**NLRP7** mutations in women with diploid androgenetic and triploid moles: a proposed mechanism for mole formation

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Hydatidiform mole is an aberrant pregnancy with abnormal embryonic development and hydropic placental villi. Common moles are sporadic, not recurrent and affect one in every 1500 pregnancies in Western societies. Approximately, half of common moles are complete and mostly diploid androgenetic, whereas the remaining are partial and mostly triploid diandric. NLRP7 has been found to be responsible for a recurrent form of molar pregnancies. Recently, we showed that patients with NLRP7 mutations have an impaired inflammatory response to various stimuli. To date, molar tissues analyzed from patients with NLRP7 mutations have been found to be diploid and biparental. In this study, we report 10 new non-synonymous variants and one stop codon found in patients and not in controls. We demonstrate the presence of different types of moles, diploid biparental, diploid androgenetic, triploid and tetraploid conceptions, in patients with NLRP7 variants. We document *in vitro* and *in vivo* early embryo cleavage abnormalities in three patients. We propose a two-hit mechanism at the origin of androgenetic moles. This mechanism consists of variable degrees of early embryo cleavage abnormalities leading to chaotic mosaic aneuploidies, with haploid, diploid, triploid and tetraploid blastomeres. Surviving embryonic cells that reach implantation are then subject to the maternal immune response. Because of the patients’ impaired inflammatory response, androgenetic cells, which are complete allograft, are able to grow and proliferate. In women with normal immune system, chaotic mosaic aneuploidies may also occur during early cleavage, however, androgenetic cells would die after implantation or stay undetected, confined to a small portion of the placenta.

**INTRODUCTION**

Hydatidiform mole (HM) is an abnormal human pregnancy characterized by absence of, or abnormal embryonic development and hydropic degeneration of chorial villi. The common form of this condition is sporadic, not recurrent, and occurs once in every 1000–1500 pregnancies in western countries, but at two to 10 times higher frequencies in underdeveloped and developing countries (1). Among women with sporadic moles, 1–6% will have a second mole (2–7) and about 10–20% will have a second non-molar reproductive wastage, mostly as spontaneous abortion (5,7–9). The frequency of familial recurrent HMs (RHMs) is not known, but such cases are believed to be very rare. So far, approximately 20 familial cases have been reported in the recent English literature in PubMed.

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At the histopathological level, HMs are divided into two types, complete (CHM) and partial (PHM) based on the extent of trophoblast proliferation and the absence or presence of embryonic tissues other than the chorionic villi. At the karyotype and genotype levels, most sporadic CHMs are diploid androgenetic, but may also have any of the following genotypes, diploid biparental, tetraploid androgenetic or biparental, aneuploid (non-triploid/tetraploid aneuploid), triploid diandric or dygenic, or mosaic with two cellular populations (10–15). Among androgenetic moles, the majority is monospermic and 5–20% are dispermic. PHMs are mostly triploid diandric, but may also be diploid biparental, triploid dygenic or aneuploid (11,13,15). To date, all characterized recurrent HMs (RHMs) from patients with no family history of moles have been found to be diploid and biparental with the exception of two cases where one mole in a patient and three in another were shown to be diploid androgenetic (16). Also, all analyzed molar tissues from familial cases have been found diploid biparental (17–23).

By studying rare families of RHMs, a defective gene, NLRP7, responsible for this condition has been identified (24) and mutations in this gene have been found in women from several ethnic groups. Eleven NLRP7 variants found only in patients are now listed in INFEVERS (http://fmf.igh.cnrs.fr/ISSAID/inf-). Some of their products of conception are again by day 5 (25). To date, it is not clear how NLRP7 defects lead to RHMs and associated reproductive wastage. As a maternal effect gene, NLRP7 could cause recurrent fetal loss by leading to defective oocytes or/and by creating a hostile maternal environment for embryonic development in the Fallopian tubes and the uterine cavity. We recently demonstrated that patients with NLRP7 mutations have impaired inflammatory response against various stimuli, such as lipopolysaccharides, various microbial products and synthetic compounds, which induce strong inflammatory response in normal individuals (Deveault, manuscript in preparation).

