NLRPs, the subcortical maternal complex and genomic imprinting

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Abstract

Before activation of the embryonic genome, the oocyte provides many of the RNAs and proteins required for the epigenetic reprogramming and the transition to a totipotent state. Targeted disruption of a subset of oocyte-derived transcripts in mice results in early embryonic lethality and cleavage-stage embryonic arrest as highlighted by the members of the subcortical maternal complex (SCMC). Maternal-effect recessive mutations of *NLRP7*, *KHDC3L* and *NLRP5* in humans are associated with variable reproductive outcomes, biparental hydatidiform moles (BiHM) and widespread multi-locus imprinting disturbances. The precise mechanism of action of these genes is unknown, but the maternal-effect phenomenon suggests a function during early pre-implantation development, while biochemical and genetic studies implement them as SCMC members or interacting partners. In this review article, we discuss the role of the NLRP family members and the SCMC proteins in the establishment of genomic imprints and post-zygotic methylation maintenance, the recent advances made in the understanding of the biology involved in BiHM formation and the wider roles of the SCMC in mammalian reproduction.

Reproduction (2017) **154** R161–R170

Oocyte development

During folliculogenesis, the growth of the oocytes is linked to the proliferation and differentiation of the surrounding granulosa cells (Fig. 1). Follicle development is a prolonged and complex process during which time female primordial germ cells enter meiosis and arrest at the diplotene stage of meiotic prophase I. These primary oocytes, contained within the primordial follicle, then periodically enter the growth phase in response to the luteinizing hormone surge, which culminates in meiotic maturation, expulsion of the first polar body and ovulation. Meiosis is then resumed and finally completed when a sperm penetrates the oocyte at fertilization, which triggers Ca²⁺-induced cyclin degradation allowing for the completion of meiosis II and the exclusion of the second polar body (Amleh & Dean 2002). During the process of oogenesis, there is continual cross-talk between the nucleus and cytoplasm, which is essential for oocyte maturation, epigenetic patterning and the developmental potential of pre-implantation embryo. In addition, there is the accumulation of maternal proteins that are of importance not only for the oocyte, but also for the cellular processes that occur before embryonic genome activation (EGA) (Table 1 for full list of abbreviations) during the cleavage stages of pre-implantation development (Clift & Schuh 2013).

Methylation acquisition in the oocyte

The correct establishment of DNA methylation is essential for the successful development of the embryo following fertilization, primarily due to the necessity for epigenetic marking of genomic imprints (Monk 2015). Following the widespread epigenetic erasure that occurs in the primordial germ cells, oocytes acquire a unique methylation landscape compared to somatic cells (Smallwood *et al.* 2011, Kobayashi *et al.* 2012, Sanchez-Delgado *et al.* 2016). Since many methylated domains are located in genes expressed in the oocyte, many of which are solely observed in the developing oocyte, the process of transcription is thought to be a prerequisite for methylation establishment (Chotalia *et al.* 2009, Veselovsha *et al.* 2015). Data from mice have shown that DNA methylation is fully established in the germinal vesicle stage-ovulated metaphase II oocytes, but that there is a degree of asynchrony at earlier stages (Gahurova *et al.* 2017). Such asynchrony may reflect the requirement for specific factors in addition to the core *de novo* complex comprising DNA methyltransferase 3A (DNMT3A) and its obligated partner DNMT3-like (DNMT3L) (Bourc'his *et al.* 2001, Kaneda *et al.* 2004). The acquisition of *de novo* methylation in the female germline depends upon the absence of H3K4 methylation (Ooi *et al.* 2007), as

Figure 1 Overview of oocyte maturation and early embryo development in humans. From birth the ovaries contain a decreasing number of immature primordial follicles that each contain an immature primary oocyte. These oocytes have undergone meiotic DNA replication, are arrested in meiotic prophase and are surrounded by somatic cells. Sporadically, some of these primordial follicles initiate a prolonged growth phase in which the supporting cells proliferate and oocytes increase in size, accumulating the maternal factors necessary to support the early embryo. From this time onwards, DNA methylation imprints are progressively established. Every menstrual cycle, in response to the gonadotropin surge, meiosis resumes to metaphase II resulting in a mature oocyte. Meiosis is only completed on fertilization, which initiates a cascade of events in the early embryo that includes mitotic cleavage division and embryonic genome activation.

