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Biallelic *NLRP7* variants in patients with recurrent hydatidiform mole: A review and expert consensus

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Abstract

Hydatidiform mole (HM) is an abnormal human pregnancy characterized by excessive growth of placental trophoblasts and abnormal early embryonic development. Following a first such abnormal pregnancy, the risk for women of successive molar pregnancies significantly increases. To date variants in seven maternal-effect genes have been shown to cause recurrent HMs (RHM). *NLRP7* is the major causative gene for RHM and codes for NOD-like receptor (NLR) family pyrin domain containing 7, which belongs to a family of proteins involved in inflammatory disorders. Since its identification, all *NLRP7* variants have been recorded in Infevers, an online registry dedicated to autoinflammatory diseases (<https://infevers.umai-montpellier.fr/web/>). Here, we reviewed published and unpublished recessive *NLRP7* variants associated with RHM, scored their pathogenicity according to the American College of Medical Genetics classification, and recapitulated all functional studies at the level of both the patients and the conceptions. We also provided data on further variant analyses of 32 patients and genotypes of 36 additional molar pregnancies. This comprehensive review integrates published and unpublished data on *NLRP7* and aims at guiding geneticists and clinicians in variant interpretation, genetic counseling, and management of patients with this rare condition.

KEYWORDS

biallelic variants, diploid biparental moles, expert consensus, Infevers, *NLRP7*, recurrent hydatidiform mole, subcortical maternal complex

1 | BACKGROUND

Hydatidiform mole (HM), or molar pregnancy, is an abnormal human pregnancy characterized by trophoblastic proliferation and limited fetal development. HMs usually result from chromosome abnormalities that lead to an excessive contribution of the paternally derived genome. Complete HMs (CHMs) are diploid and androgenetic (Kajii & Ohama, 1977; Wake et al., 1978). The majority develop following loss of the maternal genome from the ovum and doubling of the paternal genome (Jacobs et al., 1982; Lawler et al., 1982), with approximately 15% developing following fertilization of an ovum by two sperms and loss of the maternal chromosomes (Xing et al., 2021). Partial HMs (PHMs) almost always arise following fertilization of an ovum by two sperms, with retention of the maternal chromosomes, and are consequently triploid. These unusual conceptions are clinically important because of the significant risk, 13.6% after a CHM and 1.1% after a PHM, of the molar tissue progressing to a malignant gestational trophoblastic neoplasia (GTN) which would require chemotherapy (Savage et al., 2013).

Following a pregnancy with an HM, women experience an increased risk of another molar pregnancy, a risk which increases with successive molar pregnancies (Eagles et al., 2015; Savage et al., 2013). RHM are estimated to affect 1%–4% of women with a prior HM, most of whom have no family history of HMs while the remaining are familial (Berkowitz et al., 1998; Horn et al., 2006; Kim et al., 1998; Lurain et al., 1982; Sand et al., 1984; Sebire et al., 2003; Yapar et al., 1994). Studies of the latter cases led to the mapping of a locus in 19q13.4, in which biallelic variants in the mother are responsible for familial RHM (Moglabey et al., 1999). While successive molar pregnancies may be triploid dispermic or diploid androgenetic, most molar pregnancies from familial cases have a diploid, biparental genome (Helwani et al., 1999).

To date, variants in seven maternal-effect genes, *NLRP7* (Murdoch et al., 2006), *KHDC3L* (Parry et al., 2011), *PADI6* (Qian et al., 2018), *NLRP5* (Rezaei et al., 2021), *MEI1* (Dong et al., 2021), *TOP6BL*, and *REC114* (Nguyen et al., 2018; W. Wang et al., 2020) have been shown to underlie the genetic etiology of RHM. Of these genes, *NLRP7* (previously named *NALP7*) is the major gene, in which variants explain the etiology of 55% of patients with at least two HMs (Nguyen et al., 2018). *NLRP7* is a member of the NOD-like receptor (NLR) protein family that consists of 14 members in humans (*NLRP1*, *NLRP2*, *NLRP3*, *NLRP5*, etc.) (Zambetti et al., 2012). Loss of one of these proteins, *NLRP5*, had been shown to be responsible for female infertility in mice due to early embryonic arrest at the two-cell stage (Tong et al., 2000). *NLRP3*, had been shown to play a causal role in cryopyrin-associated autoinflammatory diseases (Hoffman et al., 2001). The second gene in which variants are responsible for familial RHM is *KHDC3L* (Parry et al., 2011), but explains only 5% of RHM cases. Since the identification of *NLRP7*, all variants reported in this gene in patients with RHM and other rare or common nucleotide substitutions in the general population, have been recorded in Infevers, an online registry of autoinflammatory variants (<https://infevers.umai-montpellier.fr/web/>) (Milhavet et al., 2008).

The current study was undertaken to review the spectrum of *NLRP7* variants associated with RHM, classify them according to the

American College of Medical Genetics recommendations (den Dunnen et al., 2016), recapitulate all functional studies at the level of both the patients and their conceptions, and examine the correlation between the nature of the variants in the patients and the phenotype of their conceptions. Our objective is to integrate published and unpublished data on *NLRP7* in a comprehensive review to better guide variant interpretation, genetic counseling, and management of patients with this rare condition.

2 | MATERIALS AND METHODS

2.1 | Variant collection and classification

The different steps of data collection used in this review are summarized in Figure 1. A systematic review of *NLRP7* variants (NM_001127255.1) recorded in Infevers was undertaken and completed (1) through a PubMed search with the term “*NLRP7*” to identify missing or recently reported variants, and (2) inclusion of genetic data from 32 additional patients analyzed in the authors' (R. S., I. T., R. F.) laboratories, but not yet published. All *NLRP7* variants located in exons or intronic boundaries, and regulatory regions, were then downloaded from Infevers, inserted in a sheet specifically created for variant classification, and the data were analyzed using an original workflow as previously described (Van Gijn et al., 2018). In brief, five possible pathogenicity scores, namely benign, likely benign, variant of uncertain significance, likely pathogenic and pathogenic, were blindly and independently assigned to *NLRP7* variants by R. S., R. F., S. R., and I. T. through sequential rounds of voting and/or discussions. The classification was established according to the American College of Medical Genetics and Genomics recommendations and integrated data from both reports and the authors' own unpublished studies using Varsome and the Genetic Variant Interpretation Tool of the University of Maryland School of Medicine. A classification was considered validated if at least 75% of the votes were consistent, provisional if between >50% and 75% of the votes were consistent, and unsolved otherwise. Pathogenicity scores with their respective status were then uploaded to Infevers and made publicly available.

