Genetics and Epigenetics of Hydatidiform Moles

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A hydatidiform mole (HM) is an abnormal human pregnancy characterised by absence of, or abnormal, embryonic development, excessive trophoblastic proliferation and hydropic degeneration of placental villi. The common types of moles are sporadic, not recurrent, and affect 1 in 1000 pregnancies in western countries. HM may recur in the same patient, which is referred to as recurrent HM (RHM), and indicates that the patient is genetically susceptible to HM. Through the examination of rare familial cases of RHM, two maternal-effect genes, *NLRP7* and *KHDC3L*, responsible for this condition have been identified. Pathogenic variants in these genes appear to impair imprinting establishment during oogenesis. Herein, we review current knowledge on the genetics and epigenetics of RHM, and highlight the benefits of testing patients for pathogenic variants in the known genes.

Introduction

The clinical manifestations of hydatidiform mole (HM) have greatly evolved throughout the years. The first clinical descriptions were made by Hippocrates in 400 BC who described the presence of intrauterine vesicles. It was only in 1276 that a more 'precise' clinical description appeared. At that time, according to the medieval legend of Countess Margaret of Henneberg, who had most probably spontaneously ejected an HM, the vesicles were believed to be the immature birth of '365 children' (Bondeson and Molenkamp, 1996). Unfortunately, the Countess died the next day of heavy bleeding. This surely is the first description of a spontaneous evolution of a complicated HM.

The clinical manifestation of moles by the spontaneous ejection of vesicles, heavy bleeding and uterine rupture is still seen nowadays in developing countries where medical follow-up of pregnancies does not start at the eighth week of gestation, and consequently HM can freely evolve. However, in developed countries, the current clinical signs are usually vaginal bleeding, sensation of heaviness in the pelvic region, enlargement of the uterus and excessive nausea and vomiting (hyperemesis). Because these symptoms are frequent in the first stages of normal pregnancies, most clinicians discover moles by ultrasound performed on cases of abnormal evolution of a pregnancy in the first 8 weeks of gestation or at the first gynaecological visit (between 6 and 8 weeks) (Hou *et al*., 2008; Mangili *et al*., 2008; Sebire *et al*., 2001).

Clinical presentation

An HM is suspected when ultrasonography reveals echogenic structures all over the uterus and this is referred to as a 'snow storm' with the absence of a defined gestational sac or with the presence of a gestational sac as well as some embryonic structures. Uncommon clinical manifestations can develop in advanced diseases such as hyperthyroidism when human chorionic gonadotropin (hCG) is above 100 000 U mL^{-1} (Amir *et al*., 1984). Luteinising cysts of the ovarian theca cells may also be seen as a result of ovarian hyperstimulation due to the rise in hCG. In the majority of cases, the cysts will disappear when the mole is removed. However, in rare cases, the cysts may twist and induce violent abdominal pain (Osathanondh *et al*., 1986). Preeclampsia has also been described around 20 weeks of gestation in some patients with HM, but this clinical manifestation is rare in developed countries (Ramsey *et al*., 1998).

The clinical evaluation of a patient with a suspected HM by ultrasound will include a detailed history of all systems, in particular those related to obstetrics and gynaecology. A pelvic medical

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examination will show an enlarged uterus and eventually the presence of cysts in the adnexaes. This initial suspicion of the presence of an HM is followed by a laboratory evaluation of the level of hCG, which is higher in molar than in normal pregnancies of a similar gestational stage; an assessment of thyroid functions and an X-ray of the lungs to search for embolus moles or pleural effusions (accumulation of fluid around the lung). Abnormal values for these tests, combined with the ultrasound result, are in favour, but not diagnostic, of an HM. However, when the pregnancy is not viable, the clinician will perform dilatation and curettage suction of the product of conception. This product of conception will then be sent for histopathological examination, based on which the final diagnosis is established.

Epidemiology

HM is a disease that displays a wide geographic distribution in its frequencies with a gradient of increasing frequencies from West to East. The highest frequencies reach 1 in 60 pregnancies and are found in Southeastern Asia such as in Indonesia and the Philippines, and the lowest frequencies, 1 in 1000 to 1 in 1500 pregnancies, are found in the United States, Canada and Europe (Grimes, 1984). These frequencies mostly represent the common sporadic HM as recurrent hydatidiform moles (RHMs), defined by the occurrence of at least two HM in the same patient, are rare and account for only 1.4–9.4% of all HM. Similar to sporadic HM, RHM have been reported to be more frequent in Morocco (9.4% of all HM) (Boufettal *et al*., 2011), Lebanon (6%) (Kronfol *et al*., 1969), and Korea (4.3%) (Kim *et al*., 1998). The lowest frequencies of RHM have been reported in the United States (1.4–1.5%) (Berkowitz *et al*., 1998) and Europe (1.8%) (Sebire *et al*., 2003).