Table 1 A list of abbreviations used in this review.

Abbreviation	Description
5mC	5-Methylcytosine
5hmC	5-Hydromethylcytosine
BiHM	Biparental hydatidiform moles
CHM	Complete hydatidiform moles
EGA	Embryonic genome activation
FRHM	Familial recurrent hydatidiform moles
H3K	Histone 3 lysine
IVF	<i>In vitro</i> fertilization
ICSI	Intracytoplasmic sperm injection
MLID	Multi-locus imprinting disturbance
SCMC	Subcortical maternal complex
Gene abbreviation	Gene name
<i>CDKN1C</i>	Cyclin-dependent kinase inhibitor 1C
<i>DNMTs</i>	DNA methyltransferases
<i>DPPA3</i>	Developmental pluripotency associated 3
<i>GNAS</i>	Guanine nucleotide binding protein
<i>IGF1R</i>	Insulin-like growth factor 1 receptor
<i>KCNQ1OT1</i>	KCNQ1 Opposite Strand/Antisense Transcript 1
<i>KDM1B</i>	Lysine-specific histone demethylase 1B
<i>KHDC3L</i>	KH Domain-Containing 3-Like
<i>MEST</i>	Mesoderm-Specific Transcript
<i>NAP1L5</i>	Nucleosome Assembly Protein 1 Like 5
<i>NLRP</i> family	NLR family, Pyrin domain containing
<i>OOEP</i>	Oocyte Expressed Protein
<i>PEG3</i>	Paternally expressed gene 3
<i>PEG10</i>	Paternally expressed gene 10
<i>RB1</i>	Retinoblastoma 1
<i>SNRPN</i>	Small Nuclear Ribonucleoprotein Polypeptide N
<i>TETs</i>	10–11 translocation proteins
<i>TLE6</i>	Transducin-Like Enhancer of Split 6
<i>TRIM28</i>	Tripartite motif containing 28
<i>YY1</i>	Yin Yang 1
<i>ZBTB16</i>	Zinc finger and BTB domain containing 16
<i>ZFP57</i>	Zinc finger protein 57

revealed by the essential role of lysine-specific histone demethylase 1B (KDM1B) (Ciccone *et al.* 2009), and probably the deposition of H3K36me3 (Baubec *et al.* 2015), a histone modification associated with the elongating PolIII complex. It is currently unknown if the mechanism of methylation establishment is the same in humans as several differences have been observed. For example, despite DNMT3L having no catalytic

activity, this co-factor is essential for the establishment of all germline-derived maternally methylated imprinted regions in mice, but DNMT3L is not expressed in human oocytes (Petrussa *et al.* 2014) (Table 2). It is therefore assumed that methylated loci in human oocytes recruit DNMT3A directly via their PWWP domain that recognizes H3K36me3 (Dhayalan *et al.* 2010).

Genomic imprinting

Genomic imprinting refers to the epigenetic mechanism that results in the parent-of-origin monoallelic expression of autosomal genes (Monk 2015). It is a particularly unique epigenetic mechanism, since an individual somatic cell has both active and repressed alleles of the same gene. Imprinted differentially methylated regions (DMRs) are established in the male and female gametes. However, the specific aspect that makes imprinted DMRs unique is not their germline status, but their ability to maintain parent-of-origin allelic methylation during pre-implantation reprogramming (Smallwood *et al.* 2011, Smith *et al.* 2014). To date, there are 38 known germline-derived imprinted DMRs in humans, the majority originating in the oocyte, that retain life-long allelic methylation in adult tissues (Monk *et al.* 2016). It has been shown that imprinted DMRs are selectively protected from the demethylation that occurs in the zygote immediately after fertilization. The paternal pronucleus is rapidly demethylated by the oxidative action of 10–11 translocation protein 3 (TET3) that converts 5-methylcytosine (5mC) to 5-hydromethylcytosine (5hmC), in a process that is finished before the completion of the first mitotic division (Wossidlo *et al.* 2011). Conversely, the demethylation process in the maternal pronucleus is more gradual and replication dependent. The maternal genome, including maternally methylated imprinting DMRs, is protected from TET3-mediated 5mC oxidation by developmental pluripotency-associated 3 (DPPA3) (also known as Stella or Pgc7), which specifically interacts with H3K9me2 enriched in the maternal, but not paternal, pronucleus

Table 2 Summary of the differences between mouse and human SCMC and imprinting highlighted in this review.

