NLRP7 and the genetics of hydatidiform moles: recent advances and new challenges

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HISTORICAL VIEW ABOUT HM

Hydatidiform mole (HM) is an aberrant human pregnancy with no embryo that has fascinated and puzzled scientists in all civilizations. The recognition and description of this condition is very ancient and appears in Hippocrates’ manuals under the name “dropsy of the uterus” (1). In addition, a famous physician in the Byzantine period, Aetius of Amida, who was the private physician of Emperor Julian, wrote about moles and interestingly used the term inflammation to describe them, “an inflammation or strenuous walking” (2, 3). The etiology of HM continues to fascinate scientists in several aspects. HM is the only disease or conception (POC). A non-viable pregnancy accompanied with

CLINICAL AND ULTRASOUND MANIFESTATIONS

The clinical manifestation of moles has changed with the advances of medicine, largely because of the introduction of ultrasonography in the second half of the twentieth century as a routine exam to monitor all pregnancies starting from the eighth week of gestation. Consequently, many moles are now detected by ultrasound examination at the first gynecological visit or even earlier in cases of vaginal bleeding, which is the most common presenting symptom that would precipitate early medical consultation and diagnosis. Ultrasound indications of moles include the presence of echogenic structures in the placenta, the absence of a gestational sac, and/or the absence of fetal heart activity. These initial ultrasound observations are followed by a blood test of the human chorionic gonadotropin (hCG), the pregnancy hormone that is secreted mainly by syncytiotrophoblast cells of the chorionic villi (CV) into the intervillus space, whereupon it is carried to the maternal systemic blood circulation. HCG is much higher in women with molar pregnancies than in women with normal pregnancies of matching gestational stages, which is believed to be the consequence of the increased proliferation of syncytiotrophoblast cells. Depending on ultrasound findings, the gestational stage of the pregnancy, and the level of blood hCG, further ultrasound examinations and hCG follow-up tests may be required before a clinical decision is reached regarding the arrest of the pregnancy and the requirement of a surgical evacuation of the product of conception (POC). A non-viable pregnancy accompanied with
a high hCG test will necessitate dilatation and curettage suction of the POC. The evacuated tissues (Figure 1) are submitted for histopathological examinations and the diagnosis is made based on histopathological findings and criteria.

**HISTOPATHOLOGY AND DIAGNOSIS**

The original definition of HM was a pregnancy devoid of a fetus in which the chorion is replaced by grape-like vesicles. A long time ago, the severe form of this condition was believed to originate from pathologic ovaries (15) and was originally called “true moles” or “classical moles,” which correspond to what we now call complete HMs. This classification evolved and other terms emerged later to describe milder forms of the same condition such as “transitional,” “partial,” and “incomplete” moles (16–18). The current histopathological classification of spontaneously arrested pregnancies includes three entities designated complete HM (CHM), partial HM (PHM), and non-molar spontaneous abortion (SA) (19).

CHMs display circumferential trophoblastic proliferation affecting most CV (Figure 2) and do not contain extra-embryonic membranes (chorion and amnion), a fetal cord, fetal nucleated red blood cells, or any other embryonic tissue of inner cell mass origin. Both SAs and PHMs may contain extra-embryonic membranes (chorion and/or amnion), a fetal cord, fetal nucleated red blood cells, other embryonic tissues (cartilage, bones, etc.), or even a normal or an abnormal embryo in a single cavity (Figure 2). PHMs display mild and focal trophoblastic proliferation that can be observed on some CV and in several microscopic fields, whereas SAs do not display abnormal circumferential trophoblastic proliferation to warrant close hCG follow-up (Figure 2). The histopathological subdivision of spontaneously arrested pregnancies into CHMs, PHMs, and SAs has always been challenging and several scientists have noted the continuous variation in the molar degeneration (18). This challenge is more problematic nowadays because of the early evacuation of arrested pregnancies based on ultrasonography and before the manifestations of all their histopathological features. Consequently, there is a wide inter-observer and intra-observer variability in distinguishing non-molar SAs from PHMs and in distinguishing PHMs from CHMs (20–22). Practically, the difficulty for the pathologists is to know where to draw the lines of separation between the three entities due to the continuous spectrum of abnormalities and due to the fact that histopathology is a qualitative descriptive science (mild, excessive, focal, occasional, etc.) that lacks quantitative measurements to assess the degree and extent of trophoblastic proliferation.