2.2 | Patient data collection

The study protocols were approved by the Institutional Review Board of McGill University (A01_M07_03A) and the Joint Research Compliance Office of Imperial College London and Imperial College Healthcare NHS Trust (reference number 15HH2513). Patients with RHM were identified from publications accumulated over the years and unpublished work performed in our three laboratories (Figure 1, right panel). Inclusion criteria were patients with two or more HMs in whom sequencing of *NLRP7* showed homozygosity or compound heterozygosity for variants in *NLRP7*, of which at least one is pathogenic or likely pathogenic. While biallelic variants could not always be phased by sequencing samples from other family members,

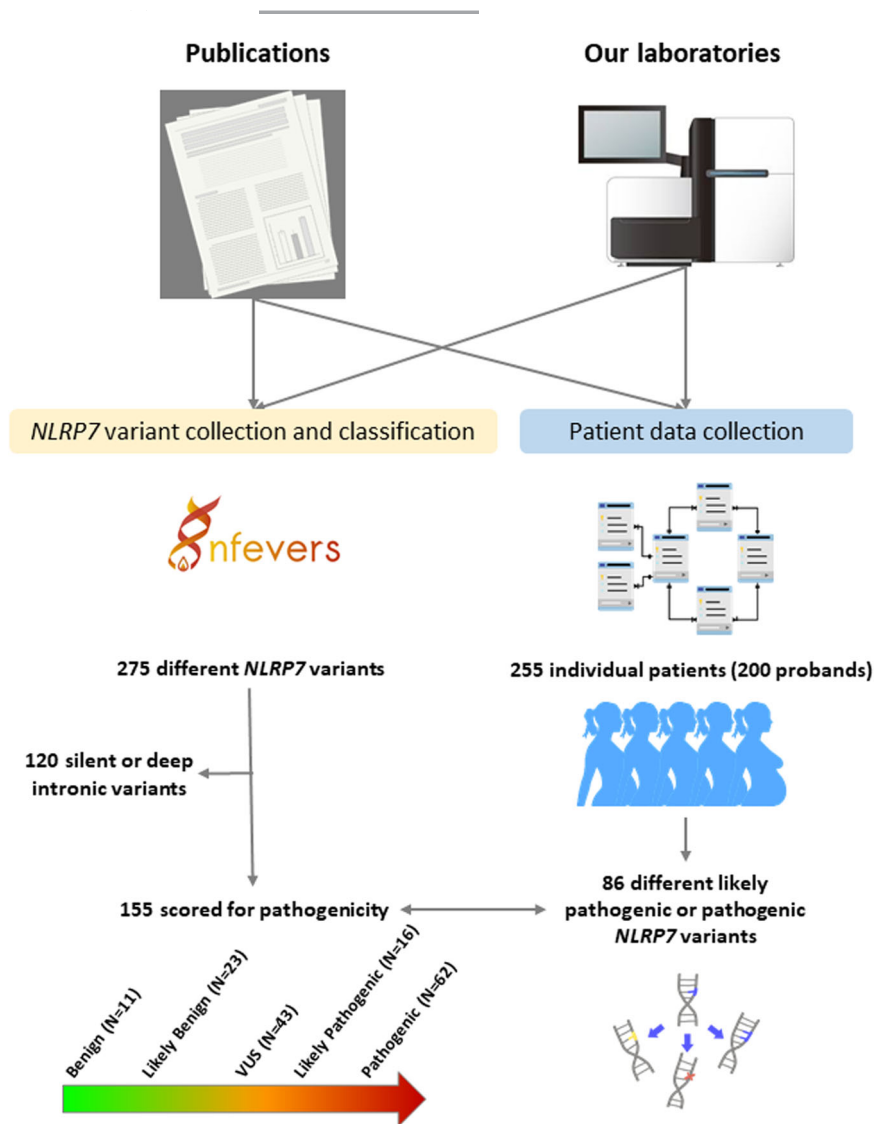


FIGURE 1 Workflow used to collect *NLRP7* and patient data for this review. *Left panel*: classification of *NLRP7* variants according to their pathogenicity score. After completing the list of variants included in the Infevers registry (275 different variants at the end of the process) (<https://infevers.umai-montpellier.fr/web/search.php?n=8>), 155 relevant variants were extracted and a workflow, previously established for autoinflammatory diseases, was used to assign them a score ranging from benign to pathogenic. *Right panel*: collection of patient data. In parallel, we reviewed the literature and our own records to extract clinical and genetic information from patients meeting the criteria defined in patients and methods. The information collected was included anonymously and homogeneously in a specific database to facilitate statistics and graph generation. The 255 patients thus identified had at least one of the 86 different *NLRP7* class 4 or 5 variants identified in this review.

they were assumed to be in trans based on their scarcity in the general population (<https://gnomad.broadinstitute.org>). A specific anonymized database in Microsoft Access was built to collect the distribution of *NLRP7* variants in the patients, the reproductive outcomes of the patients, the number of conceptions genotyped or analyzed for DNA methylation, ex-vivo cytokine secretion by patient's cells, and other in vitro assays in cell lines that support the pathogenicity of the variants. Where available, details of pathology, including p57 immunostaining of products of conception were recorded. Nine sisters of patients, who also experienced RHM, but from whom blood samples were not available for DNA sequencing were assumed to have the same variants as the probands and included to provide further data in relation to reproductive outcomes in women with RHM.

2.3 | Genotyping of molar tissues

A total of 36 additional archived formalin-fixed paraffin-embedded (FFPE) molar tissues from patients with biallelic variants in *NLRP7*

were genotyped. The tissues were sectioned, and the sections were stained with hematoxylin and eosin, and examined using a stereomicroscope. Chorionic villi were separated and their DNA was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen). Maternal DNA was extracted similarly from endometrial tissues present on archived FFPE products of conception or from fresh blood. Genotyping was performed using of the following multiplex short tandem repeat (STR) markers, the PowerPlex 16 HS System (Promega, Corporation) or the AmpFISTR Identifier Plus kit (Applied Biosystems) as previously described (Fisher et al., 2014; Khawajkie et al., 2019).