Histopathology

The classical macroscopic features (visible to the naked eyes) of a typical HM used to include severe oedematous and hydropic chorionic villi (**Figure 1**) that have a grape-like appearance. However, because of the standard use of ultrasonography in current medical practice, most HM are diagnosed and evacuated at earlier stages, before the excessive hydropic degeneration of the chorionic villi and their characteristic grape-like appearance. Consequently, the obvious manifestation of HM by macroscopy, as shown in **Figure 1**, is becoming much less frequent. Evacuated products of conception are sent to histopathology laboratories where they are fixed and embedded into paraffin blocks. The tissues are then sectioned, mounted on microscopic slides, stained with hematoxilin and eosin, and examined by light microscopy by pathologists. Microscopic examination of a product of conception clinically suspected to be an HM has two goals – the first is to determine whether the product of conception corresponds to an HM or a nonmolar arrested pregnancy and the second is to classify the HM into two histopathological types, complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM), because these two types have different risks of malignant degeneration into neoplasias.

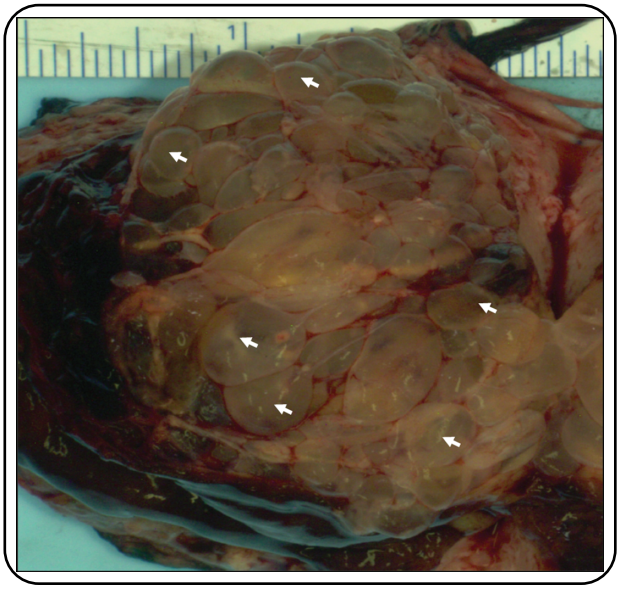

Figure 1 Gross morphology of a hydatidiform mole. The photo was taken directly after the evacuation of the HM by curettage and suction. Arrows indicate some vesicles.

Complete hydatidiform mole

On the basis of the histopathological features, HM are divided into CHM and PHM. Microscopic features of CHM consist of clubbing of the chorionic villi, the presence of apoptotic bodies referred to as karyorrhexis, uniformity in the shape of chorionic villi, stromal hypercellularity and nonpolarised, circumferential trophoblastic hyperplasia with atypia (cells with abnormal nuclear morphology) (**Figure 2a**) (Szulman and Surti, 1978). In general, all the chorionic villi display oedema and cisterns with unpolarised and haphazard trophoblastic proliferation. The rim of stroma at the periphery of chorionic villi is often rich in cells admixed with apoptotic bodies (**Figure 2b**). Trophoblastic inclusions may be seen but are not a common feature of CHM. Exaggerated placental site is frequently associated with CHM but rarely with PHM. The presence of foetal tissues or nucleated red blood cells inside the chorionic villi excludes the diagnosis of CHM, except in rare cases of twin pregnancies (consisting of a foetus and a mole), mosaicism and chimerism. In conclusion, the specific features of CHM are club-shaped chorionic villi, oedematous chorionic villi with cistern, karyorrhexis and excessive circumferential trophoblastic proliferation.

Partial hydatidiform mole

PHM macroscopically displays two populations of oedematous and small fibrotic chorionic villi (**Figure 2c**). Upon microscopic evaluation, apoptotic bodies are usually absent but might be seen focally in some cases. Cisterns could be present in some chorionic villi, but they are not as frequent as in CHM. Excessive circumferential trophoblastic proliferation and atypia are absent in PHM. Extraembryonic tissues (chorion and amnion, which constitute the foetal membranes) (**Figure 2d**) and/or embryonic tissues such