Here we report 11 new NLRP7 variants found only in patients with recurrent reproductive wastage and the characterization of some of their products of conception. We show that NLRP7 variants are present, not only in patients with diploid biparental moles as previously shown, but also in patients with diploid androgenetic moles, triploid moles and tetraploid spontaneous abortions. Furthermore, we document in vivo and in vitro early embryo cleavage abnormalities in three patients with NLRP7 variants. Our findings shed new lights on the mechanism of mole formation and expand the involvement of NLRP7 to a wider range of recurrent and non-recurrent reproductive wastage.

RESULTS

New NLRP7 variants in patients with recurrent reproductive wastage

The search for mutations revealed two previously reported mutations, a missense, c.2248C>G, p.Leu750Val (26), in a homozygous state and a splice mutation, c.2471+1G>A, p.L825X (24), in a heterozygous state in sisters from families MoUs99 and MoCh77, respectively (Table 1). Eleven other variants, 10 leading to non-synonymous amino acid changes and one leading to a stop codon were also found. These variants were not found in 50–289 control subjects from various ethnic groups indicating their association with the disease phenotype (Supplementary Material). Patient 428 had also a rare variant, c.1460G>A, p.Gly487Glu, in a heterozygous state that was found in trans with p.Cys399Tyr. Variant p.Gly487Glu is reported in public databases and we found it in heterozygous state in 4.6% of control women (with five to nine children) of European descent and at lower frequencies in subjects from other ethnic groups (Supplementary Material). All the other variants that we found are known frequent polymorphisms in the general population. In five patients, 492, 501, 647, 565 and 636, only one variant which is not present in controls was found. Available parents were tested for their offspring variants. This analysis revealed the inheritance of p.Cys84Tyr to patient 492 and of p.Ala719Val to patient 636 from their fathers. Variants p.Arg156Gln and p.Lys511Arg were inherited to patients 647 and 565, respectively, from their mothers. For patient 501, only the mother was available for genetic testing and does not carry p.Lys379Asn (Fig. 1). Haplotype establishment for coding and intronic DNA variants found in NLRP7 demonstrated the absence of a common haplotype in patients, 492, 501 and 636 (Supplementary Material), which is not in favor of a common rearrangement in the three patients. In patients 492 and 428, long range polymerase chain reaction (PCR) was also used to amplify the entire genomic region from the start to the stop codons, in five fragments, and did not reveal any rearrangement. In patient 492, the five amplified fragments contained heterozygous single nucleotide polymorphisms (SNPs) demonstrating the amplification of the two parental alleles. cDNA sequencing from patient 428, from which an Epstein-Barr virus (EBV)-transformed cell line is available, revealed normal splicing of all exons. The variant found in patient 662, p.Leu964Pro, is the first in exon 10, present only in splice isoform v2 (27), and highlights the importance of screening the 11 NLRP7 exons (NM_001127255.1) in the search for mutations.