3 | RESULTS

3.1 | Scoring the pathogenicity of *NLRP7* variants

As of 18/06/2021, 275 *NLRP7* variants were included in the Infevers database. Where necessary, we have curated the names of the variants at the protein and/or cDNA level to comply with recent

recommendations of the Human Genome Variant Society (HGVS) (den Dunnen et al., 2016; Richards et al., 2015). A total of 151 variants, located in exons or intronic boundaries and expected to affect the gene function, were blindly evaluated (Figure 1). After two rounds of voting and a consensus meeting, all but four variants achieved a validated score (<https://infevers.umai-montpellier.fr/web/search.php?n=8>).

3.2 | Patient and variant characteristics

A total of 255 patients (200 probands) corresponding to our inclusion criteria were identified (Supporting Information: Table S1), of which 32 (13%) have not been previously published. The largest series came from a recent publication describing a cohort of patients of Mexican origin (Aguinaga et al., 2021). One hundred and eighty-four of the

246 patients tested (75%) were homozygous for the identified variant (s), although consanguinity was reported or known for only 30% of the cases. The 200 probands (400 alleles) exhibited 86 different variants, with p.Leu750Val being by far the most represented (96/400, 24%), followed by p.Arg693Pro (35/400, 8.8%) and p.Leu825* (19/400, 5%) (Figure 2 and Supporting Information: Table S2). Sixty-one of the 86 different variants found in these patients were classified as pathogenic (71%), 14 (16%) as likely pathogenic, and the others (13%) as variants of unknown significance.

NLRP7 variants that are pathogenic or likely pathogenic have been described in all its coding exons with the exception of exon 11 (Figure 2). Of the 86 variants observed, 63 (73.2%) are protein-truncating and 23 (26.7%) are missense (Figure 2). This distribution is significantly different from the frequencies of protein-truncating and missense variants in all other human genes, 43.4% and 44.3%, respectively ($p=0.000011$) (Stenson et al., 2017). In addition, while

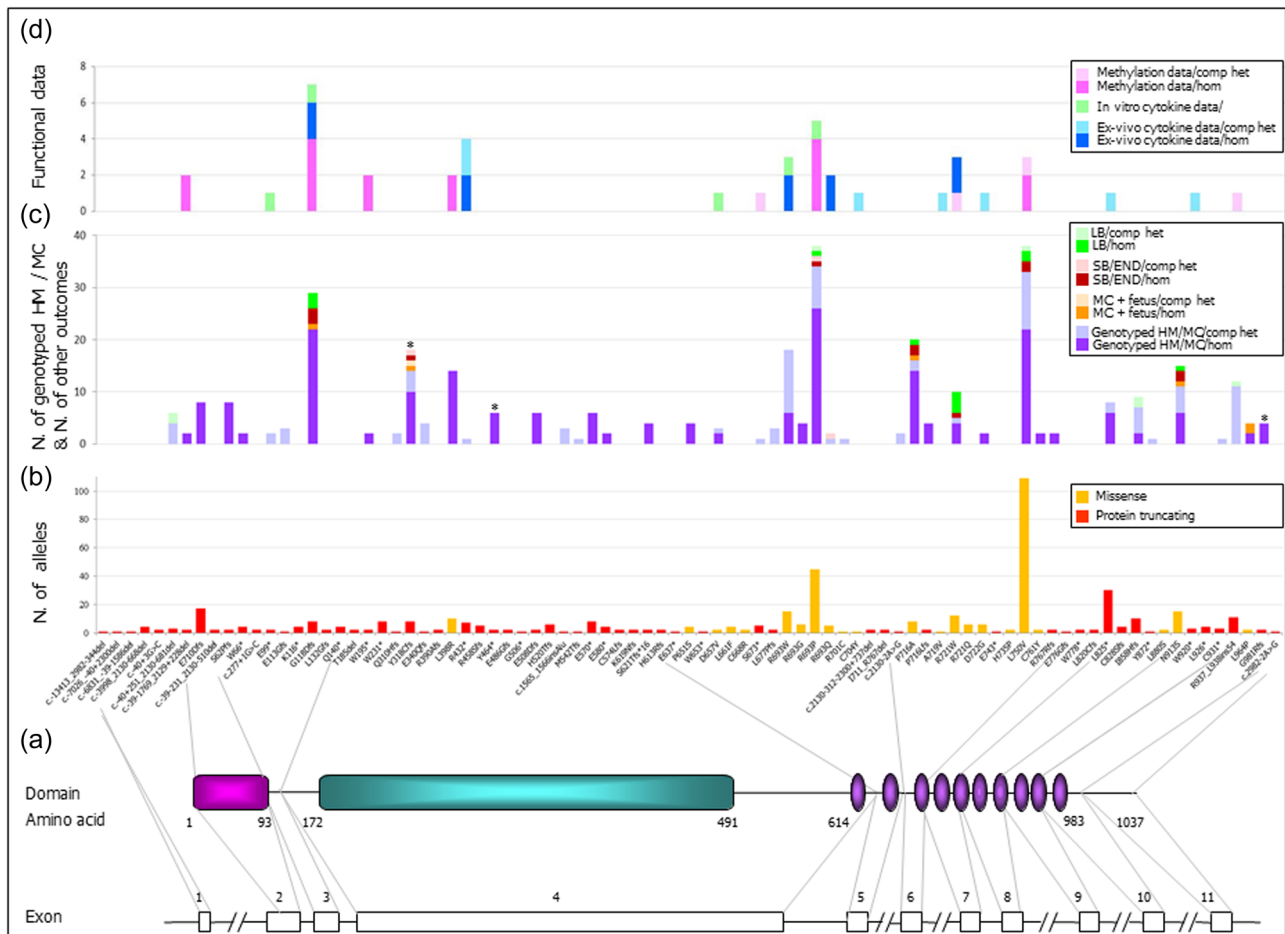


FIGURE 2 NLRP7 genomic and protein structures with variants observed in biallelic state and data used to score the pathogenicity of the variants. From bottom to top, (a) NLRP7 genomic structure according to RefSeq NC_000019.9 and protein structure according to Q8WX94; (b) number of alleles for each variant; (c) number of genotyped HMs or miscarriages and number of other reproductive outcomes (miscarriages with well-formed fetuses, stillbirths or early neonatal deaths, and live births); and (d) number of alleles for each variant for which there are functional data (ex-vivo cytokine secretion by patient cells, in vitro cytokine data on various cell lines and methylation data on the molar conceptions for each variant. Note that in all histograms, the number of alleles for homozygous variants was counted twice and for heterozygous variants once. comp, compound; END, neonatal death; het, heterozygous; HM, hydatidiform mole; hom, homozygous; LB, live birth; MC, miscarriage; SB, stillbirth.

protein-truncating variants are distributed throughout the protein, missense variants that are pathogenic or likely pathogenic are clustered in the leucine-rich repeat (LRR). To date, only one such missense, p.Leu398Arg, found in a homozygous state in four families of Asian origin, has been reported outside the LRR domain (Figure 2 and Supporting Information: Table S2).

