations are in large part epigenetic. Global epigenetic reprogramming occurs twice: once in the germline and once in the very early zygote (Seisenberger et al, 2012; Smallwood et al, 2011; Smith et al, 2012; Arand et al, 2015). Germline cells essentially ‘wipe the slate clean’ – they undergo almost complete loss of DNA methylation, before the eggs and sperm acquire their highly specialised and divergent epigenetic marks. In the cleavage-stage embryo there is a second wave of epigenetic reprogramming; both egg and sperm epigenetic marks are erased, with to a minimum of DNA methylation at around the blastula stage, before lineage-specific DNA methylation arises with the onset of differentiation (FIGURE 1A).

A small number of genomic regions evade the zygotic wave of epigenetic reprogramming. Their epigenetic marks are not demethylated during the first cell divisions of the cleavage-stage embryo; nor are they altered by any cell fate decisions during subsequent differentiation (FIGURE 1B). Thus, these regions retain the epigenetic marks of the eggs and sperm that constituted the zygote, an epigenetic ‘memory’ of their parent of origin. This phenomenon is termed genomic imprinting (Barlow and Bartolomei, 2014; Kelsey and Feil, 2013). Recently, epigenomic analysis of embryonic and extraembryonic tissues has revealed widespread transient and placental imprinting, but these are outside the scope of this review (Hanna et al, 2016, Sanchez-Delgado et al, 2016A, Duffié et al, 2014).

## 2. Putting principles into practice: imprinting and imprinting disorders

In humans over 40 genomic regions, distributed across the genome, show imprinting – that is, DNA methylation (and other epigenetic marking) on imprinting control regions. Comprehensive genomewide analyses suggest that all germline imprints of somatic tissues have now been identified (Court et al, 2013), but it remains possible that more remain to be found. All currently-known imprinting control regions (ICRs) include differentially methylated regions (DMRs). These ICRs restrict the expression of genes under their control, according to their parent of origin. Imprinting marks are permanent and ubiquitous: that is, they persist essentially throughout the lifecourse and in essentially all cells of the body.

Most ICRs are methylated on the maternal allele, and overlap with transcriptional start sites in the oocyte (Chotalia et al, 2009), whereas the known paternally-methylated ICRs are intergenic and form chromatin boundary elements.

Genes in imprinted loci include both protein-coding and non-coding genes. Some ICRs control the expression of only one or two genes, while other imprinted loci include numerous transcripts, both translated and untranslated. The ‘classical’ definition of an imprinted allele is the allele whose expression is downregulated epigenetically from the specified parent of origin. However, the complexity of some imprinted loci limits the value of such definitions; for example, the ICR on chromosome 14q32 is paternally methylated, but both maternally- and paternally-imprinted genes are under its control. Also, imprinted genes may be regulated by other DNA sequences besides ICRs, so their *expression* may not be ubiquitously imprinted: for example, the Angelman syndrome gene *UBE3A* shows imprinted expression only in the brain (Buiting, 2010); and *GNAS* is expressed from the maternal allele in only certain tissues such as kidney tubule and pituitary gland, and this accounts for the clinical features of PHP1b (Mantovani, 2016).

Despite these caveats, the asymmetrical regulation of imprinted genes makes them important in human development. At least at some critical developmental times and places, imprinted genes are expressed hemizygously (from one parental allele only). Some of these genes are regulators of growth and development, with tightly regulated expression, and altered expression of these genes has deleterious effects on growth and development. Additionally, imprinting effects are increasingly being recognised in population-level traits in growth and metabolism (Kong et al, 2009; Horikoshi et al, 2016; Benonisdottir et al, 2016). Many other imprinted genes are not currently associated with phenotypic traits.

In principle, either increased or decreased dosage of imprinted genes may be deleterious. And indeed, eight of the nine currently-defined primary imprinting disorders are ‘mirror’ disorders – pairs of disorders caused by opposing molecular alterations of specific imprinted loci, with broadly ‘opposing’ effects on development. Imprinting disorders may occur through three broad mechanisms:

-changes affecting the *expressed* allele of the gene in question. This includes coding mutations, and copy number changes (deletions or duplications). Such variations are genetic and therefore heritable, but whether a phenotype results depends on the parental allele on which the change is present.

-chromosomal errors. This includes large-scale reorganisation of genetic material, normally without overall change in copy number, including translocation, uniparental disomy (upd), and the more recently-described genomewide uniploidy. Translocations altering regulation of imprinted genes are a rare but recognised cause of imprinting disorders, and are in principle heritable, but whether a phenotype results depends on the parental allele on which the change is present. In most IDs, upd is a meiotic error, and is therefore not generally heritable, unless a predisposing parental chromosomal rearrangement is present. In Beckwith-Wiedemann and Silver-Russell syndromes, upd arises post-fertilisation, and almost always appears in mosaic and segmental form; it carries a low recurrence risk.

-epigenetic errors. This encompasses changes to the DNA methylation of DMRs controlling imprinted genes. While some have detectable underlying genetic causes, either in-cis or in-trans, it is generally believed that the majority of primary epimutations are purely epigenetic and stochastic in origin, and are not heritable.

## 3. Practical problems with imprinting disorders

Currently nine ‘classical’ imprinting disorders (IDs) are recognised: Classical imprinting disorders clinical syndromes caused by errors in the effective gene dosage of imprinted genes, with features affecting growth, development, metabolism and behaviour. In addition, a new growing class of disorders is being recognised, associated specifically with coding mutations of genes regulated by imprinting, such as MAGEL2 mutations causing Schaaf-Yang syndrome and MKRN3 mutations causing central precocious puberty type 2 (Schaaf et al, 2013; Abreu et al, 2013).

Individual imprinting disorders have been very well reviewed in published literature, so are summarised for reference in Table 1. Here I will focus on some of the characteristics that make IDs distinctive as a group of disorders. These include: heterogeneous and overlapping clinical features; heterogeneous molecular aetiology, including somatic mosaicism; and multi-locus imprinting disorder.

-heterogeneous and overlapping clinical features. Classical definitions of clinical IDs include groups of clinical features, very often featuring early growth, development and metabolism. Some of these features develop or disappear at different times in development, which can challenge clinical diagnosis. Not every child with an ID presents with sufficient cardinal features to secure a clinical diagnosis. Moreover, some clinical features – notably posnatal growth restriction – are shared by different IDs. The heterogeneity of presentation means that a child with certain features may warrant testing for more than one ID. For example, the cardinal features of Silver Russell syndrome (SRS) are pre- and postnatal growth restriction, relative macrocephaly, protruding forehead, asymmetry, and feeding difficulties (Wakeling et al, 2016). A clinical diagnosis of SRS requires four of these six features to be present, while molecular testing is warranted when three of six features are present. A minority of children with SRS do not have sufficient presenting features to trigger molecular testing. On the other hand, children with other IDs, notably Temple syndrome, but also upd20mat and even PWS, can be clinically diagnosed as SRS, particularly by clinicians less experienced with IDs (Poole et al, 2013; Kagami et al, 2015; Mulchandani et al, 2016). Moreover, a wide range of CNVs (affecting non-imprinted genes) is found in children clinically overlapping with SRS (reviewed in Wakeling et al, 2016). This is likely because numerous CNVs may have deleterious effects on pre- and postnatal growth. In summary, the heterogeneous and overlapping clinical features of IDs suggest a need for comprehensive approaches to maximise diagnosis.