Difference	Observation in mouse	Observation in humans
<i>DNMT3L</i> expression in oocytes	Expressed in oocytes and essential for the establishment of maternal methylation	<i>DNMT3L</i> is not expressed in human oocytes
Phenotype associated with mutations in SCMC members	Primarily cause early embryonic arrest at early cleavage stages, no evidence of molar pregnancies	Early cleavage-stage arrest or hydatidiform moles, depending on which member of the complex is affected
<i>NLRP7</i>	No <i>Nlrp7</i> in the murine genome	<i>NLRP7</i> is a paralogue of <i>NLRP2</i> arising from evolutionary duplication
Phenotype associated with <i>NLRP2</i>	Mid-gestation embryo lethality in pregnancies of <i>Nlrp2</i> ^{-/-} females	Rare cases of MLID and Beckwith–Wiedemann Syndrome in children of women carrying mutations
Phenotype associated with <i>NLRP5</i>	Embryos of female <i>Nlrp5</i> ^{-/-} mice arrest at the pre-implantation cleavage stages	Women with mutations have varied reproductive histories, suffering from recurrent pregnancy loss, hydatidiform moles as well as live-born children with MLID
Placenta-specific imprinting	No placenta-specific maternally methylated imprinted DMRs	Oocyte-derived methylation associated with maternally methylated placenta imprints

(Nakamura *et al.* 2012). Both maternally and paternally methylated imprinted DMRs are further protected from pre-implantation reprogramming by the sequence-specific factor zinc finger protein 57 (ZFP57) (Li *et al.* 2008b). This kruppel-associated box-containing zinc finger protein recruits the Tripartite motif-containing 28 (TRIM28) repressor complex (Quenneville *et al.* 2011) and is therefore responsible for targeting DNMT1 to specific loci at a time when most of this protein is located in the cytoplasm.

The subcortical maternal complex

The subcortical maternal complex (SCMC) is a multi-protein complex expressed in oocytes and pre-implantation embryos, essential for zygote progression beyond the first embryonic cell divisions (Bebbere *et al.* 2016). In humans, it is currently composed of at least four known oocyte-derived proteins: oocyte-expressed protein (OOEP; also known as Factor Located in Oocytes Permitting Embryonic Development, FLOPED), Transducin-Like Enhancer of Split 6 (TLE6), NLR family, Pyrin domain-containing 5 (NLRP5; also known as Maternal Antigen That Embryos Require, MATER) and KH Domain Containing 3-Like (KHDC3L; also known as ES Cell-Associated Transcript 1, ECAT1, C6ORF221 or FILIA) (Zhu *et al.* 2015). These proteins localise to the subcortical region in mouse and human oocytes and pre-implantation embryos are excluded from regions of cell-to-cell contact from the zygote onwards and are devoid from the inner cell mass of the blastocysts (Li *et al.* 2008a).

All transcripts of the SCMC are encoded by maternal-effect genes, a class of genes coding for transcripts expressed exclusively in oocytes and early embryos and essential for the early embryonic development (Bebbere *et al.* 2016). They are highly abundant in the oocyte and early embryo and usually degraded by the time of EGA without transcriptional compensation from the embryonic genome (Tong *et al.* 2004, Ohsugi *et al.* 2008, Li *et al.* 2010). Consistent with their spatial and temporal expression profiles, targeted mice lacking individual proteins of the SCMC arrest between zygotic and cleavage stages (Tong *et al.* 2000, Li *et al.* 2008a, Tashiro *et al.* 2010). Therefore, the resulting female sterility is not due to aberrant folliculogenesis, ovulation, fertilization or uterine receptivity but the lack of maternal SCMC RNA/protein reserves required by the zygote to survive until EGA. However, despite the recent focus, the precise biological roles of the components of the SCMC are largely unknown.