**KARYOTYPE AND GENOTYPE DATA**

Karyotype and genotype analyses have shown that sporadic moles may have different genotypic types with the majority of CHMs being diploid androgenetic and the majority of PHMs being triploid diandric dispermic. Among androgenetic moles, the majority are monospermic and 10–20% are dispermic (23–27). In a minority of cases, some CHMs have been reported to be diploid biparental (25), triploid diandric dispermic (23), tetraploid triandric (3 paternal and 1 maternal sets of chromosomes) (28) or digynic (29), aneuploid, or mosaic with two cellular populations. Sporadic PHMs are mostly triploid diandric dispermic, but they have also been reported with diploid biparental, triploid digynic (29), triploid monospermic (30), tetraploid triandric (31, 32), or aneuploid genomes. Based on the major categories of complete and partial moles, different hypothetical models at the origin of moles’ formation were proposed and have been accepted by the scientific community over the last 30 years. One of these models postulates that an androgenetic monospermic mole results from the fertilization of an empty oocyte by a haploid sperm that undergoes an endoreduplication of its genome to form the diploid androgenetic monospermic mole. Similarly, androgenetic dispermic moles would result from the fertilization of an empty oocyte by two spermatzoa, while triploid diandric (or dispermic) moles would result from the fertilization of a haploid oocyte by two different haploid spermatzoa. These accepted models were recently challenged by Golubovsky (33) who refutes the existence of empty oocytes at the origin of androgenetic moles. Instead, he proposes that dispermic fertilization followed by complex postzygotic abnormalities and diplodization is at the origin of the various genotypic types of moles as well as mixoploidies, trisomies, and various aneuploidies. These different models and their implications in the genesis of HMs are discussed below.

**POST-EVACUATION hCG SURVEILLANCE AND MALIGNANCIES**

Molar pregnancies are the most common gestational tumors and are benign in about 80% of cases. In these cases, hCG falls to non-pregnant levels after the surgical evacuation of the molar conception. However, in about 20% of cases in western countries, elevated hCG levels persist for several weeks post-evacuation or rise after falling down, which indicates the retention of some trophoblastic tissues. Such conditions are termed persistent gestational trophoblastic diseases (PGTDs) or gestational trophoblastic neoplasias (GTNs) and may necessitate a second surgical evacuation and/or chemotherapy treatments. GTNs occur most commonly after CHMs (15–29%), less frequently after PHMs (0.5–4%), and rarely after SAs, ectopic pregnancies, or normal pregnancies (34–36). Several classification systems of GTNs have been elaborated.
over time and are used to help standardize and optimize treatments of these conditions. For good reviews on these topics see (37–39).

In recent times, the most commonly used guidelines are those of the World Health Organization (WHO) and the Fédération Internationale de Gynécologie et d’Obstétrique (FIGO).

The most common malignant degeneration of HMs or GTNs are invasive moles and gestational choriocarcinomas (CCs). The diagnosis of invasive moles is based on persistent or rising levels of hCG and histopathological identification of CV within the myometrium (the deep layer of uterine tissues beneath the endometrium), maternal blood vessels, or within extrauterine tissues. Invasive moles affect approximately 20 and 2–4% of patients with CHMs and PHMs, respectively (34).

CCs may occur after any type of pregnancy in the following proportions: 35–60% after CHMs, 0.5–2% after PHMs, 15–20% after SAs, 1–2% after ectopic pregnancies, and 25–42% after normal pregnancies (40, 41). The diagnosis of CC is based on high hCG levels and both clinical and laboratory evidence demonstrating the presence of tumor cells in distant maternal tissues such as the lung, lower genital tract, brain, liver, kidney, gastrointestinal tract, and spleen. A definitive diagnosis of CC is based on histopathological findings demonstrating the presence of cytotrophoblastic and syncytiotrophoblast cells, without organized villous structures in distant maternal tissues (42). CCs are the most aggressive GTNs because of their ability to spread hematogenously. They may be fatal in the absence of appropriate follow-up and management. Again, both invasive HMs and CCs have higher frequencies in both developing and underdeveloped countries than in developed countries (40).