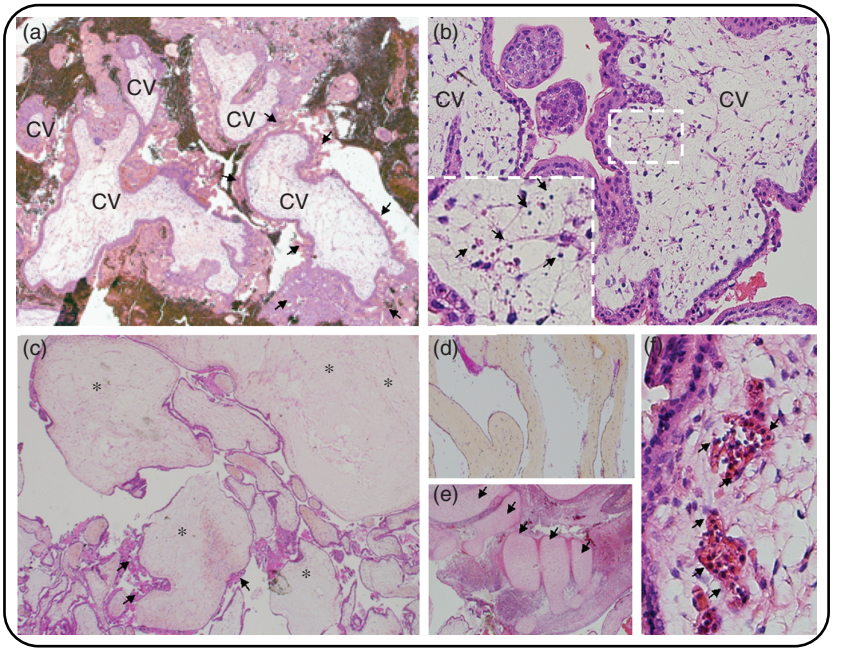

Figure 2 Histopathology of complete and partial hydatidiform moles. (a) A microphotograph of a CHM. CV stands for chorionic villi and arrows indicate circumferential trophoblastic proliferation around one chorionic villous. (b) Two chorionic villi displaying important karyorrhexis. The inset shows a higher magnification of karyorrhectic debris (arrows). (c) A microphotograph of a partial hydatidiform mole showing the presence of two populations of chorionic villi, the large hydropic chorionic villi (asterisk) and the small ones. Focal trophoblastic proliferation around one chorionic villous is indicated by arrows. A microphotograph of a different PHM showing foetal membranes in (d) and some skeletal bones of embryonic origin (arrows) in (e). (f) A microphotograph of a PHM showing nucleated red blood cells (arrows) inside a chorionic villous.

as skeletal bones (**Figure 2e**), cartilages and nucleated foetal red blood cells inside the chorionic villi may be present (**Figure 2f**) (Szulman and Surti, 1978). In addition, a complete foetus with normal or abnormal morphology may be present in PHM. In conclusion, the most characteristic features of PHM include the presence of two populations of chorionic villi, irregular contour of chorionic villi and moderate trophoblastic proliferation around the chorionic villi.

Genotypic Types of HM

Methods

The methods used to determine the genotypes of HM and the parental contribution to their genomes have evolved with time from karyotype analysis by classical culture-based cytogenetics to a wide range of DNA (deoxyribonucleic acid)-based methods that include flow cytometry, fluorescent *in situ* hybridisation with probes against various centromeric or heterochromatin repeats, and multiplex and/or simplex microsatellite genotyping. Another method, $p57^{KIP2}$ immunohistochemistry, introduced in 2001 (Castrillon *et al*., 2001), has become an important one and is being performed on a routine basis in many laboratories as part of HM assessments. This method is currently used to identify, indirectly, the presence of the maternal genome in molar tissues. $P57^{KIP2}$ is the protein coded by cyclin-dependent kinase inhibitor 1C, *CDKN1C*, which is paternally imprinted and expressed only from the maternal genome in the nuclei of normal first trimester cytotrophoblastic and villous stroma cells (Castrillon *et al*., 2001). Therefore, the presence of nuclear staining for $p57^{KIP2}$ in these cells indicates that these cells contain at least one copy of the maternal genome. The advantage of $p57^{KIP2}$ immunohistochemistry is that it is simple, inexpensive and can be performed on sections of formalin-fixed paraffin embedded (FFPE) tissues that are prepared systematically from all molar pregnancies as part of patient care.

The other DNA-based methods used to be performed only on fresh tissues. However, nowadays, all can be performed on FFPE tissues. Importantly though, one needs to keep in mind that working with FFPE tissues is technically more challenging than fresh tissues. Therefore, when genotyping FFPE tissues, it is advisable to use more than one method to minimise mistakes due to technical difficulties caused by the low quality of DNA from archived FFPE tissues and the difficulty in some cases in isolating chorionic villi and separating them from maternal endometrial cells. From our experience, we find that using several methods and reconciling their data allow us, in addition to minimising potential mistakes, to confirm the genotypes of unusual HM cases. This will consequently improve our understanding of this pathology by drawing better and more accurate correlations between the genotype of the product of conception and their histopathological features.