3.3 | Ethnic distribution of *NLRP7* pathogenic variants associated with RHM

A consistent and intriguing observation since the identification of *NLRP7* is that the gene displays several founder variants in various populations (Aguinaga et al., 2021; Slim et al., 2009). The distribution of the variants according to the ethnic origin of the patients is shown in Figure 3. Among the founder variants, p.Leu750Val is the most frequent and accounts for 79% of the alleles found in Mexican cases, while the patients in this population reported less than 10% consanguinity, arguing for an ancient founder effect (Aguinaga et al., 2021). Another founder variant in the Mexicans is p.Arg937_Leu938ins54, which has been reported in 10 unrelated patients, and this variant along with p.Leu750Val, appears to be restricted to Mexicans and have not been seen yet in other populations (Aguinaga et al., 2021).

A mutational hotspot affecting two adjacent nucleotides and changing codon 693 to four different amino acids was observed in several populations, that is, p.Arg693Trp in Turks and other Caucasians from various countries; p.Arg693Gly in one Caucasian family; p.Arg693-Pro, which is the main pathogenic variant in Indo-Pakistanis (39%); and

p.Arg693Gln reported so far only in Chinese (11%) (Supporting Information: Table S1). Another variant, p.Asn913Ser, was first observed in the Indo-Pakistani patients (Akoury et al., 2015; Murdoch et al., 2006; Slim et al., 2009), mostly in a compound heterozygous state with p.Arg693Pro, and later in two patients of African descent who carried this variant in a homozygous state despite the absence of consanguinity between their parents (Akoury et al., 2015; Brown et al., 2013). However, among the general healthy population, the frequency of this variant is highest in Africans (minor allele frequency of 0.0015). This suggests its African ancestral origin and later introduction to the Indo-Pakistani population via migration. The p.Arg721Trp and p.Leu825* variants were present mainly in Indo-Pakistani (Kou et al., 2008; Murdoch et al., 2006; Sanchez-Delgado et al., 2015; C. M. Wang et al., 2009) and Chinese (Qian et al., 2011; Zhang et al., 2020) patients and to a lesser extent in other populations. In Egyptians, variant p.Glu710Aspfs slightly dominated the others (32% of the alleles) and again this variant has not been reported in other populations (Kou et al., 2008; Mahadevan et al., 2013; Panichkul et al., 2005). A large panel of different private variants were present in Chinese, Middle-Easterners, and Caucasians.

3.4 | Roles and functions of *NLRP7*

3.4.1 | Diagnosis of HMs

The diagnosis of HMs was originally based on microscopic morphological evaluation (Szulman & Surti, 1978). Another important technique for molar diagnosis is immunohistochemistry (IHC) for the expression of

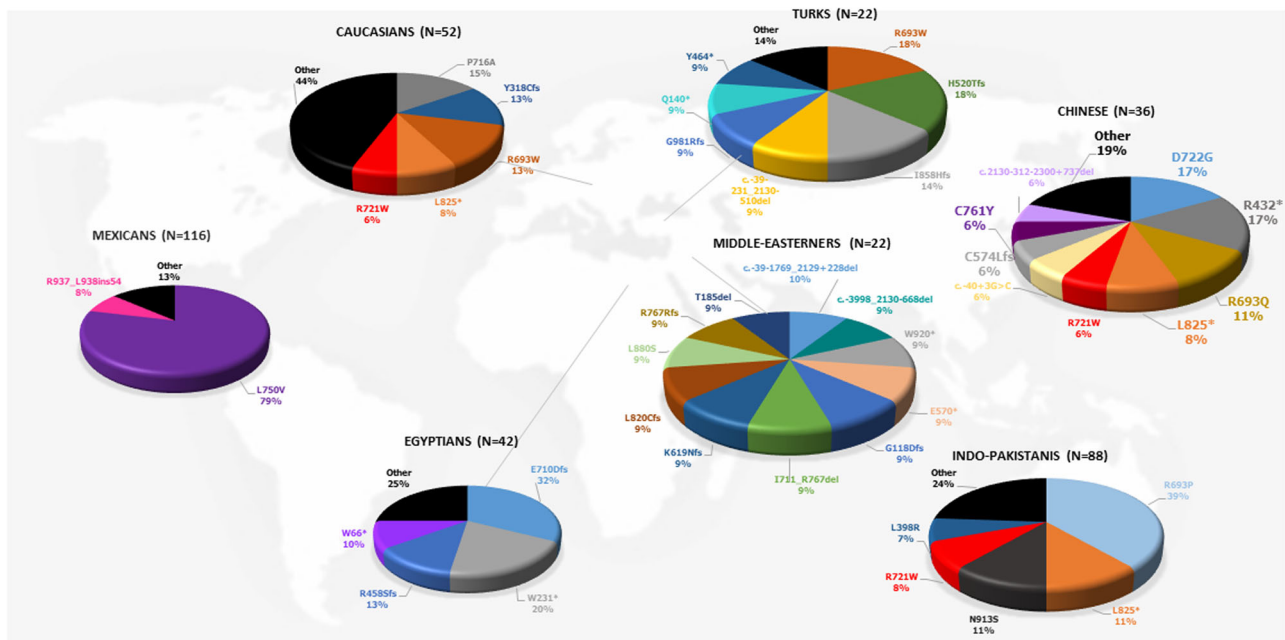


FIGURE 3 Ethnic distribution of *NLRP7* pathogenic variants. Using data on the probands, the frequency of each variant was calculated in different populations. Only variants with a frequency greater than 5% in a given population are represented in the pie charts. Caucasians: England: N = 10; France: N = 8; Not defined: N = 6; New Zealand: N = 4; United States: N = 4; Armenia, Canada, Estonia, Greece, Ireland, Italy, Germany, Spain, and Sweden: N = 2, each. Middle Easterners: Iran: N = 6; Saudi Arabia, N = 4; Algeria, Cyprus, Lebanon, Qatar, Syria, Tunisia: N = 2, each.