**FIGURE 2** | (A) Hematoxilin and eosin (H&E) staining of a section of chorionic villi (CV) from a CHM. Note the presence of excessive circumferential trophoblastic proliferation around all CV (arrows) and the beginning of hydropic degeneration in two CV (asterisks). (B) H&E staining of a section of CV from a PHM. Note the presence of circumferential trophoblastic proliferation (arrows) around one chorionic villus (indicated by CV) while the others have no or few sprouts of trophoblastic proliferation (arrows). Note the presence of nucleated red blood cells inside the chorionic villus (on the right corner) in the conception that led to PHM. (C) One CV from a PHM displaying trophoblastic inclusions (arrows and magnified view on the right corner). (D) A view from a PHM showing phalanges of fetal foot. (E) Another view from a PHM showing fetal membranes. (F) H&E staining of a section of CV from a spontaneous abortion. Note the absence of trophoblastic proliferation around the CV.

**THE IMPORTANCE OF CROSSING OUR DISCIPLINE**

Despite the ancient clinical recognition of HMs and the presence of several reports describing cases of recurrent moles (15, 43–46) no attempts were made to identify causative genes for the recurrent form until the report by Seoud et al. (47) that led to the mapping of the first maternal locus to 19q13.4 (48). At that time, only six other familial cases of RHMs had been reported in the English PubMed literature since 1980 (49–52). Consequently, we and others believed that the familial form of moles was extremely rare. However, this was not true and approximately 30 new familial cases of RHMs had been reported in the English PubMed literature since 1980 (49–52). Consequently, we and others believed that the familial form of moles was extremely rare. However, this was not true and approximately 30 new familial cases have been reported since 1999 (47, 53–69) indicating that familial RHMs are not extremely rare as originally believed, but were probably under-reported. In addition, about 88 singleton cases of RHMs have been described since 1999. The importance of the case reported by Seoud and his collaborators (47) is in the fact that the authors crossed the boundaries of their disciplines, a common practice in many medical specialties, but a rare one in the field of Obstetrics and Gynecology. These authors sought the help of scientists from other disciplines at a time where small nuclear consanguineous families were an opportunity for gene mapping by homozygosity analysis. This original family as well as another (51) led to the mapping of the first maternal-effect locus responsible for recurrent moles to 19q13.4 (48) and opened a new avenue of research aimed at identifying maternal genes causing RHMs and recurrent fetal loss.

**LESSONS FROM STUDYING EXTREME PHENOTYPES**

One difficulty associated with homozygosity mapping and studying rare families is in narrowing down the size of the candidate...
intervals. This was the case of 19q13.4 candidate region, which was originally four megabases and is a gene-dense region. Consequently, the identification of the causative gene, NLRP7, was tedious and required the screening of 80 different genes until the first causative mutations were identified (70). The mutations segregated in the studied families and each patient had two defective alleles, each inherited from one parent as expected for an autosomal recessive disease. Later, others and we confirmed the causality of NLRP7 mutations in patients from different populations (54, 60, 63, 66, 67, 71, 72), demonstrating that NLRP7 is a major gene for RHMs. To date, approximately 42 different mutations have been reported in patients with two defective alleles (Figure 3) (73) Of these mutations, 65% are protein-truncating (stop codon, splice mutations, small insertions and deletions, and large rearrangements) and 35% are missense mutations, which are, respectively, higher and lower than the frequencies of these two categories of mutations observed in all human diseases, 56 and 44% (http://www.hgmd.cf.ac.uk/ac/index.php). Although, this difference is not statistically significant, it indicates that patients with RHMs and two mutations may represent the most severe phenotype of the disease.