One of the first characterised maternal-effect genes in mice was *Nlrp5* (Tong *et al.* 2000), which was later found to physically interact with KHDC3L (Ohsugi *et al.* 2008). Subsequent screening for additional maternal-effect genes identified *Dppa3* (Payer *et al.* 2003), peptidyl

arginine deiminase 6 (PADI6) (Esposito *et al.* 2007) and *Ooep* (Li *et al.* 2008a). *Dppa3* is not a member of the SCMC complex since the protein is found in both the cytoplasm and nucleus of oocytes with pronounced localisation in the pronuclei following fertilization (Sato *et al.* 2002). Recent detailed protein–protein interaction studies have confirmed that, in addition to oocytes and embryos co-localisation, that OOEP physically binds to NLRP5 and to TLE6, whilst KHDC3L interacts independently with NLRP5 to collectively form the SCMC (Li *et al.* 2008a,b). However the combined molecular weight of the known SCMC proteins (NLRP5 ~125 kDa; KHDC3L ~38 kDa; OOEP ~18 kDa; TLE6 ~65 kDa) is less than that observed by fast protein liquid chromatography gel filtration (between 669 and 2000 kDa) suggesting that there are additional SCMC proteins and interacting partners to be identified, the subcortical localisation of PADI6 implicating it as a potential member (Li *et al.* 2008a,b). With the exception of KHDC3L, genetic ablation of other SCMC components destabilises the complex, resulting in dispersed protein localisation that suggests these proteins are tightly regulated and function as an entire complex. This is further endorsed by the demonstration that both KHDC3L and the OOEP, NLRP5 and TLE6-complex play an important role in correct spindle assembly, the OOEP, NLRP5 and TLE6-complex controlling spindle position to ensure symmetrical cell division by regulating the dynamics of F-actin in the mouse zygotes (Zheng & Dean 2009, Yu *et al.* 2014) (Fig. 2).

Maternal-effect mutations in members of the SCMC

Consistent with the early cleavage arrest observed in mouse mutants, recessive mutations in *TLE6* and *PADI6* have been identified in females undergoing fertility treatment. Women carrying mutated alleles were identified during a cohort screening of couples

Figure 2 The hypothetical structure of the SCMC complex localised adjacent to the oocyte membrane. The SCMC interacts with both F-actin and the oocyte cytoplasmic lattices. The protein–protein interactions shown are deduced from co-immunoprecipitation experiments, although not all interactions for NLRP2 and NLRP7 have been assessed.

who had multiple failed IVF and ICSI cycles specifically associated with recurrent embryonic developmental arrest (pre-implantation embryonic lethality (MIM: 616814)). Mutations in *TLE6* were shown to cause the earliest known human embryonic lethality by affecting progression of oocyte meiosis II to zygote formation (Alazami *et al.* 2015), individuals with the mutations having a markedly impaired binding capacity for SCMC component proteins. Mutations in the *PADI6* gene were shown to cause early embryonic arrest by the 4-cell stage, potentially impairing EGA (Xu *et al.* 2016). Women with loss-of-function mutations in *NLRP5* have varied reproductive histories, suffering from recurrent pregnancy loss, possible molar pregnancies as well as live-born children with abnormal epigenetic profiles at imprinted loci (Docherty *et al.* 2015). Interestingly, women with *KHDC3L* mutations also experience multiple reproductive failures predominantly a rare recurrent form of biparental hydatidiform mole (BiHM) (HYDM1; OMIM# 231090) (Parry *et al.* 2011).

Biparental hydatidiform moles

Complete hydatidiform moles (CHM) are an abnormal human pregnancy classically characterised by the absence of embryonic development, hydropic degeneration of the chorionic villi of the placenta and hyperplasia of the villous trophoblast. CHM are commonly sporadic and diploid androgenetic with the majority resulting from a monospermic fertilization event followed by genome duplication without cytokinesis and a minority, 10–20%, by a dispermic event (Hoffner & Surti 2012). The fate of the maternal chromosomes in CHM is unclear. An error during meiosis could give rise to an anucleate egg. However, it has been argued that evidence for a reservoir of anuclear oocytes is lacking and that CHM might actually arise as a result of post-zygotic diploidisation of a triploid conceptus.