-molecular heterogeneity. Children with IDs are molecularly as well as clinically heterogeneous. As mentioned above, IDs are caused by altered gene dosage of imprinted genes, which can arise through multiple causes: genetic changes including single nucleotide variants (SNV) or copy number variants (CNV), uniparental disomy (UPD), and epimutations (Eggermann et al, 2015). Careful phenotyping has shown that different molecular aetiologies can confer slightly different presenting features and management issues (eg le Fevre, 2017; Elli et al, 2016; Wakeling et al, 2016). Because of this, care must be taken with molecular diagnosis, (a) to include the range of epigenetic tests warranted in order to maximise diagnostic yield, and (b) to determine the aetiology accurately in order to support accurate counselling – particularly the different recurrence risks between genetic and other changes, and the different management risks, eg for neoplasm.

-mosaicism. Some molecular defects in IDs are mosaic, ie, not present in every cell of the body (reviewed in Sanchez-Delgado et al, 2016B). This strongly suggests that mosaic events arise after fertilisation, in very early development. Mosaicism is particularly associated with epimutations, but has been found rarely with genetic changes and with UPD. Mosaic presentation has been observed in most IDs, but most frequently in Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS), which involve the imprinted loci on chromosome 11p15.

Mosaicism represents a challenge for both clinical and molecular diagnosis, to the point where affected individuals may not be clinically recognisable, and thus may not be referred for appropriate molecular testing. For example, children with Angelman syndrome and mosaic imprinting disturbance generally have clinical features (including a degree of speech and balance) highly atypical of the ‘classical’ phenotype, and in some cases have been diagnosed because they were referred for molecular diagnosis of Prader-Willi syndrome. (le Fevre et al, 2017). Not all children with Beckwith-Wiedemann syndrome (BWS) show the classical BWS phenotype, probably because of (a) the heterogeneity of molecular causes and (b) the heterogeneity of somatic mosaicism. The recent BWS consensus meeting has recommended redefining BWS as a spectrum (Beckwith-Wiedemann spectrum or BWSp), to reflect this heterogeneity. It also recommends a low threshold of clinical suspicion to trigger molecular testing, to diagnose these atypical cases and instigate appropriate tumour surveillance **(BWSp 2017 – in preparation**).

Molecular mosaicism also can impede accurate diagnosis. The great majority of molecular testing uses blood-derived DNA; therefore, low-level mosaicism in blood can elude detection, even though the burden of mosaicism in other tissues may be high. This is a particular challenge in BWS and SRS where positive diagnosis guides appropriate management. In children with a negative diagnosis, where a clinical suspicion of disease persists, testing of other tissues can support diagnosis (eg Azzi et al, 2009, Russo et al, 2016). Conversely, in monozygotic twins affected by BWS (which occur at tenfold over the population rate, and are almost all discordant for disease) analysis of blood DNA may detect molecular anomalies in both twins, even though only one is clinically affected; this is because of the sharing of blood between twins in utero. In such twins, analysis of other tissues may be needed to *exclude* diagnosis in the unaffected twin. (Weksberg et al, 2002, Bliek et al, 2009)

Multi-locus involvement. Over the past ten years it has become apparent that some children with IDs show methylation disturbance at multiple imprinted loci across the genome. Multi-locus imprinting disorder (MLID) is seen in a significant fraction of ID cases with epimutations – in fact, the more sensitive and comprehensive the epigenomic analysis, the more prevalent MLID appears to be (Table 1). The frequency of MLID in different disorders reflects the contribution of epimutations to the overall disorder – for example, epimutations are rare in PWS and AS, so MLID is too, whereas the majority of BWS and SRS are epigenetic, so MLID is more prevalent (Table 1).

Loci affected by MLID include those associated with ‘classical’ IDs, and also numerous other ICRs not currently associated with disorders (eg Court et al, 2013; Docherty et al, 2014). While some patterns are discernible, in general MLID cases are highly heterogeneous, epigenetically and clinically. Since referral for molecular diagnosis normally follows clinical diagosis, the clinical heterogeneity of MLID cases can confound diagnosis. Most cases described to date have a ‘primary presentation’ of a single ID – ie, their clinical presentation broadly consistent with one ID, such as BWS or transient neonatal diabetes mellitus (Azzi et al, 2009; Mackay et al, 2006, Bliek et al, 2008). Others have clinical features that do not align with classical IDs, and have been diagnosed adventitiously (for example, Baple et al, 2010; Docherty et al, 2015). It seems increasingly likely that the ascertainment bias of classical clinical definitions is preventing recognition of MLID in some children.

MLID is generally mosaic, with different loci showing different degrees of methylation disturbance in a given tissue; moreover, when multiple tissues are available for study, patterns of imprinting disturbance vary (Azzi et al, 2014). These factors suggest that the mosaicism may be related to developmental lineage, and this in turn suggests that DNA methylation disturbance occurs post-fertilisation, in the first few cell divisions of the embryo.

## Progress in Imprinting Disorders: MLID and the biology of the early embryo

Imprinting disturbance across multiple loci suggests trans-acting or mechanistic problems with imprint maintenance. Moreover, the (rare) finding of MLID in siblings demonstrates that, at least in some cases, MLID has an underlying genetic cause. To date, two types of genetic mutation have been identified:

-The DNA-binding factor ZFP57 operates in concert with a multimeric complex to direct methylation to hemimethylated DNA sequences. In the early embryo, ZFP57 is required to maintain methylation that would otherwise be diluted through repeated DNA replication. ZFP57 recognises a hexameric motif that is present throughout the genome, including all ICRs (Li et al, 2008A; Quenneville et al, 2011; Strogantsev et al, 2015). In humans, recessive mutations of *ZFP57* are associated with a relatively specific pattern of MLID, with loss of imprinted methylation that is complete at the *PLAGL1* ICR and variable at *PEG3* and *GRB10*; clinically, affected children present with transient neonatal diabetes, though other clinical features may be present (Mackay et al, 2008; Boonen et al, 2010). To date, no other mutations in DNA-binding factors have been associated with failure of imprint maintenance in humans, perhaps because such mutations are incompatible with life.

-Maternal-effect mutations have been identified in some cases of MLID. The first such case was identified in a rare case of siblings with BWS and MLID, whose mother had homozygous inactivating mutations of *NLRP2* (Meyer et al, 2009). The mother of a child with MLID was found to have a heterozygous mutation of *NLRP7* (Caliebe et al, 2014). More recently, maternal mutations in *NLRP5* were found in five pedigrees affected by MLID (Docherty et al, 2015). It is striking that the epigenotypes of affected children are variable, and affect both paternally- and maternally-methylated ICRs. Affected pedigrees experienced a spectrum of reproductive outcomes, including infertility, miscarriage, hydatidiform mole, and liveborn children with MLID with variable phenotypes, and no clinical phenotypes (Docherty et al, 2015 & DJGM, unpublished data). The only consistent feature of probands is MLID itself.