The identification of NLRP7 is therefore one of many examples where rare families segregating severe monogenic Mendelian forms of common conditions have led to the identification of causative genes [for an interesting review on the subject see (74)]. This raises an important question: do familial RH cases with NLRP7 mutations have more severe mutations than singleton cases? The originally reported families tended to have more protein-truncating mutations than singleton cases. However, this is no longer the case since reports of singleton cases with protein-truncating mutations have increased lately. This could be due to the fact that many singleton cases do not manifest as familial cases because of the small size of families in current societies and/or the lack of other female siblings who have tried to conceive. These factors may have prevented the familial manifestation of many singleton cases with inherited mutations from the two parents.

**NLRP7 EXPRESSION**

Before the identification of the causal link between NLRP7 and RHMs, NLRP7 transcripts were shown to be expressed in a large number of human tissues including liver, lung, placenta, spleen, thymus, peripheral blood leukocytes, testis, and ovaries (75, 76). After our group linked NLRP7 to RHMs, we investigated its expression in oocytes and detected its transcripts in all stages of immature oocytes, fertilized eggs, and early embryo cleavage stages (70). These data were later confirmed in an interesting study that showed that NLRP7 transcripts decrease progressively during oocyte maturation and reach their lowest level on day 3 post-fertilization, which corresponds to the morula stage, then increase sharply from day 3 to day 5, which corresponds to the blastocyst stage and the activation of the fetal genome transcription (77).

At the protein level, NLRP7 expression was shown in all stages of growing follicles and in all these stages, its expression was restricted to oocytes (72). In another study reported by our group, we detected variable levels of NLRP7 protein in seven analyzed hematopoietic cells: Epstein Barr virus transformed

![FIGURE 3 | Schematic representation of NLRP7 protein structure and identified mutations and variants in patients with hydatidiform moles and reproductive loss. PYD, stands for the pyrin domain, NACHT, stands for found in the NAIP, CIITA, HET-E, and TP1 family proteins; ATP for adenosine 5-triphosphate binding motif; and LRR, for leucine rich repeats. The ATP binding domain is a small motif of 8 amino acids and starts at position 178. Mutations found in patients with two defective alleles are in red and include nonsense, frameshift, invariant splice site, and missense mutations. Variants found in patients in a heterozygous state and not in controls are mostly missenses and are in blue. Variants found in patients and in subjects from the general population are in black. Mutation nomenclature is according to the Human Genome Variation Society (HGVS) guidelines (http://www.hgvs.org/mutnomen/recs.html).](http://www.frontiersin.org)
B-lymphocytes, BJAB, Raji and Ramos (all of B-cell origin), Jurkat (of T-cell origin), and THP1 and U937 (both of monocytic origin) (78).

**NLRP7 DOMAINS**

The NLRP7 protein consists of three domains: (i) an N-terminal pyrin, (ii) a NACHT termed after four proteins containing an NTPase domain with significant similarities, neuronal apoptosis inhibitor protein (NAIP), MHC class II transcription activators (CIITA), incompatibility locus protein from Podospora anserine (HET-E), and mammalian telomerase-associated proteins (TP1); and (iii) a C-terminal stretch of 9 or 10 leucine rich repeats (LRRs) depending on splice isoforms (Figure 3). The pyrin domain is a small domain (92 amino acids) found in all NLRPs and apoptotic proteins. The pyrin domain functions as an adaptor that helps to connect proteins of the programmed death machinery. Pyrin domains can self-associate to form homodimers or associate with other proteins containing structurally related domains to form heterodimers. Domains known to interact with the pyrin domain include the death domain (DD), the death-effector domain (DED), and the caspase activation and recruitment domain (CARD). These pyrin-mediated associations result in the formation of protein complexes and networks that transmit signals from receptors to downstream effectors that function in various cell-death pathways (79). The NACHT domain has an ATP/GTPase-specific P-loop domain, which is a very ancient domain found in bacteria, plants, and all eukaryotes. NTPase domains are found in both apoptotic and anti-apoptotic proteins; they control programmed cell-death during development by regulating cytochrome c efflux from the mitochondria, which stimulates apoptosis (80). The LRR domain is found in other proteins with divergent functions such as Toll-like receptors (TLRs), Ras GTPase, and RNase inhibitor proteins. TLRs are components of the innate immune system, from which the LRR extends into the extracellular milieu where it senses extracellular danger signals and transmits the signals to cytoplasmic proteins. Ran GTPases are essential for transporting RNAs and proteins through the nuclear pore complex by interacting with shuttling transport proteins and changing their ability to bind or release cargo molecules. Finally, RNase inhibitor proteins bind RNase A and angiogenin and regulate RNA degradation and angiogenesis (81).