p57, the protein coded by the cyclin-dependent kinase inhibitor gene, *CDKN1C* (Castrillon et al., 2001). This gene is paternally imprinted, maternally expressed, in the nuclei of the villous cytotrophoblast and stromal cells of first-trimester placenta. Since the discovery of the association of its differential expression with CHMs and PHMs (Chilosi et al., 1998), p57 IHC has been widely used to classify HMs into CHMs and PHMs and sometime to distinguish early CHMs from nonmolar miscarriages. Since androgenetic CHMs lack a copy of the maternal genome, they lack p57 expression while triploid dispermic PHMs, which have one copy of the maternal genome, express p57. At the genotype level, various methods have been introduced, with multiplex STR markers being the most accurate, to determine the parental contribution to the conceptions mainly because it uses several markers from different chromosomes and is suitable for use with degraded DNA extracted from FFPE tissues that are systematically prepared for histopathological diagnosis of all molar conceptions. To date, STR markers and, in most cases, p57 IHC have been used to examine a total of 150 products of conception from patients with recessive *NLRP7* variants (Figure 2).

3.4.2 | HMs from patients with biallelic *NLRP7* pathogenic or likely pathogenic variants are mostly diploid biparental

By morphology, conceptions from patients with biallelic pathogenic or likely pathogenic *NLRP7* variants have been diagnosed as either a CHM, PHM, atypical HM, or miscarriage. A total of 150 products of conception have been genotyped from 75 different patients. Of these products of conception, the genotypes of 114 tissues have been reported and those of 36 additional ones were added to this study. These 150 products of conception have all been found to be diploid biparental with the exception of three HMs that were found to be triploid. In one of these three cases, the HM tissue was found triploid by karyotype and diandric by STR. However, this tissue was genotyped at only two loci, and consequently, its genotype is not convincing (Ulker et al., 2013). The remaining two HM tissues were analyzed at several STR, were well-documented to be triploid digynic (Allias et al., 2020; Fallahian et al., 2013), and are indicated by asterisks above their associated variants in Figure 2. In one of these two HM tissues, the triploid digynic genotype was found to have resulted from the failure of the extrusion of the second polar body during meiosis II (Allias et al., 2020). Data on these two triploid digynic HMs demonstrated, therefore, another meiotic abnormality, that can occur, although rarely, in the oocytes of patients with biallelic *NLRP7* variants. These abnormalities also demonstrate that *NLRP7* defects in the oocytes are the main contributor to the molar phenotype, independent of the zygotic genome (Allias et al., 2020).

3.4.3 | Diploid biparental CHMs have milder morphological features than androgenetic CHMs

To better understand the morphological variability of HMs from patients with biallelic *NLRP7* variants, two comprehensive

studies have examined this question using different approaches. One study compared 51 diploid biparental CHMs from patients with *NLRP7* variants to 27 CHMs from patients with androgenetic RHM, by assessing 11 morphological features (Sebire et al., 2013). Of these 11 features, 6 were significantly different between the 2 groups and all the 6 were milder in diploid biparental RHM from patients with recessive *NLRP7* variants. Of these features, the most significant were budding villous architecture and the extent of trophoblastic proliferation, with the latter being a feature used by most pathologists to identify HMs and categorize them into CHMs or PHMs. The conclusion of this study was that diploid biparental molar tissues caused by *NLRP7* variants have the same, but milder, morphological features than androgenetic CHMs.

3.4.4 | Correlation between the variants in the patients and the phenotypes of the conceptions

The second study investigated genotype phenotype correlation between the nature of the variants, protein truncating versus missense, in patients with biallelic *NLRP7* variants and the morphological features of 32 of their conceptions that are diploid biparental (Nguyen et al., 2014). Because RHM is a loss-of-function disease, protein-truncating variants, in general, are expected to have a more severe impact on the protein's function than missense variants. This study showed that protein-truncating variants are associated with features of a CHM, namely excessive trophoblastic proliferation, lack of embryonic tissues of inner cell mass origin (fetal nucleated red blood cells, well-formed vessels, fetal membranes, or others), and negative p57 expression in the cytotrophoblast. However, missense variants were associated with some features of a PHM, specifically, mild trophoblastic proliferation, presence of embryonic tissues derived from the inner cell mass (such as fetal nucleated red blood cells within the chorionic villi, well-formed vessels, fetal membranes, and other tissues), and positive p57 expression in the cytotrophoblast, albeit to variable extent.

Applying the same reasoning to the reproductive outcomes of the patients revealed that variants with milder functional consequences, such as missense variants (p.Arg693Pro, p.Pro716Ala, p.Arg721Trp, and p.Asn913Ser), and some hypomorphic splice variants (p.Gly118Aspfs) or protein-truncating variants located toward the C-terminal end of the protein (p.Ile858Hisfs and p.Tyr872*), are permissive for some embryonic tissue differentiation and fetal development to, or beyond, the second trimester, and are associated with late miscarriage with a well-formed fetus, stillbirth, early neonatal death, and live birth (Akoury et al., 2015; Nguyen et al., 2014). A recapitulation of the reproductive outcomes of all published or unpublished (from our groups) cases is provided in Figure 2 and shows the association of fetal development (HM and fetus) and live birth with missense variants, one hypomorphic splice variant, or protein-truncating variants located in the C-terminal part of the protein.