The most extreme manifestation of MLID is caused by maternal-effect mutation of *NLRP7*, and is not compatible with life. Maternal inactivation of *NLRP7* causes hydatidiform mole, an adverse pregnancy outcome resembling overproliferation of placenta without formation of a foetus (Judson et al, 2002, Murdoch et al, 2006). Hydatidiform moles caused by maternal mutations have a normal biparental genomic constitution, but complete loss of maternal imprinting marks.

NLRP genes form a large family in mammals, some of which are critical for humoral immunity, and some (including *NLRP7*, *NLRP2* and *NLRP5*) expressed only in the oocyte and early embryo (Tian et al, 2009). NLRP5 and its associated proteins are referred to as the subcortical maternal complex (SCMC; Li et al, 2008B; Zhu et al, 2015; reviewed in Zhou and Dean, 2015). Human maternal-effect mutations of SCMC genes are associated with a range of adverse pregnancy outcomes including mole and infertility as well as miscarriage and MLID (Meyer et al, 2009; Parry et al, 2011; Caliebe et al, 2014; Docherty et al, 2015; Alazami et al, 2016; Xu et al, 2016). Their precise roles in the embryo are unknown. They are among the most highly expressed genes in the oocyte; but their mRNA and protein abundance decline to undetectable levels by blastulation (Virant-Klun et al, 2016). Pre- and post-ovulatory oocyte ageing are associated with increased rate of decline in transcript levels (Dankert et al, 2014). Loss of function models in mice show a range of adverse effects on the onset of development, including maintenance of genome integrity and ploidy, mitochondrial function, and gene transcription and translation (Tong et al, 2000; Li et al, 2008B; Yurttas et al, 2008; Zheng et al, 2009; Fernandes et al, 2012; Zhao et al, 2016; Mahadevan et al, 2017).

This is striking, because oocyte transcription in humans ceases upon maturation and onset of meiosis (Bouniol-Baly et al, 1999). After fertilisation, the first transcription from the zygote genome is non-canonical, and involves DUX-family transcription factors (de Iaco et al, 2017); embryonic transcription is fully activated only at the 8-cell stage (Braude et al, 1988). Transcription and translation are tightly and independently regulated in the zygote and very early embryo (Nothias et al, 1996; reviewed in Zhou and Dean, 2015). At the same time, the embryo undergoes comprehensive epigenetic remodelling, including passive demethylation of genomic DNA to a minimum level around the time of blastulation (Smith et al, 2012). Embryonic stem cell populations recapitulating this developmental stage likewise undergo passive DNA demethylation, and this is associated with decline in protein levels of DNMT1, the methyltransferase that protects DNA methylation from dilution due to DNA replication (Eckersley-Maslin et al, 2016, von Meyenn et al, 2016).

Taken together, these observations strongly suggest that epigenetic reprogramming and zygotic genome activation (ZGA) must occur on linked timescales to enable the embryo to grow and develop (Figure 2A, 2B). Maternal genetic variations, or environmental effects (including maternal age) – or both – may impede the embryo from co-ordinating these two programmes, and this in turn may lead to a ‘crisis’ in the embryo, with delayed ZGA and degradation of imprinted DNA methylation (Figure 2C). If the zygote is severely compromised, the crisis becomes a catastrophe and the embryo dies – depending on the nature of the compromise, between the 1-cell stage and the blastocyst. But if the embryo survives the crisis, it continues to develop. Ongoing differentiation erases the majority of early epigenetic errors. But imprinting marks are not overwritten, and therefore imprinting disturbance may persist as evidence of an embryonic crisis. Thus, ‘embryo crises’ are predicted to lead to a range of consequences, from embryo death and apparent maternal infertility, through nonviable outcomes such as hydatidiform mole or miscarriage, to imprinting disorders and MLID, and offspring with no imprinting disturbance, or epigenetic disturbance with no apparent phenotypic effect.

It follows that MLID, and arguably the majority of primary imprinting epimutations, are no more nor less than relics of developmental crises in early embryos. The contribution of maternal-effect mutation is hard to estimate, because (a) reproductive problems are not generally quantified or analysed in clinical practice, (b) maternal genomes are not routinely analysed with reference to offspring genetics, and (c) imprinting disturbance is not analysed in the normal or disease population. The role of environmental influences is also hard to estimate. It is well recognised that the frequencies of fertility problems, aneuploidies and upd rise dramatically with increasing maternal age, suggesting compromise of the oocyte under these conditions (Eichenlaub-Ritter, 2012). Moreover, it is recognised that assisted reproductive technology (ART) is associated with adverse outcomes, including growth disturbance (Qin et al, 2016; Luke et al, 2017) and increased frequency of classical imprinting disorders (Cox et al, 2002; Mussa et al, 2017). More comprehensive, large-scale analysis is needed to indicate whether children born after ART have an increased risk of MLID or of non-classical epigenetic disturbance.

## Summary and future directions

Imprinted genes are a small subset of human genes, whose epigenetic control is not re-set in the early embryo, and thus reflects their gamete of origin. Human imprinting disorders (IDs) are caused by inappropriate expression of imprinted genes; either through genetic changes which are heritable, depending on their parental origin, or through chromosomal or epigenetic changes (UPD and imprinting errors) which are normally not heritable. IDs affect growth, development, metabolism and behaviour. They were originally defined as clinical syndromes; but recent developments in molecular genetic and epigenetic diagnosis is bringing about a re-definition of many IDs, and suggesting they may be less rare and more heterogeneous than originally thought. Beyond ‘classical’ syndromes, imprinting effects may be more prevalent than currently recognised, through population-level effects on lifecourse disease, and effects on fetal development, particularly in relation to ART.

Use of epigenomic analysis has expanded the range of known imprinted genes. The same tools are now required to determine whether any are associated with phenotypes that are currently clinically unrecognised, including the fascinating – and presently unknown – possibilities of transient and placental imprinting effects on growth and development. At the same time, integrating studies of animal models and human disease will drive progress in the biology of our early development, as both genetic and epigenetic creatures.

**References**

Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM, Gagliardi PC, Rodrigues T, Kochi C, Longui CA, Beckers D, de Zegher F, Montenegro LR, Mendonca BB, Carroll RS, Hirschhorn JN, Latronico AC, Kaiser UB. Central Precocious Puberty Caused by Mutations in the Imprinted Gene MKRN3. N Engl J Med 2013;368:2467-75. DOI: 10.1056/NEJMoa1302160

Alazami AM, Awad SM, Coskun S, Al-Hassan S, Hijazi H, Abdulwahab FM, Poizat C, Alkuraya FS. TLE6 mutation causes the earliest known human embryonic lethality. Genome Biol. 2015 Nov 5;16:240. doi: 10.1186/s13059-015-0792-0. PubMed PMID: 26537248; PubMed Central PMCID: PMC4634911.