**KNOWN FUNCTIONS AND ROLES OF NLRP7**

The most studied functions linked to the different NLRP domains are those involved in the activation of the innate immune system in response to various microbial and chemical products. With respect to NLRP7, four studies have addressed its functional roles to date and their results are recapitulated in Table 1. Using transient transfections, two studies showed that NLRP7 down-regulates the intracellular level of mature IL1B (76, 78). While the first study showed that this is due to the down-regulation of pro-IL1B processing (76), the second, by our group, showed that this is due to the lower production of intracellular pro-IL1B (78). In addition, we found that in transient transfections, NLRP7 inhibition of pro-IL1B production is mediated concomitantly by its three domains, with the strongest effect being mediated by the LRR, followed by the NACHT and the pyrin domains (78). In the study by Kinoshita et al., the authors showed that NLRP7 binds pro-IL1B and procaspase 1 and inhibits IL1B secretion induced by caspase 1, ASC, or NLRP1-delLRR. They also showed that both recombinant mouse IL1B and LPS stimulation enhance NLRP7 transcription, which in turn down-regulates IL1B secretion. They concluded that NLRP7 is a negative feedback regulator of IL1B and consequently plays an anti-inflammatory role (76).

Part of the study conducted by our group was performed on *ex vivo* LPS-stimulated peripheral mononuclear blood cells from patients with one or two mutations in NLRP7. This experiment demonstrated the requirement of wild type NLRP7 for normal IL1B secretion (78). Within monocytes, which are the main cells that secrete IL1B, NLRP7 co-localized with the Golgi apparatus and microtubule organizing center (MTOC) (Figure 4) (78). Moreover, treatment of EBV lymphoblastoid cell lines with nocodazole, a drug that depolymerizes microtubules resulted in the fragmentation of NLRP7 signal. This suggested that normal NLRP7 associates with microtubules and that its mutations may impair cytokine secretion by disrupting microtubules structures and consequently affecting intracellular trafficking of IL1B vesicles. The role of NLRP7 in IL1B secretion was confirmed in another independent study involving silencing NLRP7 in macrophages using small interfering RNA (82). In this study, the effect of silencing eight other NLRPs was also tested, but only NLRP7 knockdown significantly decreased IL1B secretion. This study by Khare et al. also confirmed the physical interaction between NLRP7, ASC, and caspase 1 via the pyrin domain, and that the LRR of NLRP7 is required for sensing bacterial acylated lipopeptides.

Khare et al. (82) also revealed another function of NLRP7 by demonstrating that NLRP7 silencing promotes intracellular growth of *Staphylococcus aureus* and *Listeria monocytogenes*. A prior study implying a role for NLRP7 in cellular proliferation, but in the opposite direction, was reported by Okada et al. (75), who showed that silencing NLRP7 reduces the proliferation of human embryonal carcinoma cell lines, suggesting that the normal protein promotes cellular growth and has an oncogenic role. The mechanisms leading to both functions are currently unclear and need to be explored in future studies. However, from the HM perspective, we tend to believe in the role suggested by Khare et al. (82), because an important feature of molar tissues from patients with two NLRP7 defective alleles, which are diploid biparental and obligate carriers of one mutated copy of NLRP7, is the excessive proliferation of their trophoblastic cells. This is in line with the data by Khare et al., and is a further indication that NLRP7 mutations promote cellular growth.