Occasionally, HM can be recurrent and familial in nature consistent with an autosomal condition resulting in a predisposition to CHM (Fisher *et al.* 2004). Cases of familial recurrent HM (FRHM) are extremely rare, but the condition can be identified by demonstrating that pregnancies which are morphologically CHM are diploid and biparental or, rarely, digynic triploids (Fallahian *et al.* 2013, Nguyen & Slim 2014) rather than androgenetic diploids. While FRHM may result from mutations in *KHDC3L* (Parry *et al.* 2011), this accounts for only 5–10% of cases, mutations in another maternal-effect gene, *NLRP7* having been shown to account for approximately 75% of cases (Murdoch *et al.* 2006). Ten years following the initial reports of *NLRP7* being responsible for recurrent BiHM, ~60 pathogenic variants have been reported in females homozygous or compound heterozygous for these defective alleles. These include missense mutations, nonsense mutations,

splice site mutations and *Alu*-mediated deletions (Wang *et al.* 2009, Dixon *et al.* 2012, Reddy *et al.* 2016).

Like mutations in *NLRP7*, mutations in *KHDC3L* may occur in single affected individuals or multiple members of a large family and affected individuals may be homozygous or compound heterozygotes. To date, most mutations in *KHDC3L* have been found to be small deletions, with two variants affecting the initiation codon and a single variant giving rise to a splice site mutation (Parry *et al.* 2011, Reddy *et al.* 2013, Rezaei *et al.* 2016).

Other pregnancy outcomes associated with *NLRP7* maternal-effect mutations

While approximately 75% of pregnancies in women with FRHM are CHM (Fisher *et al.* 2004), variable pregnancy outcomes have been reported in a minority of cases of women with recessive *NLRP7* mutations, including stillbirth, early spontaneous pregnancy loss, partial HM and androgenetic CHM. Similarly, spontaneous abortions have been reported in women with *KHDC3L* mutations. Intriguingly, occasional live births have been documented to date in women with homozygous *NLRP7* mutations (Akoury *et al.* 2015b), while no live births have been reported among women with two defective *KHDC3L* alleles (Parry *et al.* 2011, Reddy *et al.* 2013, Rezaei *et al.* 2016). For women with *NLRP7* mutations who wish to achieve a normal pregnancy, *in vitro* fertilization with ovum donation has been shown to provide a viable option (Fisher *et al.* 2011, Akoury *et al.* 2015b).

It has recently been suggested that heterozygous mutations in *NLRP7* may be associated with other forms of reproductive failure other than BiHM, such as recurrent miscarriage and sporadic CHM (Messaed *et al.* 2011). However, mutation screening in women with a diagnosis of primary unexplained infertility or with unexplained recurrent miscarriage failed to identify pathological variants in *NLRP7* or *KHDC3L* suggesting that mutation in these genes are not a common cause of recurrent pregnancy loss (Aghajanova *et al.* 2015).

Widespread imprinting defects in *NLRP7*-associated moles

The characteristic morphological features of sporadic CHM result from their androgenetic origin with the consequent overexpression of paternally transcribed genes with potential loss of maternally transcribed genes. Since BiCHM are morphologically similar, and in many cases indistinguishable, from androgenetic CHM, imprinting defects are likely to underlie their development. Consistent with this is an absence of expression of p57, the product of the maternally transcribed gene *CDKN1C* in both androgenetic and BiHM (Fisher *et al.* 2002). Initial epigenetic studies in

Figure 3 Profiles at imprinted differentially methylated regions in placenta and hydatidiform moles. In normal placental tissue oocyte-derived methylation is maintained on the maternal allele at imprinted DMRs, while reciprocally sperm-derived methylation is observed exclusively on the paternal allele. In both androgenetic and NLRP7-associated molar tissues there is complete loss of methylation at the majority of maternal DMRs. However paternal DMRs can differentiate androgenetic from NLRP7-associated moles. Methylation will appear hypermethylated in androgenetic moles due to the two copies of the paternal genome and normal in NLRP7-associated moles as a result of a biparental fertilization.

molar tissue from women carrying recessive *NLRP7* mutations have revealed aberrant DNA methylation profiles at a limited number of imprinted genes (El-Maarri *et al.* 2003, Kou *et al.* 2008, Hayward *et al.* 2009). Similarly, investigation of a single BiHM from a woman, later found to be homozygous for mutations in *KHDC3L*, identified a number of imprinted genes to be aberrantly expressed (Judson *et al.* 2002). Lack of allelic methylation in *NLRP7*-associated BiHM was consistently noted for maternally methylated imprinted regions, including the differentially methylated regions (DMRs) associated with *PEG3*, *SNRPN*, *KCNQ1OT1*, *MEST* and *GNAS*. Consistent with a fertilisation event with a normal sperm, the paternally methylated *H19* region was normally methylated in BiHM. Curiously, maternal methylation at the *PEG10* DMR seems to maintain allelic methylation with a profile similar to control placentas, suggesting that imprinting at this locus is established by a different mechanism (Hayward *et al.* 2009, Sanchez-Delgado *et al.* 2015).