Ali T, Renkawitz R, Bartkuhn M. Insulators and domains of gene expression. Curr Opin Genet Dev. 2016 Apr;37:17-26. doi: 10.1016/j.gde.2015.11.009. Epub 2016 Jan 20. Review. PMID: 26802288

Arand J, Wossidlo M, Lepikhov K, Peat JR, Reik W, Walter J. Selective impairment of methylation maintenance is the major cause of DNA methylation reprogramming in the early embryo. Epigenetics Chromatin. 2015 Jan 9;8(1):1. doi: 10.1186/1756-8935-8-1. eCollection 2015. PMID: 25621012

Azzi S, Rossignol S, Steunou V, Sas T, Thibaud N, Danton F, Le Jule M, Heinrichs C, Cabrol S, Gicquel C, Le Bouc Y, Netchine I. Multilocus methylation analysis in a large cohort of 11p15-related foetal growth disorders (Russell Silver and Beckwith Wiedemann syndromes) reveals simultaneous loss of methylation at paternal and maternal imprinted loci. Hum Mol Genet. 2009 Dec 15;18(24):4724-33. doi: 10.1093/hmg/ddp435. Epub 2009 Sep 14. PMID: 19755383

Azzi S, Blaise A, Steunou S, Harbison MD, Salem J, Brioude F, Rossignol S, Abi Habib W, Thibaud N, Das Neves C, Le Jule M, Brachet C, Heinrichs C, Le Bouc Y, Netchine I. Complex Tissue-Specific Epigenotypes in Russell–Silver Syndrome Associated with 11p15 ICR1 Hypomethylation. Hum Mut. 2014. 35:1211-1220.

Baple EL, Poole RL, Mansour S, Willoughby C, Temple IK, Docherty LE, Taylor R, Mackay DJG. An atypical case of hypomethylation at multiple imprinted loci. Eur J Hum Genet 19:360-362 2011

Barlow DP, Bartolomei MS. Genomic imprinting in mammals. Cold Spring Harb Perspect Biol. 2014 Feb 1;6(2). pii: a018382. doi: 10.1101/cshperspect.a018382.

Benonisdottir S, Oddsson A, Helgason A, Kristjansson RP, Sveinbjornsson G, Oskarsdottir A, Thorleifsson G, Davidsson OB, Arnadottir GA, Sulem G, Jensson BO, Holm H, Alexandersson KF, Tryggvadottir L, Walters GB, Gudjonsson SA, Ward LD, Sigurdsson JK, Iordache PD, Frigge ML, Rafnar T, Kong A, Masson G, Helgason H, Thorsteinsdottir U, Gudbjartsson DF, Sulem P, Stefansson K. Epigenetic and genetic components of height regulation. Nat Commun. 2016 Nov 16;7:13490. doi: 10.1038/ncomms13490. PMID: 27848971

Bliek J, Verde G, Callaway J, Maas S, De Crescenzo A, Sparago A, Cerrato F, Russo S, Ferraiuolo S, Rinaldi M, Fischetto R, Lalatta F, Giordano L, Ferrari P, Cubellis M, Larizza L, Temple IK, Mannens M, Mackay DJG, Riccio A. Hypomethylation at multiple maternally methylated imprinted regions including PLAGL1 and GNAS loci in Beckwith-Wiedemann syndrome. 2008 17:611-619

Bliek J, Alders M, Maas SM, Oostra RJ, Mackay DM, van der Lip K, Callaway JL, Brooks A, van 't Padje S, Westerveld A, Leschot NJ, Mannens MM. Lessons from BWS twins: complex maternal and paternal hypomethylation and a common source of haematopoietic stem cells. Eur J Hum Genet. 2009 17:1625-34.

Boonen SE, Mackay DJ, Hahnemann JM, Docherty L, Grønskov K, Lehmann A, Larsen LG, Haemers AP, Kockaerts Y, Dooms L, Vu DC, Ngoc CT, Nguyen PB, Kordonouri O, Sundberg F, Dayanikli P, Puthi V, Acerini C, Massoud AF, Tümer Z, Temple IK. Transient neonatal diabetes, ZFP57 and hypomethylation of multiple imprinted loci: a detailed follow-up. Diabetes Care. 2013 Mar;36(3):505-12 PMID: 23150280

Bouniol-Baly C, Hamraoui L, Guibert J, Beaujean N, Szöllösi MS, Debey P. Differential Transcriptional Activity Associated with Chromatin Configuration in Fully Grown Mouse Germinal Vesicle Oocytes. 1999. Biol Reprod 60, 580–587.

Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988 Mar 31;332(6163):459-61. PMID: 3352746

Buiting K. Prader–Willi Syndrome and Angelman Syndrome. Am J Med Genet. 2010. 154C:365–376.

Caliebe A, Richter J, Ammerpohl O, Kanber D, Beygo J, Bens S, Haake A, Jüttner E, Korn B, Mackay DJ, Martin-Subero JI, Nagel I, Sebire NJ, Seidmann L, Vater I, von Kaisenberg CS, Temple IK, Horsthemke B, Buiting K, Siebert R. A familial disorder of altered DNA-methylation. J Med Genet. 2014 Jun;51(6):407-12. doi: 10.1136/jmedgenet-2013-102149. PMID: 24721835

Chotalia M, Smallwood SA, Ruf N, Dawson C, Lucifero D, Frontera M, James K, Dean W, Kelsey G. Transcription is required for establishment of germline methylation marks at imprinted genes. Genes Dev. 2009 Jan 1;23(1):105-17. doi: 10.1101/gad.495809.

Choufani S, Shuman C, Weksberg R. Beckwith-Wiedemann syndrome. Am J Med Genet. 2010. 154C:343–354.

Court F, Martin-Trujillo A, Romanelli V, Garin I, Iglesias-Platas I, Salafsky I, Guitart M, Perez de Nanclares G, Lapunzina P, Monk D. Genome-wide allelic methylation analysis reveals disease-specific susceptibility to multiple methylation defects in imprinting syndromes. Hum Mutat. 2013 Apr;34(4):595-602

Cox GF, Bürger J, Lip V, Mau UA, Sperling K, Wu BL, Horsthemke B. Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet. 2002 Jul;71(1):162-4.

Dang-Nguyen TQ, Torres-Padilla ME. How cells build totipotency and pluripotency: nuclear, chromatin and transcriptional architecture. Curr Opin Cell Biol. 2015 Jun;34:9-15. doi: 10.1016/j.ceb.2015.04.006. Review. PubMed PMID: 25935759.

Dankert D, Demond H, Trapphoff T, Heiligentag M, Rademacher K, Eichenlaub-Ritter U, Horsthemke B, Grümmer R. Pre- and postovulatory aging of murine oocytes affect the transcript level and poly(A) tail length of maternal effect genes. PLoS One. 2014 Oct 1;9(10):e108907. doi: 10.1371/journal.pone.0108907. PubMed PMID: 25271735; PubMed Central PMCID: PMC4182777.