**UNDERSTANDING THE VARIABILITY OF A PHENOTYPE: BACK TO THE GENETIC COMPLEXITY OF REPRODUCTIVE LOSS**

An important aspect of our understanding of any disease or system is to understand its variability and determine its extreme phenotypes with its most and less severe manifestations. Despite the fact that we named the 19q13.4 locus as responsible for RHMs, affected patients from the original family, MoLb1, experienced, in addition to their moles, other forms of reproductive loss, namely SAs, stillbirths, an early neonatal death, one malformed live birth, and two live births that led to healthy adults. This large variability...
in the reproductive outcomes of three patients from MoLb1 was intriguing because such variability is unusual in recessive diseases. However, this variability was not restricted to one family, but was observed, to a lesser extent, in other families studied by our group. Furthermore, this variability was in agreement with data from a large epidemiological study showing increased frequencies of moles, preterm births, stillbirths, and ectopic pregnancies in women with at least two SAs (83). These observations led us to extend our inclusion criteria for \textit{NLRP7} sequencing to women with at least three SAs and no moles as well as to women with the sporadic, common, non-recurrent moles. This analysis showed that two of the 26 analyzed women with recurrent SAs (8%) and eight of the 64 analyzed women with a single HM (associated with and without other forms of reproductive losses) (13%) have novel \textit{NLRP7} non-synonymous variants (NSVs), all missenses in heterozygous state, which were not found in a large number of control subjects from the same ethnicity of the patients (\textit{Figure 5}) (84). One of the two patients with >3 SAs and a missense mutation had a persistent gestational trophoblastic disease requiring chemotherapy after one of her miscarriages. Moreover, six of the patients

| Table 1 | Recapitulation of the functional roles of \textit{NLRP7} in different studies and cellular models. |
|---------|-------------------------------------------------|-------------------------------------------------|
| (75) LPS or rm-IL1B induce \textit{NLRP7} transcription in PBMC and THP1 | | |
| \textit{NLRP7} down-regulates pro-IL1B and pro-caspase 1 processing leading to lower intracellular mature IL1B | \textit{NLRP7} down-regulates pro-IL1B production leading to lower intracellular mature IL1B | \textit{NLRP7} silencing reduces IL1B secretion in macrophages |
| \textit{NLRP7} interacts with transfected pro-caspase 1 and pro-IL1B | \textit{NLRP7} and IL1B subcellular localization overlaps Cells with \textit{NLRP7} mutations have low secreted TNF | \textit{NLRP7} interacts with caspase 1 and ASC in HEK293 cells through the pyrin domain \textit{NLRP7} Silencing does not affect IL6 or TNF secretion by macrophages |
| \textit{NLRP7} silencing with siRNA reduces cellular proliferation | | |

LPS stands for lipopolysaccharides; PBMC for peripheral blood mononuclear cells; \textit{NLRP1}-delLRR stand for \textit{NLRP1} in which the leucine rich repeat is deleted; \textit{siRNA} for small interfering RNA; rm-IL1B, indicates recombinant mouse IL1B. Conclusions obtained by at least two independent studies are in bold character.

\textbf{FIGURE 4 | \textit{NLRP7} expression in monocytes using immunofluorescence.} \textit{NLRP7} stains two small dots specific for the microtubule organizing center, which is also revealed with \textit{γ}-tubulin as previously reported (78).
with one HM and a NSV in \textit{NLRP7} had at least two other reproductive losses, in addition to their HMs, indicating their genetic susceptibility to recurrent reproductive loss. In addition, patients with one defective allele statistically had less severe reproductive outcomes and more live births than patients with two defective alleles ($p$-value $= 2.809e^{-06}$) (Figure 6).

In conclusion, this analysis did provide a positive answer to our search for mutations in milder phenotype of RHMs. However, it raised challenging questions that all scientists working on complex traits are currently facing: how do we define a pathological NSV? And what tells us that these rare NSVs, found in heterozygous states in a so far believed autosomal recessive disease, have functional consequences on the protein and confer genetic susceptibility for reproductive loss?