Recently, BiHM tissues from females with recessive *NLRP7* mutations have been screened for methylation defects using high-density methylation arrays. Using this approach, Sanchez-Delgado and colleagues reported that the majority of maternally methylated DMRs are hypomethylated, with some notable exceptions. For example the DMRs associated with *IGF1R* and *RB1* maintained methylation in all BiHM samples, whereas the *PLAGL1*, *PEG10* and *NAP1L5* DMRs retained allelic methylation in a stochastic fashion (Sanchez-Delgado *et al.* 2015). Intriguingly, complete hypomethylation was also observed at 48 placenta-specific imprinted loci suggesting that the molar phenotype could largely be due to genome-wide disruption of genomic imprinting (Fig. 3).

Are other NLRP proteins SCMC members?

Remarkably, maternal loss-of-function of two highly homologous genes, *NLRP2* and *NLRP7*, which like *NLRP5* encode NLR family proteins, are highly

expressed in oocytes and pre-implantation embryos (Zhang *et al.* 2008) and associated with various forms of reproductive wastage. Maternal mutations in *NLRP2* have been described in a mother with two offspring affected with Beckwith–Wiedemann syndrome and one multi-locus imprinting disturbance (MLID), although the full extent of the methylation defects was not reported (Meyer *et al.* 2009), while mid-gestation embryos from *Nlrp2*^{-/-} female mice exhibit a range of methylation abnormalities at imprinted loci (Mahadevan *et al.* 2017). *NLRP7* is highly homologous to *NLRP2* and the two genes adjacent to each other on chromosome 19. *NLRP7* does not have an orthologue in mice, but is thought to have originated from a direct evolutionary duplication of *NLRP2* (Duñez-Guzmán & Haig 2014). As discussed earlier, maternal mutations in *NLRP7*, like those in *KHDC3L*, have been shown to be associated with poor reproductive outcomes, specifically complete molar pregnancies (Murdoch *et al.* 2006, Wang *et al.* 2009, Parry *et al.* 2011). Consistent with SCMC protein expression patterns, *NLRP7* co-localises with *KHDC3L* to the cortical region in oocyte and 2-cell embryos, but not to regions of cell-to-cell contact (Akoury *et al.* 2015a). Murine *NLRP2* has also been shown to be located in the subcortical layer and to interact with multiple SCMC proteins (Mahadevan *et al.* 2017) suggesting that, in addition to *NLRP5*, both *NLRP2* and *NLRP7* may also be *bona fide* SCMC proteins (Fig. 2).

Potential SCMC-NLRP epigenetic function

It is surprising that SCMC-NLRP proteins have potentially different roles in imprint establishment or maintenance. The broad loss of maternally methylated imprints, whilst paternally methylated DMRs are unaffected, implicate *NLRP7* in oocyte-specific methylation establishment (Sanchez-Delgado *et al.* 2015). However, both maternally and paternally methylated imprinted DMRs are affected in MLID patients resulting from *NLRP5* mutations (Docherty *et al.* 2015) and in offspring from