Table 1 Summary of the main genotypic types of sporadic and recurrent HM

	CHM	PHM
Sporadic HM	Diploid androgenetic monospermic, **XX** Diploid androgenetic dispermic, **XX** or **XY** Tetraploid 4n, **XXYY**, **XXXX**, **XXXX**, **XXXY**, **XYYY** Diploid biparental[a]	Triploid dispermic **XXX**, **XXY** and **XYY**
RHM from patients with recessive pathogenic variants in *NLRP7* or *KHDC3L*	Diploid biparental, **XX** or **XY**	

Pink and blue colours are used to indicate the maternal and paternal genetic complements.
[a]The genotypes of these HM need to be revisited based on emerging data in the field in order to validate their existence.

Genotypic types of HM

Diploid androgenetic

The use of the various methods mentioned above has shown that common sporadic nonrecurrent CHMs are mostly androgenetic. In the majority of the cases (80–90%), they are monospermic and contain two copies of a single paternal set of 23 chromosomes originating from one spermatozoid (androgenetic monospermic). Such HM are always XX because YY zygotes or embryos are not viable (**Table 1**). In up to 20% of the cases, CHMs contain two paternal sets of chromosomes deriving from two different spermatozoids. These HM are androgenetic dispermic and can be XX or XY.

Triploid dispermic

Sporadic, nonrecurrent PHMs are mostly triploid with 69 chromosomes consisting of two sets of paternal chromosomes originating from two different spermatozoids and one set of maternal chromosomes. These HM are said to be triploid dispermic and can be XXX, XXY or XYY (**Table 1**).

Unusual and rare genotypes

Other rare genotypic types of HM that have, or mimic, the morphology of CHM or PHM have also been reported. These include tetraploid HM, aneuploid diploid biparental HM and diploid biparental HM with no aneuploidies (**Table 1**). Such HM represent a small fraction of all HM and therefore will not be discussed in this article with the exception of RHM from patients with inherited recessive pathogenic variants in *NLRP7* or *KHDC3L*. These RHM have diploid biparental genome with no detectable aneuploidies and are discussed below.

Genes Responsible for RHM

NLRP7

NLRP7 maps to 19q13.4 (Moglabey *et al*., 1999) and was the first maternal-effect gene to be identified in humans (Murdoch *et al*., 2006). Maternal-effect genes are a subset of genes that are needed in the oocytes, in the form of RNA (ribonucleic acid) or proteins, to sustain normal embryonic development until the activation of the embryonic genome at both the transcriptional and translational levels. NLRP7 codes for a protein that is a member of the NOD-like receptor pyrin-containing domain 7. It has three main domains: a pyrin domain; a NACHT domain, which contains an ATPase (adenosine triphosphatase) domain, and leucine-rich repeat domains formed by 9–10 repeats, depending on splicing isoforms (**Figure 3**). The causal role of *NLRP7* in the aetiology of RHM was reported in 2006 by our group based on the identification of recessive pathogenic variants in four unrelated patients from different populations (Murdoch *et al*., 2006). To date, approximately 64 different pathogenic variants in *NLRP7* have been seen in a recessive state and in a total of approximately 150 patients (**Figure 3**) (http://fmf.igh.cnrs.fr/ISSAID/infevers/). Available reports on large cohorts indicate that recessive pathogenic variants in *NLRP7* are not present at the same frequency in all populations; while China seems to have the lowest frequency (58%) (Qian *et al*., 2011), Pakistan (Hayward *et al*., 2009), India (Slim *et al*., 2009) and Mexico (Estrada *et al*., 2013) have the highest frequencies ranging from 81% to 85%. Because *NLRP7* is a maternal-effect gene and the primary defect in patients with two defective alleles is in their oocytes, three patients have so far tried donated ova after *in vitro* fertilisation and had successful pregnancies leading to healthy children (Akoury *et al*., 2015a; Fisher *et al*., 2011). The benefit of ovum donation for patients with RHM and recessive pathogenic variants in *NLRP7* highlights the importance of offering DNA testing for these patients. Indeed, such a situation is rare in reproductive medicine where we can know the exact defect at the nucleotide level and offer the appropriate assisted reproductive technology service for the patients.