**FIGURE 5** | Summary of \textit{NLRP7} mutation and non-synonymous variants found in 135 unrelated patients with varying histories of reproductive wastage. HM stands for hydatidiform mole; SA, stands for spontaneous abortion; NSV, for non-synonymous variant. Mutations in \textit{NLRP7} were most frequently observed in patients with at least two HMs, followed by patients with one HM, and then by patients with at least three SAs (84).

**FIGURE 6** | A comparison of reproductive outcomes between women with two or one defective \textit{NLRP7} allele. In both histograms, $n$ indicates the total number of pregnancies from patients in either category. HM, hydatidiform mole; SA, spontaneous abortion; SB, stillbirth; and LB, live birth. A higher incidence of HMs and a lower incidence of live births are observed in patients with two defective alleles.
Table 2 | Examples of genes causing rare severe recessive diseases and conferring susceptibility to common or related forms of the same disease.

| Gene                  | Two defective alleles                                      | Single mutated allele                                                                 | Reference |
|-----------------------|-----------------------------------------------------------|---------------------------------------------------------------------------------------|-----------|
| PINK1                 | Autosomal recessive Parkinson disease (PD) with early onset | More rare variants in patients vs. controls (10 vs. 2) Milder phenotype and later onset in heterozygous relatives of severely affected patients in large pedigrees | (90, 92)  |
| ATP13A2               | Juvenile onset Parkinson disease <21 years                 | Young onset Parkinson disease                                                         | (93)      |
| GBA                   | Gaucher’s disease                                          | More rare variants in patients with PD vs. controls. This seems specific to some ethnic groups, e.g., Ashkenas, French | (94, 95)  |
| MEFV                  | Familial mediterranean fever                               | In 15% of patients                                                                    | (97)      |
| ABCA1                 | Familial hypoalphalipoproteinemia                          | More rare variants in individuals with low HDL-C than in those with high HDL-C (18% vs. 2%) | (98)      |

or/and age of onset or have a related condition that include some of the features of the severe disease (93–97).

With respect to RHMs, the age of onset is not an appropriate indicator of severity; however, a severe genetic defect would translate into recurrence and would be expected to lead to the same genetic defect every time a patient tries to conceive. On the contrary, a milder genetic defect, which can be modulated by other environmental factors, would be expected to lead to more variability in the reproductive outcomes of the patients. This is exactly the conclusion we reached in the last analysis performed on three categories of patients (RHM, sporadic HM, and recurrent SA), which showed that patients with RHM have the highest frequency of NLRP7 mutations (60%), and these patients had mostly two defective alleles, each. However, 13% of patients with one mole and other reproductive wastage had a single variant in a heterozygous state, while 8% of patients with at least three SAs had rare NLRP7 variants in heterozygous state (Figure 5). Similar results were obtained from patients with sporadic HM and reproductive wastage in a different population (Tunisian) and again showed the presence of NLRP7 variants in heterozygous state in 13% of the patients (59). Additional case-control studies designed to screen all NLRP7 exons in patients with sporadic HM and recurrent SAs are needed to assess whether the burden of NLRP7 mutations and rare NSVs is higher in patients than in ethnically matched controls. In the meantime, a number of other tests can be used to investigate the pathogenicity of encountered variants. These include (i) the absence of the variants in controls of matching ethnicity to the patients; (ii) the conservation of the changed amino acids throughout evolution; (iii) the predicted functional consequences of the identified variants using various algorithm; (iv) the segregation of the variants on different haplotypes when present with other known deleterious mutations; (v) the functional impact of the variants on the protein subcellular localization; and ideally (vi) the impact of the variants on the protein function in any type of cellular assays.

**GENOTYPE OF HM TISSUES IN PATIENTS WITH NLRP7 MUTATIONS**

To date, the parental contribution to approximately 70 HM tissues from patients with two defective alleles in NLRP7 have been characterized and all of them were found to be diploid biparental (55, 62, 63, 87, 98–100) with the exception of one tissue that was digynic (101). However, this is not the case for HM tissues from patients with single heterozygous rare NLRP7 variants. In this category of patients, few HM tissues were genotyped; some were found to be diploid androgenetic monospermic (67, 85, 87, 89) and others were found to be triploid diandric dispermic (102). The reason for this difference is not yet clear and needs to be addressed in future studies. Such studies may also clarify whether specific single heterozygous rare NLRP7 variants confer a genetic susceptibility to a specific genotypic type of moles. This would help elucidating the mechanisms of the formation of different genotypic types of moles. This is particularly important because the currently accepted mechanisms of mole formation are hypothetical and the emerging ideas propose a single model stemming from dispermic fertilization followed by postzygotic abnormalities (33).