De Iaco A, Planet E, Coluccio A, Verp S, Duc J, Trono D. DUX-family transcription factors regulate zygotic genome activation in placental mammals. Nat Genet. 2017 Jun;49(6):941-945. doi: 10.1038/ng.3858. Epub 2017 May 1. PMID: 28459456

Docherty LE, Rezwan FI, Poole RL, Jagoe HT, Lake HA, Lockett G, Wilson DI, Holloway J, Temple IK, Mackay DJG, Genome-wide DNA methylation analysis of patients with imprinting disorders identifies differentially methylated regions associated with novel candidate imprinted genes. J Med Genet. 2014 Apr;51(4):229-38.

Docherty LE, Rezwan FI, Poole RL, Turner CL, Kivuva E, Maher ER, Smithson SF, Hamilton-Shield JP, Patalan M, Gizewska M, Peregud-Pogorzelski J, Beygo J, Buiting K, Horsthemke B, Soellner L, Begemann M, Eggermann T, Baple E, Mansour S, Temple IK, Mackay DJ. Mutations in NLRP5 are associated with reproductive wastage and multilocus imprinting disorders in humans. Nat Commun. 2015 Sep 1;6:8086. doi: 10.1038/ncomms9086. PMID: 26323243

Duffié R, Ajjan S, Greenberg MV, Zamudio N, Escamilla del Arenal M, Iranzo J, Okamoto I, Barbaux S, Fauque P, Bourc'his D. The Gpr1/Zdbf2 locus provides new paradigms for transient and dynamic genomic imprinting in mammals. Genes Dev. 2014 Mar 1;28(5):463-78. doi: 10.1101/gad.232058.113. PMID: 24589776

Eckersley-Maslin MA, Svensson V, Krueger C, Stubbs TM, Giehr P, Krueger F, Miragaia RJ, Kyriakopoulos C, Berrens RV, Milagre I, Walter J, Teichmann SA, Reik W. MERVL/Zscan4 Network Activation Results in Transient Genome-wide DNA Demethylation of mESCs. Cell Rep. 2016 Sep 27;17(1):179-92. doi: 10.1016/j.celrep.2016.08.087. PubMed PMID: 27681430; PubMed Central PMCID: PMC5055476.

Eggermann T. Russell-Silver syndrome. Am J Med Genet. 2010. 154C:355–364.

Eggermann T, Perez de Nanclares G, Maher ER, Temple IK, Tümer Z, Monk D, Mackay DJ, Grønskov K, Riccio A, Linglart A, Netchine I. Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. Clin Epigenetics. 2015 Nov 14;7:123. doi: 10.1186/s13148-015-0143-8. Review. PubMed PMID: 26583054; PubMed Central PMCID: PMC4650860.

Eichenlaub-Ritter U. Oocyte ageing and its cellular basis. Int J Dev Biol. 2012;56(10-12):841-52. doi: 10.1387/ijdb.120141ue. Review. PMID: 23417406

Elli FM, Linglart A, Garin I, de Sanctis L, Bordogna P, Grybek V, Pereda A, Giachero F, Verrua E, Hanna P, Mantovani G, Perez de Nanclares G. The Prevalence of GNAS Deficiency-Related Diseases in a Large Cohort of Patients Characterized by the EuroPHP Network. J Clin Endocrinol Metab 16; 101: 3657–3668.

Fernandes R, Tsuda C, Perumalsamy AL, Naranian T, Chong J, Acton BM, Tong ZB, Nelson LM, Jurisicova A. NLRP5 mediates mitochondrial function in mouse oocytes and embryos. Biol Reprod. 2012 May 3;86(5):138, 1-10. doi: 10.1095/biolreprod.111.093583. Print 2012 May. PMID: 22357545

Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol. 2013 Nov;14(11):699-712. doi: 10.1038/nrm3679. Epub 2013 Oct 9. Review. PMID: 24105322

Gonzalez-Sandoval A, Gasser SM. On TADs and LADs: Spatial Control Over Gene Expression. Trends Genet. 2016 Aug;32(8):485-95. doi: 10.1016/j.tig.2016.05.004. Epub 2016 Jun 13. Review. PMID: 27312344

Hanna CW, Peñaherrera MS, Saadeh H, Andrews S, McFadden DE, Kelsey G, Robinson WP. Pervasive polymorphic imprinted methylation in the human placenta. Genome Res. 2016 Jun;26(6):756-67. doi: 10.1101/gr.196139.115. Epub 2016 Jan 14. PMID: 26769960

He Y, Ecker JR. Non-CG Methylation in the Human Genome. Annu Rev Genomics Hum Genet. 2015;16:55-77. doi: 10.1146/annurev-genom-090413-025437. Epub 2015 Jun 4. Review. PMID: 26077819

Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN et al. Genome-wide associations for birth weight and correlations with adult disease. Nature. 2016 Oct 13;538(7624):248-252. doi: 10.1038/nature19806. Epub 2016 Sep 28.

PMID: 27680694.

Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet. 2014 Jun 2. pii: jmedgenet-2014-102396. doi: 10.1136/jmedgenet-2014-102396.

Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003 Mar;33 Suppl:245-54. Review. PMID: 12610534

Judson H, Hayward BE, Sheridan E, Bonthron DT. A global disorder of imprinting in the human female germ line. Nature. 2002. 416:539-542.

Kagami M, Mizuno S, Matsubara K, Nakabayashi K, Sano S, Fuke T, Fukami M, Ogata T. Epimutations of the IG‑DMR and the MEG3‑DMR at the 14q32.2 imprinted region in two patients with Silver–Russell syndrome-compatible phenotype. Eur. J. Hum. Genet. 23, 1062–1067 (2015).

Kagami M, Matsubara K, Nakabayashi K, Nakamura A, Sano S, Okamura K, Hata K, Fukami M, Ogata T. Genome-wide multilocus imprinting disturbance analysis in Temple syndrome and Kagami-Ogata syndrome. Genet Med. 2017 Apr;19(4):476-482. doi: 10.1038/gim.2016.123. Epub 2016 Sep 15. PMID: 27632690

Kelsey G, Feil R. New insights into establishment and maintenance of DNA methylation imprints in mammals. Philos Trans R Soc Lond B Biol Sci. 2013 Jan 5;368(1609):20110336. doi: 10.1098/rstb.2011.0336.

Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, Peshkin L, Weitz DA, Kirschner MW. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. Cell. 2015 May 21;161(5):1187-201. doi: 10.1016/j.cell.2015.04.044.

Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, Jonasdottir A, Sigurdsson A, Kristinsson KT, Jonasdottir A, Frigge ML, Gylfason A, Olason PI, Gudjonsson SA, Sverrisson S, Stacey SN, Sigurgeirsson B, Benediktsdottir KR, Sigurdsson H, Jonsson T, Benediktsson R, Olafsson JH, Johannsson OT, Hreidarsson AB, Sigurdsson G; DIAGRAM Consortium, Ferguson-Smith AC, Gudbjartsson DF, Thorsteinsdottir U, Stefansson K. Parental origin of sequence variants associated with complex diseases. Nature. 2009 Dec 17;462(7275):868-74. doi: 10.1038/nature08625. PMID: 20016592

Roadmap Epigenomics Consortium, Kundaje A et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015 Feb 19;518(7539):317-30. doi: 10.1038/nature14248. PMID: 25693563.