KHDC3L

The analysis of patients with RHM who were negative for pathogenic variants in *NLRP7* led to the identification of a second maternal-effect gene, *KHDC3L*, for this condition (Parry *et al*., 2011). KHDC3L maps to human 6q13 and is a small protein of

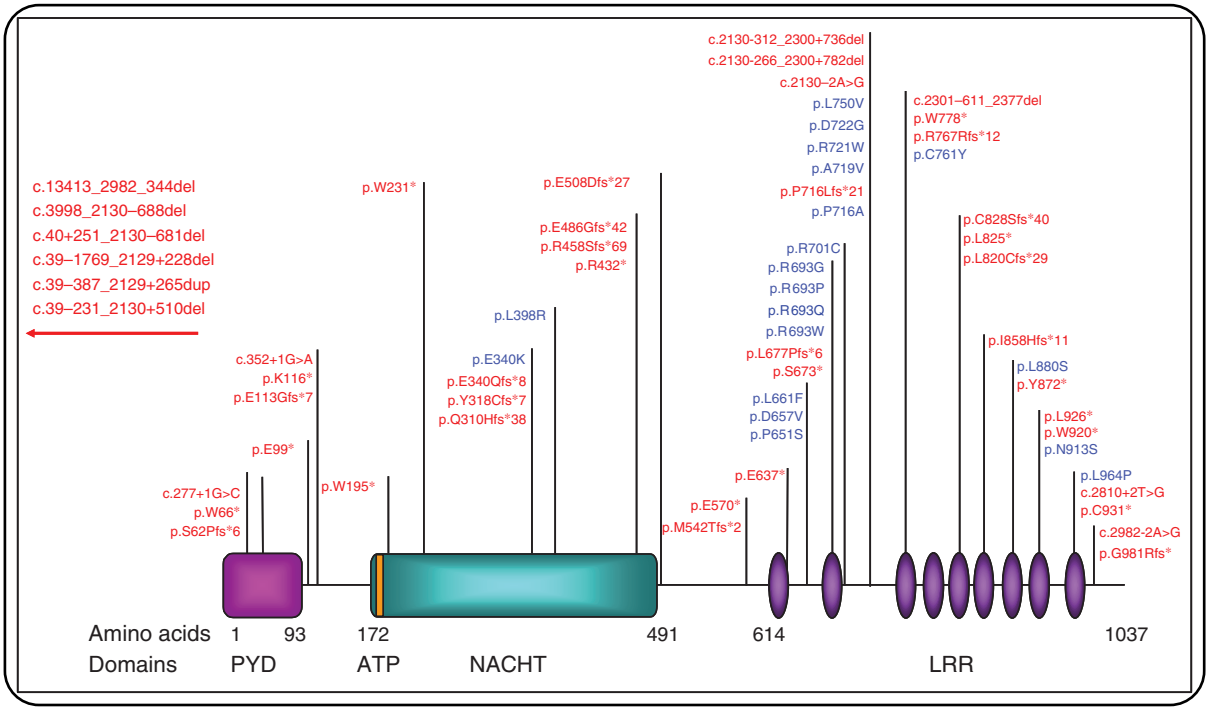

Figure 3 NLRP7 protein structure and reported pathogenic variants observed in a recessive state by various groups (http://fmf.igh.cnrs.fr/ISSAID/infevers/). NLRP7 protein has mainly three domains. Protein truncating variants (stop codon, deletions, insertions and invariant splice mutations) are in red and missense variants are in blue. The large deletions that begin before the start codon are indicated by an arrow towards the 5′ untranslated region.

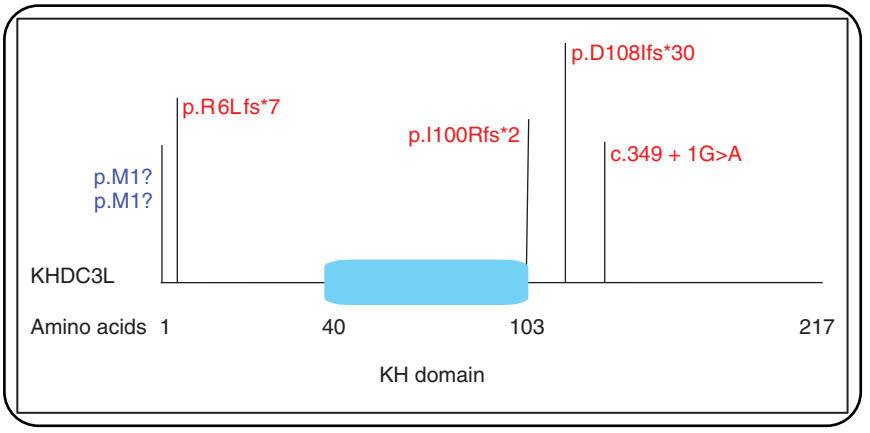

Figure 4 KHDC3L protein structure and reported pathogenic variants in recessive state by various groups. The two pathogenic variants affecting the start codon are indicated by question marks because their consequences on the protein are not known (http://databases.lovd.nl/shared/genes/KHDC3L).