NLRP7 variants are responsible for several types of hydatidiform moles

To better understand the mechanism(s) responsible for molar pregnancies caused by NLRP7 mutations/variants, we used several methods to characterize available products of conception (POC) from six patients (Fig. 1). Ploidy analysis was performed either by karyotyping or by flow cytometry and demonstrated that nine moles were diploid, one had a triploid peak, one spontaneous abortion had a tetraploid peak and one mole was inconclusive because of strong fixation of the tissues (Fig. 1 and Supplementary Material). We note that flow cytometry analysis on embedded tissues does not allow distinguishing whether the POC are only triploid or tetraploid or whether they are mosaic and contain other diploid cells because of the presence of endometrial diploid maternal tissues. Immunohistochemistry with p57KIP2 antibody demonstrated the absence of its expression in four diploid moles, two CHMs (Fig. 2) and two PHMs.
<table>
<thead>
<tr>
<th>ID</th>
<th>Family</th>
<th>Patient DNA</th>
<th>Protein</th>
<th>Reproductive history, gynecological morbidities and others</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHMs and reproductive wastage with no family history of moles</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MoCh71</td>
<td>492 Chinese</td>
<td>c.[251G&gt;A]+[?]?</td>
<td>p.[Cys84Tyr]+[?]</td>
<td>CHM, PHM, 1 failed IVF cycle blood karyotype of patient 492, 45,XX,rob (13,14)(q10;q10)</td>
</tr>
<tr>
<td>MoCh73</td>
<td>501 Chinese</td>
<td>c.[467G&gt;A]+[?]?</td>
<td>p.[Arg156Gln]+[?]</td>
<td>HM, HM (46,XX), HM operated for uterine septum between second and third HMs vitiligo, autoimmune thyroiditis and anticardiolipin blood karyotype of the patient and her partner normal</td>
</tr>
<tr>
<td>MoCa57</td>
<td>428 Moroccan and Algerian</td>
<td>c.[1137G&gt;C]+[?]?</td>
<td>p.[Lys379Asn]+[?]</td>
<td>2 CHM, PHM</td>
</tr>
<tr>
<td>MoCa94</td>
<td>636 Italian</td>
<td>c.[2156G&gt;T]+[?]?</td>
<td>p.[Ala719Val]+[?]</td>
<td>SB, BO, twin (fetus + CHM/IM), 2 SA Asherman syndrome and uterine perforation after her third conception, adenomyoma, placenta accreta after last SA</td>
</tr>
<tr>
<td>MoCa88</td>
<td>565 Moroccan and Scottish</td>
<td>c.[1532A&gt;G]+[?]?</td>
<td>p.[Lys511Arg]+[?]</td>
<td>SA, NP, 6 SA, 1 IVF cycle led to 2 twins Hashimoto disease</td>
</tr>
<tr>
<td>Ch101</td>
<td>101 Chinese</td>
<td>c.[2101C&gt;T]+[2078G&gt;A]</td>
<td>p.[Arg701Cys]+[Arg693Gln]</td>
<td>2 CHM, 3 SA</td>
</tr>
<tr>
<td>MoCa94</td>
<td>636 Italian</td>
<td>c.[2156G&gt;T]+[?]?</td>
<td>p.[Ala719Val]+[?]</td>
<td>EFL, NP, PHM, EFL, PHM, EFL, PHM lupus and borderline anticardiolipin antibody, Crohn’s disease</td>
</tr>
<tr>
<td>Ch29</td>
<td>29 Chinese</td>
<td>c.[2165A&gt;G]+[2165A&gt;G]</td>
<td>p.[Asp722Gly]+[Asp722Gly]</td>
<td>2 SA, 2 PHM, BiCHM, CHM, SA, PHM</td>
</tr>
<tr>
<td>Familial cases of recurrent HMs</td>
<td></td>
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<tr>
<td>MoUs99</td>
<td>655 Mexican</td>
<td>c.[2248C&gt;G]+[2248C&gt;G]</td>
<td>p.[Leu750Val]+[Leu750Val]</td>
<td>2 PHM, SA, PHM</td>
</tr>
<tr>
<td>Ch77</td>
<td>77 Chinese</td>
<td>c.[2248C&gt;G]+[2248C&gt;G]</td>
<td>p.[Leu750Val]+[Leu750Val]</td>
<td>PHM, CHM, HM</td>
</tr>
<tr>
<td>MoFr101</td>
<td>662 French</td>
<td>c.[2891T&gt;C]+[2891T&gt;C]</td>
<td>p.[Leu964Pro]+[Leu964Pro]</td>
<td>PHM (46,XX), 2 SA, PHM (46,XY) uterine myoma with some chorionic villi</td>
</tr>
</tbody>
</table>