3.4.5 | Impaired de novo DNA methylation during oogenesis

Because diploid biparental RHM from familial cases are similar in morphology to androgenetic CHMs, an important hypothesis to explain their etiology emerged even before the identification of *NLRP7* or *KHDC3L*. This hypothesis suggested that causative genes of diploid biparental HMs must play a role in establishing maternal imprinting marks during oogenesis (Sunde et al., 1993) and has led to several studies to evaluate DNA methylation at differentially methylated regions (DMR) of imprinted genes and at CpG-rich regions of various nonimprinted loci in a total of 10 diploid biparental HMs from patients with the following *NLRP7* variants, c.-39-1769_2129+228del (Sanchez-Delgado et al., 2015), p.Gly118Aspfs (El-Maarri et al., 2003), p.Trp195* (Ito et al., 2016), p.Leu398Arg (Hayward et al., 2009), p.Arg693Pro (Sanchez-Delgado et al., 2015), and p.Leu750Val (Kou et al., 2008), all in homozygous state; p.Ser673* with p.Arg721Trp (Sanchez-Delgado et al., 2015); and p.Leu750Val with p.Arg937_Leu938ins54 (Kou et al., 2008). The results of these analyses were consistent between different studies and approaches with the most comprehensive being that of Sanchez-Delgado et al. (2015). This study confirmed previous data on a smaller number of loci and showed a lack or diminished maternal methylation at most imprinted, maternally methylated DMR that was associated with the biallelic transcription of all analyzed imprinted genes. Diploid biparental molar tissues had higher levels of methylation than androgenetic CHM, which completely lacked methylation marks at all maternally methylated DMR, and these data are, therefore, in agreement with the histopathological evaluation of biparental and androgenetic CHMs (Sebire et al., 2013). In addition, there was some variability between diploid biparental HMs from sisters with the same variant, or even from the same patient, which is in agreement with the variability of p57 nuclear staining in different HMs from the same patients (Nguyen et al., 2014).

3.4.6 | Mononuclear cells from patients with biallelic *NLRP7* variants have defective cytokine production

The identity of *NLRP7* as a member of the *NLRP* family of proteins with roles in inflammation came as a surprise for scientists working on RHM and those in the imprinting field. The most studied member of this family was *NLRP3* which had been shown to cause dominantly inherited auto-inflammatory conditions by upregulating interleukin (IL)-1B and tumor necrosis factor (TNF) secretion without and after ex-vivo stimulation (Aksentijevich et al., 2002). Investigating the inflammatory response of ex-vivo stimulated peripheral blood mononuclear cells from two patients with biallelic *NLRP7* variants (p.Gly118Aspfs and p.Arg693Trp, both in a homozygous state) demonstrated that these cells, as opposed to those of patients with *NLRP3* variants, have decreased IL-1B and TNF secretion in the extracellular milieu. Another recent study by Zhang et al. (2020), reproduced these results using the same assay on cells from six other

patients with biallelic *NLRP7* variants (p.Arg432*, p.Arg693Gln, and p.Arg721Trp in homozygous state and the following in compound heterozygous state, p.Asp722Gly with p.Trp920*, p.Asp722Gly with p.Leu825*, and p.Arg432* with p.Ala719Val). This study confirmed the requirement of normal *NLRP7* for normal IL-1B and TNF production. Based on the cellular localization of *NLRP7* protein in peripheral blood mononuclear cells to the Golgi apparatus and the microtubule-organizing center, defective IL-1B secretion was attributed to the impaired formation of the cellular cytoskeleton. Beside the role of *NLRP7* variants in decreasing cytokine secretion, two other studies have documented functional consequences of five of its variants (p.Glu99*, p.Gly118Aspfs, p.Arg693Trp, p.Arg693Pro, and p.Asp657Val) on inflammasome activation in vitro (Khare et al., 2012; Messaed et al., 2011), supporting the pathogenicity of these variants.

4 | DISCUSSION

RHM resulting from variants in *NLRP7* is a rare condition but important for women who are affected and wish to achieve a normal pregnancy. Analysis of variants in *NLRP7* has identified a number of protein-truncating variants distributed throughout the gene with a much smaller proportion of missense variants. While most variants are private and have been reported in a small number of patients, founder variants have been described in several populations.

Regardless of morphology, none of the 150 products of conception that were genotyped had the typical androgenetic or triploid diandric genotype normally associated with a CHM or a PHM, respectively, almost all HMs (98.6%) that were genotyped were found diploid biparental. Approximately 20% of the conceptions were described as miscarriages. Most of these may not have been examined by morphology, p57 IHC, or genotype analysis and could potentially represent additional molar pregnancies. Despite the poor reproductive outcomes for women with biallelic *NLRP7* variants, 12 patients achieved a term pregnancy with their own oocytes, while three further patients had two term pregnancies, one of which resulted in dizygotic twins. Of the 16 offspring, 2 experienced early neonatal death, and 1 exhibited intrauterine growth restriction. Where assisted reproductive technology was attempted, this was unsuccessful or resulted in a further HM until the introduction of ovum donation, which has enabled at least 11 successful live births (Aguinaga et al., 2021; Akoury et al., 2015; Cozette et al., 2020; Fisher et al., 2011; Rezaei et al., 2021; This study).

Pathogenic recessive variants in *NLRP7* are the major cause of RHM. The primary role of *NLRP7* is in the oocyte where it localizes to the subcortical region (Akoury et al., 2015) and forms the subcortical maternal complex (SCMC), a multimeric protein that is unique to mammalian oocytes (Figure 4). The SCMC consists of oocyte-specific cytoskeletal structures and is the site of ribosomes and mRNA storage (Li et al., 2008). In mice, variants in seven SCMC proteins, of which *NLRP2* (the paralog of *NLRP7*) (Meyer et al., 2009), *NLRP5* (Tong et al., 2000), *PADI6* (Xu et al., 2021), *TLE6* (Alazami et al., 2015), and *KHDC3* (the ortholog of *KHDC3L*) (Ohsugi et al., 2008) lead to

several abnormalities in the oocytes that culminate in an impaired embryonic genome activation (EGA) at the two- to four-cell stage and consequently embryonic arrest by the blastocyst stage (Li et al., 2008). *NLRP7* is the major gene for RHM but other members of the SCMC have also been shown to be responsible for RHM (Qian et al., 2018; Rezaei et al., 2021). Of these, *KHDC3L*, is the second most important gene for RHM (Parry et al., 2011). Two other genes, *PADI6* and *NLRP5*, explain extremely rare cases (Qian et al., 2018; Rezaei et al., 2021) (Figure 5). Patients with recessive *NLRP7* or *KHDC3L* variants produce normal numbers of oocytes, both spontaneously and after ovulation induction. However, their conceptions display variable degrees of hypomethylation at the DMR of several imprinted, maternally methylated genes that acquire their methylation marks during oogenesis (El-Maarri et al., 2003; Hayward

et al., 2009; Ito et al., 2016; Kou et al., 2008; Sanchez-Delgado et al., 2015). This hypomethylation was originally believed to be restricted to imprinted genes, but a recent elegant study on single oocytes from a patient with biallelic variants in *KHDC3L* demonstrated that this hypomethylation affects de novo DNA methylation of the entire oocyte genome (Demond et al., 2019). Despite the hypomethylation defect, oocytes from patients with biallelic *NLRP7* or *KHDC3L* variants are capable of normal fertilization since almost all their products of conception are diploid biparental. However, the abnormalities appear to start during preimplantation development, some of which may manifest at the morphological or genetic level.