**NLRPs and Reproduction**

**Nlrp5**

Nlrp5 (originally called Op1 then Mater, and lately Nlrp5) is the first NLRP gene shown to play a causative role in mammalian reproduction (103). Nlrp5 was isolated from a mouse model of autoimmune oophoritis (also termed premature ovarian failure) generated by neonatal thymectomy. Female mice thymectomized in the third day after birth spontaneously develop autoimmune disorders characterized by organ-specific inflammation and lymphocyte infiltration (104). In some mouse strains, the predominant autoantibody is directed against the ovary where it reacts with NLRP5. To gain insights about the role of NLRP5 in autoimmune oophoritis, the authors generated knockout null females, NLRP5−/−, and found that these females ovulate normally and their oocytes fertilize in vivo with no apparent abnormalities. However, their embryos stop developing at the two-cell stage, a time at which major embryonic genome activation takes place. The role of NLRP5 in preimplantation embryonic development was also confirmed in monkeys where its knockdown in MII oocytes resulted in a significant reduction in the number of embryos that reached the blastocyst stage (105). In mouse oocytes, NLRP5 is part of specialized oocyte cytoskeletal structures (called cytoplasmic lattices) that are responsible for the distribution of organelles, maternal mRNA, and maternal proteins in the oocytes (106–108). Also, previous studies on NLRP5 showed that within...
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CONCLUSION

Since the identification of Nlrp5 and NLRP7, the list of NLR genes with maternal-effects continues to grow. We expect this list to expand even further because of the presence of four additional NLRPs besides NLRP4 and NLRP2 that show oocyte-specific expression and have not yet been linked to reproduction in any organism: NLRP8, 9, 11, and 13. All of these NLRPs are highly expressed in germinal vesicle oocytes and decrease during preimplantation development to reach their lowest levels at the blastocyst stage, which is in favor of their maternal-effect role. With respect to NLRP7, we do not yet know the exact role of its protein in human oocytes. However, based on several observations, we believe that oocytes from patients with mutations are defective at several levels and are not able to sustain early embryonic development. Consequently, the embryos stop developing very early in these conceptions. Because these patients also have decreased cytokine secretion, we believe that they fail to mount an appropriate inflammatory response to reject these arrested pregnancies as normal women would. As a result, the retention of these dead pregnancies with no embryos to later gestational stages leads to the hydropic degeneration of CV. This, combined with the potential role of NLRP7 mutations in enhancing proliferation, may lead to the three fundamental aspects of moles: aberrant human pregnancies with no embryo, abnormal excessive trophoblastic proliferation, and hydropic degeneration of CV.

We believe that fully understanding the three aspects of the pathology of HM would greatly benefit from collaborations between scientists in various medical fields.

ACKNOWLEDGMENTS

We thank all our patients for their participation in our studies. We thank Phuong Ngoc Minh Nguyen for histopathology photos and Elie Akoury for the immunofluorescence photos. I am indebted to all members of my laboratory for their work and discussions. Evan P. Wallace is supported by a CREATE fellowship from the Réseau Québécios en Reproduction. Rima Slim is supported by the following grants (MOP102469, MOP86546, PPP122897, and CCI125687) from the Canadian Institutes of Health Research.
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any com- mercial or financial relationships that could be construed as a potential con- flict of interest.

Received: 20 June 2013; paper pending published: 21 July 2013; accepted: 05 August 2013; published online: 20 August 2013.
Citation: Slim R and Wallace EP (2013) NLRP7 and the genetics of hydatidiform moles: recent advances and new challenges. Front. Immunol. 4:242. doi: 10.3389/fimmu.2013.00242
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