217 amino acids. It contains an atypical KH domain. To date, six different pathogenic variants in *KHDC3L* have been reported in a total of 10 patients (**Figure 4**) (Parry *et al*., 2011; Reddy *et al*., 2013; Rezaei *et al*., 2016). To our knowledge, none of the reported patients with pathogenic variants in *KHDC3L* have tried *in vitro* fertilisation with donated ova. However, by analogy to *NLRP7*, we expect that this assisted reproductive technology would rescue the defects in these patients.

Functions of NLRP7 and KHDC3L

RHM genotypes

The existence of RHM was described a long time ago, but it was only in recent years that RHM cases were characterised at the genomic DNA level. The parental contribution to the first case of RHM was described by in 1991 by Vejerslev *et al*. (1991) and

Table 2 Recapitulation of HM genotypes from patients with recessive pathogenic variants in *NLRP7* or *KHDC3L*

	Diploid biparental	Triploid dispermic	Triploid digynic
NLRP7	118 (98%)	1 (0.008%)	1 (0.008%)
KHDC3L	9 (90%)		1 (10%)

these RHM were found diploid biparental with a normal parental contribution to the molar genomes as opposed to sporadic CHM or PHM. This RHM case, in addition to others from familial and nonfamilial cases, contributed to the identification of *NLRP7* and *KHDC3L.*

Now, a decade after the identification of *NLRP7*, the parental contribution to approximately 118 HM tissues from patients with recessive pathogenic variants in *NLRP7* have been reported. All these tissues were found diploid biparental with the exception of two tissues that were found triploid, one dispermic (Ulker *et al*., 2013) and one digynic (Fallahian *et al*., 2013) (**Table 2**). For *KHDC3L*, the parental contribution to only 10 HM from patients with recessive pathogenic variants has been reported. Nine of these tissues were found diploid biparental (Fallahian *et al*., 2013; Hayward *et al*., 2009; Judson *et al*., 2002; Reddy *et al*., 2013) and one triploid digynic (Fallahian *et al*., 2013) (**Table 2**). Despite their diploid biparental genome, at the histopathological level, RHM caused by pathogenic variants in *NLRP7* or *KHDC3L* mimic the sporadic HM and some are diagnosed by histopathology as CHM (Helwani *et al*., 1999; Messaed *et al*., 2011b; Sebire *et al*., 2013; Zhao *et al*., 2006), PHM (Helwani *et al*., 1999; Vejerslev *et al*., 1991), or atypical HM (Sebire *et al*., 2013). Studies on two large cohorts of RHM agree on the facts that HM from patients with inherited defects in either gene have, in general, less trophoblastic proliferation than androgenetic CHM (Messaed *et al*., 2011b; Sebire *et al*., 2013).

Not all RHM are diploid biparental, some are androgenetic monospermic (Dixon *et al*., 2012; Eagles *et al*., 2016) and others are triploid dispermic (Eagles *et al*., 2016; Slim *et al*., 2011). However, highly RHM are mostly diploid biparental and most are from patients with recessive pathogenic variants in *NLRP7* and few from patients with recessive pathogenic variants in *KHDC3L.*

Imprinting

Before the identification of *NLRP7* as a causative gene for RHM, the demonstration that RHM are diploid biparental and are morphologically similar to diploid androgenetic HM raised the hypothesis about the potential role of wild-type NLRP7 in establishing imprinting marks during oogenesis or maintaining them during early embryonic development (Sunde *et al*., 1993). This interesting hypothesis was at the origin of several studies that assessed DNA methylation of several differentially methylated region (DMR) associated with imprinted genes in diploid biparental HM from patients with pathogenic variants in *NRLP7* or *KHDC3L.* The results were as expected for imprinted, maternally methylated DMRs, and these DMRs were found to lack their methylation marks, therefore mimicking their corresponding paternal DMR. This finding was originally documented by Judson *et al*. (2002) and was since then replicated in every analysed tissue from patients with recessive pathogenic variants in *NLRP7* or *KHDC3L* (El-Maarri *et al*., 2003; Hayward *et al*., 2009; Ito *et al*., 2015; Kou *et al*., 2008; Sanchez-Delgado *et al*., 2015).