Lee MT, Bonneau AR, Giraldez AJ. Zygotic genome activation during the maternal-to-zygotic transition. Annu Rev Cell Dev Biol. 2014;30:581-613. doi: 10.1146/annurev-cellbio-100913-013027. Review. PubMed PMID: 25150012; PubMed Central PMCID: PMC4303375.

Le Fevre A, Beygo J, Silveira C, Kamien B, Clayton-Smith J, Colley A, Buiting K, Dudding-Byth T. Atypical Angelman Syndrome Due to a Mosaic Imprinting Defect: Case Reports and Review of the Literature. 2017. Am J Med Genet Part A 173A:753–757.

Li X, Ito M, Zhou F, Youngson N, Zuo X, Leder P, Ferguson-Smith AC. A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. Dev Cell. **2008A** Oct;15(4):547-57. doi: 10.1016/j.devcel.2008.08.014. PubMed PMID: 18854139; PubMed Central PMCID: PMC2593089.

Li L, Baibakov B, Dean J. A subcortical maternal complex essential for preimplantation mouse embryogenesis. Dev Cell. **2008B** Sep;15(3):416-25. doi: 10.1016/j.devcel.2008.07.010. PubMed PMID: 18804437; PubMed Central PMCID: PMC2597058.

Li W, Notani D, Rosenfeld MG. Enhancers as non-coding RNA transcription units: recent insights and future perspectives. Nat Rev Genet. 2016 Apr;17(4):207-23. doi: 10.1038/nrg.2016.4. Epub 2016 Mar 7. Review. PMID: 26948815

Luke B, Brown MB, Wantman E, Stern JE, Toner JP, Coddington CC 3rd. Increased risk of large-for-gestational age birthweight in singleton siblings conceived with in vitro fertilization in frozen versus fresh cycles. J Assist Reprod Genet. 2017 Feb;34(2):191-200. doi: 10.1007/s10815-016-0850-x. Epub 2016 Dec 1. PMID: 27909843

Mackay DJG, Boonen SE, Clayton-Smith J, Goodship J, Hahnemann JMD, Kant SG, Njølstad PR, Robin NH, Robinson DO, Siebert R, Shield JPH, White HE, Temple IK. A maternal hypomethylation syndrome presenting as transient neonatal diabetes mellitus. 2006. Hum Gen 120:262-269.

Mackay DJ, Callaway JL, Marks SM, White HE, Acerini CL, Boonen SE, Dayanikli P, Firth HV, Goodship JA, Haemers AP, Hahnemann JM, Kordonouri O, Masoud AF, Oestergaard E, Storr J, Ellard S, Hattersley AT, Robinson DO, Temple IK. Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. Nat Genet. 2008 Aug;40(8):949-51. doi: 10.1038/ng.187. PubMed PMID: 18622393.

Mackay DJ, Temple IK. Transient neonatal diabetes mellitus type 1. Am J Med Genet C Semin Med Genet. 2010 Aug 15;154C(3):335-42. doi: 10.1002/ajmg.c.30272. Review. PMID: 20803656

Mahadevan S, Sathappan V, Utama B, Lorenzo I, Kaskar K, Van den Veyver IB. Maternally expressed NLRP2 links the subcortical maternal complex (SCMC) to fertility, embryogenesis and epigenetic reprogramming. Sci Rep. 2017 Mar 20;7:44667. doi: 10.1038/srep44667. PMID: 28317850

Mantovani G, Spada A, Elli FM. Pseudohypoparathyroidism and Gsα–cAMP-linked disorders: current view and open issues. Nat Rev Endo. 2016. 12:347-356 doi:10.1038/nrendo.2016.52.

Meyer E, Lim D, Pasha S, Tee LJ, Rahman F, Yates JR, Woods CG, Reik W, Maher ER. Germline mutation in NLRP2 (NALP2) in a familial imprinting disorder (Beckwith-Wiedemann Syndrome). PLoS Genet. 2009 Mar;5(3):e1000423. doi: 10.1371/journal.pgen.1000423.PMID: 19300480

Mulchandani S, Bhoj EJ, Luo M, Powell-Hamilton N, Jenny K, Gripp KW, Elbracht M, Eggermann T, Turner CL, Temple IK, Mackay DJ, Dubbs H, Stevenson DA, Slattery L, Zackai EH, Spinner NB, Krantz ID, Conlin LK. Maternal uniparental disomy of chromosome 20: a novel imprinting disorder of growth failure. Genet Med. 2015 Aug 6. doi: 10.1038/gim.2015.103.

Murdoch S, Djuric U, Mazhar B, Seoud M, Khan R, Kuick R, Bagga R, Kircheisen R, Ao A, Ratti B, Hanash S, Rouleau GA, Slim R. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. Nat Genet. 2006 Mar;38(3):300-2. PubMed PMID: 16462743.

Mussa A, Molinatto C, Cerrato F, Palumbo O, Carella M, Baldassarre G, Carli D, Peris C, Riccio A, Ferrero GB. Assisted Reproductive Techniques and Risk of Beckwith-Wiedemann Syndrome. Pediatrics. 2017 Jul;140(1). pii: e20164311. doi: 10.1542/peds.2016-4311. Epub 2017 Jun 20. PMID: 28634246

Nothias JY, Miranda M, DePamphilis ML. Uncoupling of transcription and translation during zygotic gene activation in the mouse. EMBO J. 1996 Oct 15;15(20):5715-25. PubMed PMID: 8896464; PubMed Central PMCID: PMC452315.

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

Parry DA, Logan CV, Hayward BE, Shires M, Landolsi H, Diggle C, Carr I, Rittore C, Touitou I, Philibert L, Fisher RA, Fallahian M, Huntriss JD, Picton HM, Malik S, Taylor GR, Johnson CA, Bonthron DT, Sheridan EG. Mutations causing familial biparental hydatidiform mole implicate c6orf221 as a possible regulator of genomic imprinting in the human oocyte. Am J Hum Genet. 2011 Sep 9;89(3):451-8. doi: 10.1016/j.ajhg.2011.08.002. PubMed PMID: 21885028; PubMed Central PMCID: PMC3169823.

Poole RL, Docherty LE, Al Sayegh A, Caliebe A, Turner C, Baple E, Wakeling E, Harrison L, Lehmann A, Temple IK, Mackay DJ; On behalf of the International Clinical Imprinting Consortium. Targeted methylation testing of a patient cohort broadens the epigenetic and clinical description of imprinting disorders. Am J Med Genet A. 2013 Aug 2. doi: 10.1002/ajmg.a.36049.

Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. Fertil Steril. 2016 Jan;105(1):73-85.e1-6. doi: 10.1016/j.fertnstert.2015.09.007. Epub 2015 Oct 9. Review. PMID: 26453266

Quenneville S, Verde G, Corsinotti A, Kapopoulou A, Jakobsson J, Offner S, Baglivo I, Pedone PV, Grimaldi G, Riccio A, Trono D. In embryonic stem cells, ZFP57/KAP1 recognize a methylated hexanucleotide to affect chromatin and DNA methylation of imprinting control regions. Mol Cell. 2011 Nov 4;44(3):361-72. doi: 10.1016/j.molcel.2011.08.032. PubMed PMID: 22055183; PubMed Central PMCID:PMC3210328.

Russo S, Calzari L, Mussa A, Mainini E, Cassina M, Di Candia S, Clementi M, Guzzetti S, Tabano S, Miozzo M, Sirchia S, Finelli P, Prontera P, Maitz S, Sorge G, Calcagno A, Maghnie M, Divizia MT, Melis D, Manfredini E, Ferrero GB, Pecile V, Larizza L. A multi-method approach to the molecular diagnosis of overt and borderline 11p15.5 defects underlying Silver-Russell and Beckwith-Wiedemann syndromes. Clin Epigenetics. 2016 Mar 1;8:23. doi: 10.1186/s13148-016-0183-8. eCollection 2016. Erratum in: Clin Epigenetics. 2016;8:40. PMID: 26933465

Sanchez-Delgado M, Court F, Vidal E, Medrano J, Monteagudo-Sánchez A, Martin-Trujillo A, Tayama C, Iglesias-Platas I, Kondova I, Bontrop R, Poo-Llanillo ME, Marques-Bonet T, Nakabayashi K, Simón C, Monk D. Human Oocyte-Derived Methylation Differences Persist in the Placenta Revealing Widespread Transient Imprinting. PLoS Genet. **2016A** Nov 11;12(11):e1006427. doi: 10.1371/journal.pgen.1006427. eCollection 2016 Nov. PMID: 27835649

Sanchez-Delgado M, Riccio A, Eggermann T, Maher ER, Lapunzina P, Mackay D, Monk D. Causes and Consequences of Multi-Locus Imprinting Disturbances in Humans. Trends Genet. **2016B** Jul;32(7):444-55. doi: 10.1016/j.tig.2016.05.001. Review. PubMed PMID: 27235113.

Schaaf CP, Gonzalez-Garay ML, Xia F, et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. Nat Genet 2013;45:1405–1408.

Schübeler D. Function and information content of DNA methylation. Nature. 2015 Jan 15;517(7534):321-6. doi: 10.1038/nature14192. Review. PMID: 25592537

Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, Popp C, Thienpont B, Dean W, Reik W. The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. Mol Cell. 2012 Dec 28;48(6):849-62. doi: 10.1016/j.molcel.2012.11.001. Epub 2012 Dec 6. PMID: 23219530

Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G. Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet. 2011 Jun 26;43(8):811-4. doi: 10.1038/ng.864. PubMed PMID: 21706000; PubMed Central PMCID: PMC3146050.

Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, Meissner A. A unique regulatory phase of DNA methylation in the early mammalian embryo. Nature. 2012 Mar 28;484(7394):339-44. doi: 10.1038/nature10960. PubMed PMID: 22456710; PubMed Central PMCID: PMC3331945.

Strogantsev R, Krueger F, Yamazawa K, Shi H, Gould P, Goldman-Roberts M, McEwen K, Sun B, Pedersen R, Ferguson-Smith AC. Allele-specific binding of ZFP57 in the epigenetic regulation of imprinted and non-imprinted monoallelic expression. Genome Biol. 2015 May 30;16:112. doi: 10.1186/s13059-015-0672-7. PubMed PMID: 26025256; PubMed Central PMCID: PMC4491874.

Su Z, Denu JM. Reading the Combinatorial Histone Language. ACS Chem Biol. 2016 Mar 18;11(3):564-74. doi: 10.1021/acschembio.5b00864. Epub 2015 Dec 21. Review. PMID: 26675328

Tian X, Pascal G, Monget P. Evolution and functional divergence of NLRP genes in mammalian reproductive systems. BMC Evol Biol. 2009 Aug 14;9:202. doi: 10.1186/1471-2148-9-202. PubMed PMID: 19682372; PubMed Central PMCID: PMC2735741.

Tong ZB, Gold L, Pfeifer KE, Dorward H, Lee E, Bondy CA, Dean J, Nelson LM. Mater, a maternal effect gene required for early embryonic development in mice. Nat Genet. 2000 Nov;26(3):267-8. PubMed PMID: 11062459.

Virant-Klun I, Leicht S, Hughes C, Krijgsveld J. Identification of Maturation-Specific Proteins by Single-Cell Proteomics of Human Oocytes. Mol Cell Proteomics. 2016 Aug;15(8):2616-27. doi: 10.1074/mcp.M115.056887. PubMed PMID: 27215607; PubMed Central PMCID: PMC4974340.

von Meyenn F, Iurlaro M, Habibi E, Liu NQ, Salehzadeh-Yazdi A, Santos F, Petrini E, Milagre I, Yu M, Xie Z, Kroeze LI, Nesterova TB, Jansen JH, Xie H, He C, Reik W, Stunnenberg HG. Impairment of DNA Methylation Maintenance Is the Main Cause of Global Demethylation in Naive Embryonic Stem Cells. Mol Cell. 2016 Jun 16;62(6):983. doi: 10.1016/j.molcel.2016.06.005.

Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Bliek J, Canton AP, Chrzanowska KH, Davies JH, Dias RP, Dubern B, Elbracht M, Giabicani E, Grimberg A, Grønskov K, Hokken-Koelega AC, Jorge AA, Kagami M, Linglart A, Maghnie M, Mohnike K, Monk D, Moore GE, Murray PG, Ogata T, Petit IO, Russo S, Said E, Toumba M, Tümer Z, Binder G, Eggermann T, Harbison MD, Temple IK, Mackay DJ, Netchine I. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. Nat Rev Endocrinol. 2016 Sep 2. doi: 10.1038/nrendo.2016.138

Weksberg R, Shuman C, Caluseriu O Smith AC, Fei Y, Nishikawa J, Stockley TL, Best L, Chitayat D, Olney A, Ives E, Schneider A, Bestor TH, Li M, Sadowski P, Squire D. Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith –Wiedemann syndrome. Hum Mol Genet 2002; 15:1317– 1325.

Xu Y, Shi Y, Fu J, Yu M, Feng R, Sang Q, Liang B, Chen B, Qu R, Li B, Yan Z, Mao X, Kuang Y, Jin L, He L, Sun X, Wang L. Mutations in PADI6 Cause Female Infertility Characterized by Early Embryonic Arrest. Am J Hum Genet. 2016 Sep 1;99(3):744-52. doi: 10.1016/j.ajhg.2016.06.024. PubMed PMID: 27545678; PubMed Central PMCID: PMC5010645.