Despite the ancient recognition of HMs and the increasing use of assisted reproductive technologies in the past 40 years, until 2017, no one has ever reported how a HM develops during the preimplantation

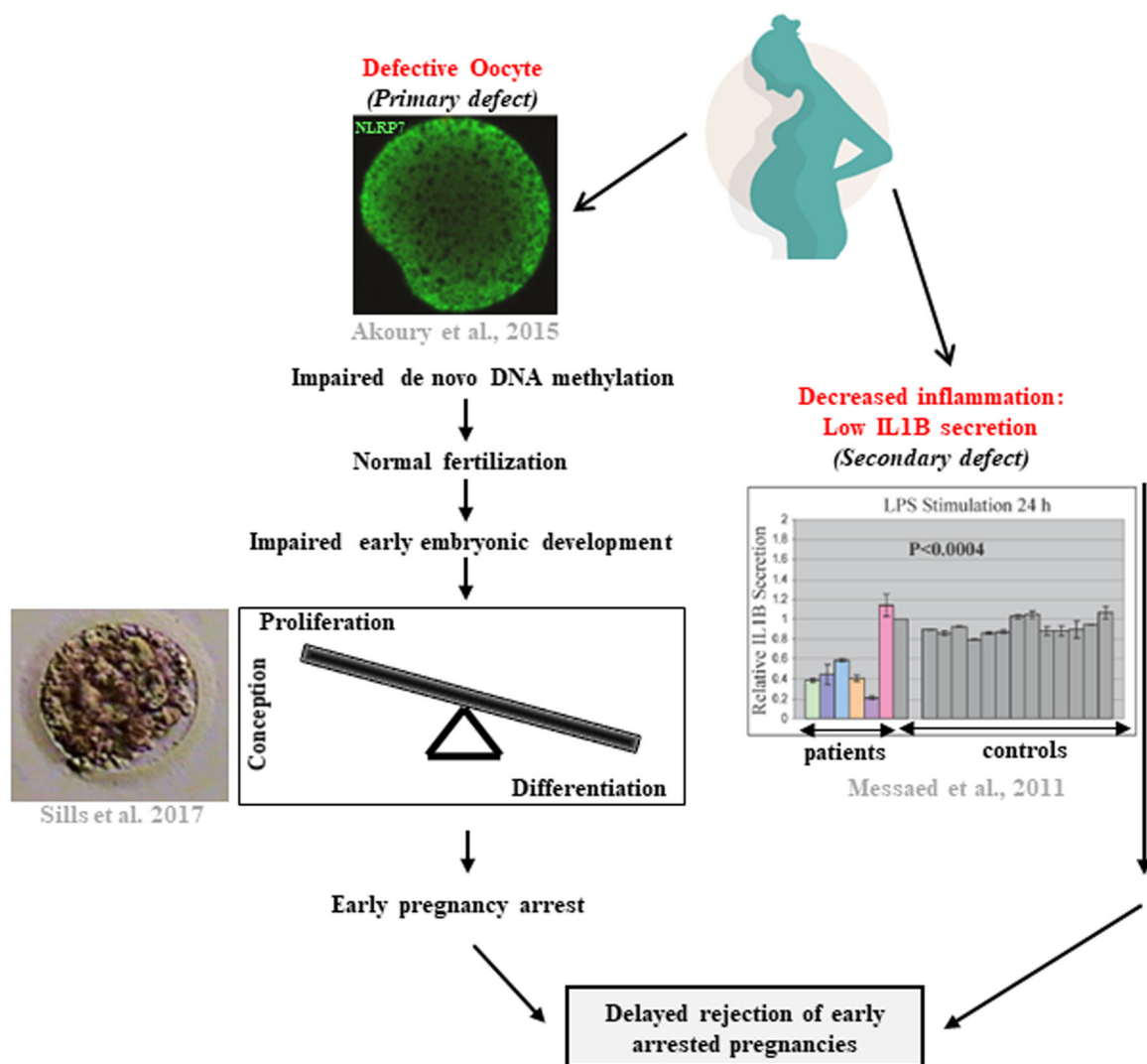


FIGURE 4 Mechanism of RHM associated with biallelic *NLRP7* variants. A suggested model that recapitulates the various roles of *NLRP7* deficiency in the pathology of RHM. The primary defect is in the oocyte that fails to acquire normal methylation marks all over the maternal genome. This defect does not affect fertilization, which appears normal. However, early preimplantation development is impaired and the molar tissues display a shift from tissue differentiation toward proliferation. Because blood mononuclear cells from the patients fail to secrete normal amounts of IL-1B, the patients fail to mount an appropriate inflammatory response to reject their arrested pregnancies and the delayed rejection of these pregnancies contributes to the molar phenotype. IL1B, interleukin 1 beta; RHM, recurrent hydatidiform mole.