The proposed explanations of such lack of DNA methylation is that the establishment or setting of methylation imprinting marks during oogenesis did not occur because of the pathogenic variants in *NLRP7* or *KHDC3L.* All these studies investigated the methylation at few DMR (4–9) (El-Maarri *et al*., 2005; Hayward *et al*., 2009; Ito *et al*., 2015; Judson *et al*., 2002; Kou *et al*., 2008) with the exception of a recent genome-wide DNA methylation study using the Illumina Infinium HumanMethylation450 Bead-Chip arrays (Sanchez-Delgado *et al*., 2015). This comprehensive and important study confirmed the lack of methylation on several maternally methylated DMR and expanded this observation to several additional DMR. The authors proposed an interesting mechanism involving a disruption in the selection and recruitment of follicles at a specific time during the foetal life of patients with pathogenic variants in *NLRP7* or *KHDC3L* that coincides with the time of methylation mark establishment, and would result in the ovulation of oocytes with inappropriate methylation marks (Sanchez-Delgado *et al*., 2015). This suggestion is interesting and plausible because it was found that in mice, several *Nlrp* genes are under the transcriptional control of factor in the germ line, alpha (FIGLA) whose knockout leads to female mice with no primordial follicles at birth (Joshi *et al*., 2007). It is therefore possible that the oocyte defect in women with recessive pathogenic variants in *NLRP7* or *KHDC3*L occurred earlier during folliculogenesis. In addition, the study by Sanchez-Delgado *et al*. (2015) is the only one that checked the transcriptional consequences of the lack of methylation marks and demonstrated the biallelic expression of all analysed genes. Moreover, this study confirmed a previous observation that the methylation defect in RHM from patients with recessive pathogenic variants in *NLRP7* is restricted to imprinted genes (Djuric *et al*., 2006) and only affects the conceptions of these patients (El-Maarri *et al*., 2005).

In conclusion, studies from various groups are concordant with the lack of DNA methylation only on the DMR of most imprinted, maternally methylated genes, probably due to a defect during folliculogenesis or oogenesis, and as a consequence of recessive pathogenic variants in *NLRP7* or *KHDC3L.*

Other roles of NLRP7 and KHDC3L

Functional studies aimed at understanding the role of NLRP7 in the pathogenesis of RHM have been hampered by the lack of an animal model to study this disease as NLRP7 does not have orthologues in rodent or bovine. In humans, the closest gene to *NLRP7* is *NLRP2*, which lies 25 kb distal to *NLRP7* in a head-to-head orientation. *NLRP7* and *NLRP2* share a similar genomic structure and richness in Alu elements (Reddy *et al*.,

2016). In addition, the same richness in Alu elements is found in all their primate orthologues from chimpanzee to marmoset, indicating that these Alu elements have been inserted in the common *NLRP2/7* ancestor before its duplication into two genes. These recent observations corroborate a previous suggestion on the duplication of the common, *NLRP2/7*, ancestor into two separate genes in primates (Tian *et al*., 2009). Because of the absence of a rodent or bovine orthologues of *NLRP7*, functional studies to dissect its function have all been carried out on cellular models.

Regulation of inflammation. NLRP7 is a member of the NLRP protein family that consists of 14 members in humans, named NLRP1, NLRP2, NLRP3, and so on up to NLRP14. Two of these proteins, NLRP3 and NLRP12, have been shown to play a causal role in inflammatory diseases. When mutated, these genes lead to abnormal excessive activation of the inflammasome, a large multiprotein complex that results in the production of the proinflammatory cytokines IL1B and IL18. Studies from various groups and in different cellular models demonstrated that NLRP7 forms an inflammasome in response to stimulation by bacterial-derived products (acylated lipoproteins, lipospolysaccharides, etc.) (Khare *et al*., 2012; Singer *et al*., 2014; Zhou *et al*., 2016) and downregulates pro-IL1B secretion in stably transfected monocytic cell lines (Khare *et al*., 2012) as well as in peripheral blood mononuclear cells from patients with pathogenic variants (Messaed *et al*., 2011a). In addition, in transient transfections, overexpressing NLRP7 downregulates pro-IL1B production (Kinoshita *et al*., 2005; Messaed *et al*., 2011a).

Trophoblastic differentiation and proliferation

The role of NLRP7 in trophoblastic differentiation was first described in human H9 embryonic stem cells, where *NLRP7* knockdown led to accelerated expression of trophoblastic differentiation markers (Mahadevan *et al*., 2014). The same conclusion was also reached in a different study designed by our group to look for a correlation between HM features and the nature of the pathogenic variants in the patients. We found that severe (protein-truncating) pathogenic variants in the patients are associated with the absence of embryonic tissues of inner cell mass origin in the molar conception and excessive trophoblastic proliferation (Nguyen *et al*., 2014). However, milder (missense) pathogenic variants were associated with the presence of some embryonic tissues and mild trophoblastic proliferation.

Oocyte Cytoskeleton

In human oocytes, where NLRP7 and KHDC3L proteins play their primary roles, the two proteins colocalise perfectly, form a cytoskeleton that is different from that of α-tubulin, and are more abundant at the cytocortex (Akoury *et al*., 2015b). After fertilisation, both proteins move to the outer cortical region and are not present at the cell-to-cell contact regions. Basically, the localisation of NLRP7 and KHDC3L in human oocytes mimics that of KHDC3 (the mouse orthologue of human KHDC3L, also called FILIA) and NLRP5 in mouse oocytes and early embryos; the only difference is that human KHDC3L enters the nuclei starting from the morula stage where it remains until the blastocyst stage (Akoury *et al*., 2015b).