Yurttas P, Vitale AM, Fitzhenry RJ, Cohen-Gould L, Wu W, Gossen JA, Coonrod SA. Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. Development. 2008 Aug;135(15):2627-36. doi: 10.1242/dev.016329. Epub 2008 Jul 3. PMID: 18599511

Zhao B, Zhang WD, Duan YL, Lu YQ, Cun YX, Li CH, Guo K, Nie WH, Li L, Zhang R, Zheng P. Filia Is an ESC-Specific Regulator of DNA Damage Response and Safeguards Genomic Stability. Cell Stem Cell. 2015 Jun 4;16(6):684-98. doi: 10.1016/j.stem.2015.03.017. Epub 2015 Apr 30. PMID: 25936915

Zheng P, Dean J. Role of Filia, a maternal effect gene, in maintaining euploidy during cleavage-stage mouse embryogenesis. Proc Natl Acad Sci U S A. 2009 May 5;106(18):7473-8. doi: 10.1073/pnas.0900519106. Epub 2009 Apr 17. PMID: 19376971

Zhou LQ, Dean J. Reprogramming the genome to totipotency in mouse embryos. Trends Cell Biol. 2015 Feb;25(2):82-91. doi: 10.1016/j.tcb.2014.09.006. Review. PubMed PMID: 25448353; PubMed Central PMCID: PMC4312727.

Zhu K, Yan L, Zhang X, Lu X, Wang T, Yan J, Liu X, Qiao J, Li L. Identification of a human subcortical maternal complex. Mol Hum Reprod. 2015 Apr;21(4):320-9. doi: 10.1093/molehr/gau116. PubMed PMID: 25542835.

Figure Legends

**Figure 1: Epigenetic reprogramming during reproduction.**

**A: Somatic epigenetic marks.** In cell populations destined to become germ cells, epigenetic marks (including DNA methylation) are removed to reach an epigenetic ‘clean slate’. The developing eggs and sperm then establish divergent epigenetic marks, reflecting their very different developmental programmes. At fertilisation, the sperm genome enters the egg. The newly-formed zygote undergoes epigenetic reprogramming during zygotic cleavage stages: paternal DNA methylation is removed principally by active demethylation in the one-cell embryo, while successive cell divisions dilute maternal DNA methylation. Reaching a minimum in late cleavage stages, DNA methylation then evolves through subsequent differentiation to re-establish normal somatic patterns.

**B: Imprinted epigenetic marks.** Germline epigenetic reprogramming proceeds as for all other epigenetic marks. However, after fertilisation, imprints evade zygotic reprogramming: they are not removed in the zygote, and not overwritten by subsequent developmental marks. Thus they retain an epigenetic ‘memory’ of their parent of origin.

**Figure 2: Hypothesis: epigenetic reprogramming, zygotic genome activation and embyro crisis.**

**A:** The five cartoons represent the growth of the embryo from a fertilised one-cell zygote, through the 2-cell and 8-cell embryo, and the morula, to the blastocyst, where different cell lineages of trophectoderm and inner cell mass are well established.

**B**: The schematic represents the changes in transcription (black lines) and DNA methylation (as a proxy for epigenetic reprogramming, green lines) in a healthy embryo. Most DNA methylation (solid green line) is progressively lost in cleavage stages, reaching a minimum around the time of blastulation, but then re-established in tissue-specific patterns. Imprinted DNA methylation (dashed green line) is unaffected. After a transient burst of non-canonical transcription mediated by pioneer factors (dashed black line) the zygote genome is activated from the 8-cell stage.

**C**: The schematic hypothesises an embryo crisis resulting from a genetic or environmental lesion. In this hypothetical crisis, onset of transcription is delayed. As a result, maternal factors required to maintain imprinting become insufficient, and imprinting is progressively degraded. If this crisis is prolonged, epigenetic dysregulation or delayed zygotic genome activation may lead to embryo death. If the embryo survives the crisis, it may have mosaic loss of imprinted DNA methylation, with or without other developmental dysregulation.

Table 1: Imprinting disorders

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Disorder | chromosome(s) | prevalence | OMIM | % genetic error(SNV / CNV)  | % chromosomal error (UPD) | % imprinting error (% MLID) | references |
| Angelman Syndrome | 15q11.2 | 1:15000 | #105830 | 70% CNV (del15mat)15% SNV (*UBE3A*) | <5% (upd15pat) | <5% (rare) | Buiting, 2010 |
| Prader-Willi syndrome | 15q11.2 | 1:15000 | #176270 | 70% CNV (del15pat) | <30% (upd15mat) | <1% (nk) | Buiting, 2010 |
| Beckwith-Wiedemann syndrome | 11p15.5 | 1:10500 | #130650 | 5% SNV (*CDKN1C*)<5% CNV and SNV of H19/IGF2 IG-DMR | 20%  | 10% H19/IGF2 IG-DMR hypermethylation (rare)60% KCNQ1OT1 TSS-DMR hypomethylation (30%) | Choufani et al, 2010 |
| Silver-Russell syndrome | 11p15.5, chr7 | 1:50000? | #180860 | <1% | 10% (upd7mat)<1% (upd11mat) | 40% (15-38%)  | Eggermann 2010, wakeling et al, 2016 |
| Pseudohypoparathyroidism type 1b | 20q13.3 | ? | #603233 | 27% CNV (delSTX16mat)3% SNV (GNAS) | 5% (upd20mat) | 61% (rare) | Mantovani et al, 2016; Elli et al, 2016 |
| Transient neonatal diabetes mellitus type 1 | 6q24 | 1:300000 | #601410 | 40% CNV (dup6pat) | 40% (upd6pat) | 20% (50%) | Mackay and Temple, 2010 |
| Kagami-Ogata syndrome | 14q32 | ? | #608140 | 15% CNV (del14mat) | 65% (upd14pat) | 20% (nk) | Ogata et al, 2016; Kagami et al, 2017 |
| Temple syndrome | 14q32 | ? | #616222 | 10% CNV (del14pat) | 78% (upd14mat) | 12% (rare) | Ioannides et al, 2014; Kagami et al, 2017 |
| Mulchandani-Bhoj-Conlin syndrome | chr20 | ? | #617352 | nk | 100% (upd20mat) | nk (nk) | Mulchandani et al, 2016 |
| Schaaf-Yang syndrome | chr15 | ? | #615547 | 100% inactivation of *MAGEL2* (SNV / CNV) | - | - | Fountain et al, 2017 |
| Central precocious puberty 2 (CPPB2) | chr15 | ? | #615436 | 100% inactivation of *MKRN3* (SNV) | - | - | Abreu et al, 2013 |

nk: not known; SNV: single nucleotide variant; CNV: copy number variant; upd: uniparental disomy. References here are reviews of individual imprinting disorders. References specifically concerning the frequency of multi-locus imprinting disorder in each IDs may be found in Sanchez-Delgado et al 2016; only reports of MLID post-dating this review are cited in this table.

OMIM: Online Mendelian Inheritance in Man (<http://omim.org)>.