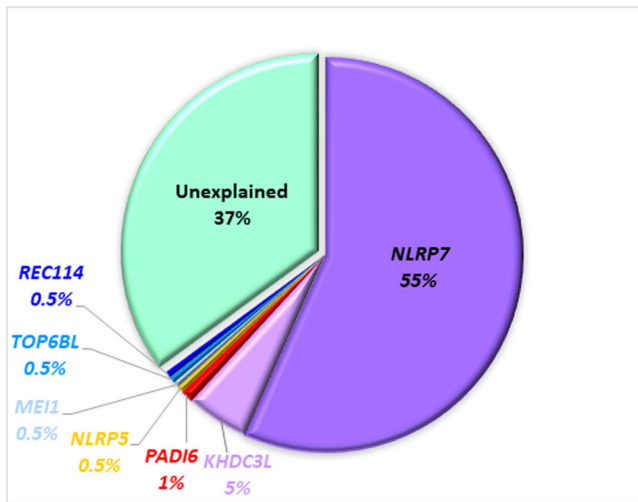


FIGURE 5 Genetic etiologies of RHM. Percentages of patients with RHM and pathogenic/likely pathogenic variants in the known genes. The violet shades are used for genes associated with diploid biparental RHM, red and orange/yellow colors for genes associated with unknown genotypic types of HM; and blue shades for genes associated with androgenetic RHM.

period. The only account of such an event was published recently by Sills on a patient with RHM caused by a homozygous variant in *NLRP7* (p.Arg937_Leu938ins54) (Sills et al., 2017). Despite appropriate counseling, the patient insisted on undergoing assisted reproductive technology with her own oocytes. Ovulation was then induced, and the patient produced a normal number of oocytes that were morphologically normal and were subject to intra-cytoplasmic sperm injection. Most injected oocytes progressed to two pronuclei but all the embryos manifested morphological abnormalities and arrested by the blastocyst stage and none of the blastocysts was judged by the embryologist to be suitable for transfer (Figure 4). Most likely, this is what happens in the natural conceptions of such patients, but nature appears to be more tolerant and less stringent than embryologists; such blastocysts reach the uterus, implant, and lead to HMs that are diploid biparental. In these HMs, there is an inverse correlation between the differentiation of embryonic tissues of inner cell mass and the proliferation of the trophoblast that seems to depend on the severity of the variants in the patients and consequently the severity of the oocyte defects. Intriguingly, this inverse correlation between the degree of embryonic tissue differentiation and trophoblastic proliferation applies to all types of moles (and products of conception), regardless of their genotype and is by itself fascinating and remains to be elucidated at the molecular level. Finally, *NLRP7* variants decrease cytokine production (Messaed et al., 2011), which reduces maternal reaction to abnormal pregnancies and makes the patients more tolerant to keep abnormal pregnancies for a longer time. The delayed rejection of these abnormal pregnancies, in which embryonic development had arrested much earlier, contributes to the molar phenotype (Figure 4).

This review recapitulates the different genetic defects that may occur in the *NLRP7* gene and provides a comprehensive and updated classification of its variants according to their pathogenicity, associated phenotypes, and available functional data. Definitive confirmation of the diagnosis at the genetic level requires the identification of two pathogenic or likely pathogenic biallelic variants. Also, private variants that are extremely rare in the general population can be considered causative if their biallelic status is demonstrated. Analysis of *NLRP7*-associated RHM variants primarily relies on bidirectional Sanger sequencing of all coding exons and flanking intronic sequences, which allows the identification of point variants, or small genetic rearrangements (that are less than 20 bp) such as duplications, deletions, and insertions. However, larger rearrangements and complex variants have also been found (Kou et al., 2008; Reddy et al., 2016), and have been explained by the particular genomic architecture of *NLRP7* and the richness of its introns in Alu repetitive elements (accounting for 48% of its intronic sequences) (Reddy et al., 2016). Therefore, a complementary approach should be performed in order not to erroneously rule out the diagnosis of *NLRP7*-associated RHM, especially in patients in whom only one pathogenic allele has been detected using routine variant analysis. Multiplex ligation-dependent Probe Amplification for the study of gene copy number variations, long-range polymerase chain reaction (PCR), or analysis of genomic DNA dosage by quantitative real-time PCR (RT-PCR) (Nguyen et al., 2018; Reddy et al., 2016) have proven successful for the detection of large deletion and complex variants. In addition, the functional impact of newly detected variants with a putative impact on gene transcription, for example, located at noncanonical splice sites, can be characterized using RT-PCR on RNA from patient cells to demonstrate the presence of abnormal transcripts or the loss of *NLRP7* transcripts.

RHM are genetically heterogeneous, implying that other known causative genes must be evaluated before excluding an inherited genetic etiology. *NLRP7* is the major gene for this condition and is the first to be analyzed for variants. When its involvement is not detected, the following genes need to be analyzed, *KHDC3L*, which explains about 5% of RHM cases (Nguyen et al., 2018), and *PADI6* and *NLRP5*, which explain approximately 1% and 0.5% of RHM cases, respectively (Figure 5). It is important to note that the main manifestations of biallelic variants in the latter two genes are female infertility, early embryonic arrest during the preimplantation period, and children with multilocus imprinting disorders particularly Beckwith-Wiedemann and Silver Russell Syndromes. Three other genes, *MEI1*, *TOP6BL*, and *REC114*, are responsible for androgenetic RHM and were identified in 2018; each explaining approximately 0.5% of patients with RHM who are negative for *NLRP7*- and *KHDC3L*-variants (Figure 5). Biallelic variants in these genes also appear to be responsible mainly for infertility and early embryonic arrest during the preimplantation period (Dong et al., 2021; Jiao et al., 2020; W. Wang et al., 2020), but it is too early to have a clear picture of their involvement in the various forms of reproductive failure. It is expected that such information will be soon available since most genetic laboratories now offer high-throughput

sequencing allowing simultaneous screening of a panel of some genes or all genes using whole exome/genome sequencing. Accurate genetic counseling of patients with RHM will, therefore, depend on the mutated gene and type of variants presented by the patients. When the molecular defect is located in the *NLRP7* gene, Figure 2 shows that, as expected, protein-truncating variants generally have a more severe impact on protein function than missense variants. The direct consequence of these observations is that patients with the latter type of variants are more likely to have a normal pregnancy. Despite this, attempting a natural pregnancy is not recommended as normal pregnancies in such patients are rare and there is a high risk of another molar pregnancy with the risk of persistent disease which may require chemotherapy. This review will help guide clinicians in managing and counseling patients and their relatives, particularly female siblings contemplating pregnancy, based on phenotype-genotype correlations now known from case accumulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. Variant data are available in the Infevers online autoinflammatory diseases registry (<https://infevers.umai-montpellier.fr/web/>). Other data are available in the article Supporting Information.

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