Nlrp2^{-/-} female mice, suggesting roles in post-zygotic maintenance (Mahadevan *et al.* 2017). In addition *NLRP2* and *NLRP7* show a different temporal expression pattern in human pre-implantation embryos from *NLRP5*, an increase in expression between days 3 and 5 being observed for the former but not *NLRP5* (Zhang *et al.* 2008). What makes the participation of these NLRPs in the imprinting process even more extraordinary is that the SCMC-NLRP proteins have an exclusive cytoplasmic localization while DNA methylation is a nuclear process. This may be explained by the additional functions of the SCMC that have been revealed in mutant mice, namely the degradation of maternal RNA and protein stores (Wang *et al.* 2012). In addition to the release of the maintenance methyltransferase, DNMT1, from its normal subcortical localization in the oocytes of *Nlrp2*^{-/-} females (Mahadevan *et al.* 2017), it could be speculated that stores of other maternal-effect proteins involved in the imprinting process, such as DPPA3, TRIM28 and ZFP57 (Nakamura *et al.* 2007, Li *et al.* 2008b, Messerschmidt *et al.* 2012), may also be mislocalized, depleted or perhaps inappropriately degraded during cleavage embryo development. Alternatively, NLRP7-SCMC may ensure the correct cellular localization and nuclear translocation of epigenetic factors during oocyte development. Immunostaining for the two mammalian *de novo* methyltransferases DNMT3A and DNMT3B has revealed that, like DNMT1, they also have a cytoplasmic localization in human oocytes (Petrussa *et al.* 2014). This suggests that low abundant protein complexes containing the DNMTs may associate to specific DNA sequences possibly by direct interaction with known NLRP7-interacting chromatin regulators YY1 (Mahadevan *et al.* 2014) or ZBTB16 (Singer *et al.* 2015).

Developmental discrepancies, speculations and conclusions

The vigilant readers of this review will have noted several developmental inconsistencies between mouse models and the equivalent human phenotypes. While maternal-effect mutations in *TLE6* and *PADI6* have similar outcomes in humans as exhibited by murine models, differences exist for other maternal-effect genes. These include (i) the offspring of *Nlrp5*^{-/-} mice, which arrest at the cleavage stage, whereas *NLRP5* maternal-effect mutations are associated with varying reproductive outcomes and MLID; (ii) female *Nlrp2*^{-/-} mice are sub-fertile, with most pregnancies ending in embryonic lethality. In the rare cases that pups are produced, they die shortly after birth. In humans, maternal-effect mutations of *NLRP2* have been shown to result in MLID. It is possible that in human cases, the true incidence of the mutations is under presented, with the majority of the conspecti being lost before pregnancy was detected. In the rare instances that live-born children are observed, the development progresses

due to the stochastic nature of the MLID. Similarly, the true impact of *Nlrp2* on imprinted methylation in mice may also be underestimated as the work reported by Mahadevan and coworkers was performed on rare embryos that developed to embryonic day 9.5 and not on pre-implantation embryos where the most extreme developmental phenotypes would be anticipated (Mahadevan *et al.* 2017). It is likely that the mid-gestation embryos reflect those with minor/viable imprinting defects and that widespread methylation anomalies would be restricted to the earlier arrested embryos.

In women with homozygous or compound heterozygous mutations in *NLRP7* most recognised pregnancies develop as BiHM with a regular diploid karyotype. In *in vitro* studies, *NLRP7* mutations have also been observed to cause early embryonic developmental arrest. Two studies have reported the *in vitro* development of embryos following assisted reproductive cycles in women carrying recessive *NLRP7* mutations (Deveault *et al.* 2009, Sills *et al.* 2017). Both studies reported high rates of mosaic, haploid-diploid and haploid-maternal aneuploid embryos, with the majority of embryos degenerating before the blastocysts stage suggesting that BiHM may result from preferential selection of cells with widespread imprinting errors without ploidy errors. Careful counselling is advised in the case of a woman with FRHM as conventional *in vitro* fertilization with PGD will be unable to distinguish a BiCHM from a normal pregnancy. For these women, the use of donor oocytes is encouraged since successful pregnancies have been achieved in women with underlying *NLRP7* mutations (Fisher *et al.* 2011, Akoury *et al.* 2015a,b).

While mutations in members of the SCMC clearly implicate the complex in establishing or maintaining maternal epigenetic marks during oogenesis and early fetal development, the mechanisms by which this is achieved remains unclear and is likely to be different in different species. While both *NLRP2* and *NLRP7* are potential members of the SCMC, the size of the SCMC suggests there may be other, as yet unidentified, members. Identification of novel members of the SCMC is needed to provide greater insight into the role of the SCMC in the establishment and maintenance of the maternal imprint in early embryogenesis and reproductive loss.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work was supported by the Spanish Ministerio de Educación y Competitividad (MINECO) (BFU2014-53093 to D M) co-funded with the European Union Regional Development Fund (FEDER).

Acknowledgements

The authors are grateful to members of the Monk laboratory for helpful discussions.

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Received 28 July 2017

First decision 4 September 2017

Revised manuscript received 6 September 2017

Accepted 15 September 2017