The localisation of NLRP7 and KHDC3L to the oocyte cytoskeleton raises an important question about how these cytoskeletal proteins affect trophoblast differentiation and proliferation. For sure, we do not know the answer to this question. However, if we were to speculate, one possibility is that the oocytes may have several other defects aside from the lack of DNA methylation on maternally imprinted DMR that would altogether be responsible for the lack of embryonic tissue differentiation. Another possibility is that NLRP7 and KHDC3L may be involved in intracellular trafficking of RNA and proteins that are essential for the activation of the embryonic genome. The fact that zygotes with different genotypes, diploid biparental, diploid androgenetic monospermic, diploid androgenetic dispermic and triploid dispermic genomes lead to HM suggests that the decision to develop into an HM is taken before the activation of the embryonic genome, which does not seem to be making a big difference.

Roles of KHDC3L

To date, no functional studies have been performed to address the role of KHDC3L protein in human cells. In mice, KHDC3 colocalises and interacts with NLRP5 in the oocyte cortical region, where the protein stability of FILIA depends on the presence of NLRP5 (Ohsugi *et al*., 2008). In another study on mice, null females for *Khdc3*$^{-/-}$ were found to have decreased fecundity due to abnormal spindle formation, chromosome misalignment in embryos, failure of the spindle assembly checkpoint and defective RhoA signalling (Zheng and Dean, 2009), which can be explained by an abnormal oocyte cytoskeleton.

Concluding Remarks

Studying rare Mendelian forms of common diseases is an opportunity to easily identify their causative genes, dissect the functions of their proteins and better understand the pathogenesis of the multifactorial forms of the diseases. Now, a decade after the identification of the first causative gene for RHM, what have we learned from the work on *NLRP7* and *KHDC3L* and how does this knowledge explain the sporadic common form of HM?

At the genotypic level, RHM from patients with recessive *NLRP7* or *KHDC3L* pathogenic variants are diploid biparental and originate from a different mechanism than sporadic androgenetic or triploid HM.

At the epigenetic level, RHM from patients with recessive pathogenic variants in *NLRP7* or *KHDC3L* lack maternal methylation marks on maternally methylated DMR, and mimic diploid androgenetic CHM, which lack a maternal genome.

RHM from patients with recessive pathogenic variants in *NLRP7* have defective oocytes and benefit from ovum donation. In human oocytes, NLRP7 and KHDC3L proteins colocalise to the cortical region. Similarly, all sporadic HM are believed to be caused by defects in the oocytes. Therefore, HM, in general, result from inherited or acquired defects affecting the oocytes.

Diploid biparental HM from patients with pathogenic variants in *NLRP7* have an imbalance between embryonic tissue differentiation and trophoblastic proliferation. This imbalance is also observed in androgenetic monospermic/dipsermic and triploid dispermic HM. The similarity between these different genotypic types of HM is certainly fascinating and remains to be elucidated in future studies.

NLRP7 is part of the innate immune system and its pathogenic variants downregulate inflammation. Similarly, patients with sporadic HM have a weak cellular-mediated immunity in response to phytohemagglutinin and concanavalin A, and delayed skin hypersensitivity to dinitrochlorobenzene, purified protein derivatives, and recall *Candida* antigens (Ho *et al*., 1980; Khanna *et al*., 1985; Tomoda *et al*., 1976)

Another similarity between inherited and sporadic HM is that most patients with pathogenic variants in *NLRP7* or *KHDC3L* originate from countries with high incidence of sporadic HM. In several of these countries, founder pathogenic variants in *NLRP7* (Estrada *et al*., 2013; Kou *et al*., 2008; Slim *et al*., 2009) and *KHDC3L* (Reddy *et al*., 2013) have been identified. In addition, variants in *NLRP7* have been shown to display a gradient of increasing frequencies from North to South (Slim *et al*., 2012). Our current explanation is related to *NLRP7*'s role in the immune system, which is known to display important differences between Northern and Southern populations. We believe that *NLRP7* defects are prevalent in Southern populations because they may have historically conferred some selective advantages for these populations, possibly against some infectious diseases. It is important to note that HM manifest in the first trimester of pregnancy (8–12 weeks), despite the fact that the embryos had stopped developing much earlier. Consequently, an important feature for the manifestation of any HM is its retention and delayed rejection by the maternal immune system.

In conclusion, the identification of two genes for RHM has, in general, advanced our understanding of the pathology of HM. The challenges ahead are to fully understand the roles *NLRP7* and *KHDC3L* and better understand the risk factors for common HM and how each risk factor predisposes to HM and to which genotypic type.

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