New variants are italicized. SA, spontaneous abortion; NP, normal pregnancy; HM hydatidiform mole; IM, invasive mole; PHM, partial HM; BiCHM, indicates a complete mole found biparental by the referring laboratory; CHM, complete HM; ART, assisted reproductive technologies; EFL, early fetal loss that manifested as heavy vaginal bleeding at the time of next menstruation and no pregnancy test was performed. HM is used when no tissues are available to re-evaluate the diagnosis and available pathology report does not distinguish between partial and complete HM. Reproductive outcomes are listed by chronological order starting from the left; the absence of a number indicates one such reproductive outcome.
genotyping of the two CHMs, from patients 428 and 492, using variable number of tandem repeats (VNTRs) and microsatellite markers from several chromosomes demonstrated their androgenetic monospermic origin (Tables 2 and 3). In patient 428, the molar and normal placentae had identical paternal alleles at 17 informative markers from 10 different chromosomes demonstrating their common origin from a single sperm and zygote (Table 2). Available parents, siblings, offsprings and materials

Figure 1. Characterization of molar tissues and conceptions from six patients with NLRP7 variants. The reproductive outcomes of the patients are listed by chronological order from left to right. Gestational age is from the last menstrual period: CHM, complete HM; PHM, partial HM; HM was used when the pathology report does not specify the type of moles and the available material is not sufficient to establish the diagnosis; BO, blighted ovum; SB, stillbirth; SA, spontaneous abortion; n, ploidy; hap, indicates biparental contribution based on haplotype analysis of several SNPs in NLRP7; + and − indicate, respectively, presence and absence of p57Kip2 expression in most of the villi; gray circles indicate women with unknown reproductive outcomes; asterisk (*) indicates that the products of conception with triploid and tetraploid peaks may also be mosaic with other diploid cells.
from POC were genotyped at several SNPs in NLRP7. Haplotype analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 436, haplotype data and microsatellite genotyping at three to four markers indicate the androgenetic origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin. In the two moles from patient 636, haplotype data and analysis of the last mole of patient 501 is in favor of her biparental origin.
result indicate that the second PHM from patient 636 is diploid biparental but do not allow clarifying the origin of the triploidy (diandric or digynic) of her first mole. A recapitulation of all data on the different moles is summarized along with the pedigree structures in Figure 1. Altogether our data demonstrate that NLRP7 mutations/variants are responsible not only for diploid biparental moles as previously demonstrated in several familial cases (18–23, 26), but also for diploid androgenetic and triploid moles, and for spontaneous abortions with tetraploid genome. Segregation of NLRP7 mutations in the haploid oocytes does not lead to hydatidiform moles

Mutation analysis of biparental POC from patients 428 and 636 demonstrated that in patient 428, the placenta of the still-born baby and the zygote that led to a mole, both inherited from their mother the rare variant, p.Gly487Glu, found in up to 4.6% of the general population and not variant, p.Cys399Tyr that was not found in controls. Also, in patient 636, a healthy 6-year-old boy inherited his mother’s mutation, p.Ala719Val (Fig. 1). These findings are in agreement with our previous report, in which we documented a healthy daughter heterozygous for her mother’s mutation (p.Gly118fs) (24), and confirm further that the development of moles is not associated with the segregation of a mutated NLRP7 allele in the haploid oocyte.

In vitro development of embryos from patients with NLRP7 mutations

Three patients described in this study have tried various types of assisted reproductive technologies (ART), patients 492 and 565 with their own oocytes and patient 517 with donated oocytes. In patient 492, seven oocytes were retrieved after ovulation induction; six were in metaphase II and were fertilized by sperm microinjection. Normal fertilization was documented in three, based on the presence of two pronuclei (PN) and two polar bodies. The three zygotes cleaved to the six-cell, but the embryos were all of poor quality (grade IV), fragmented and none was suitable for transfer (Table 4). In patient 565, 38 oocytes were collected after ovulation induction, 33 were metaphase II and were inseminated. Evidence of fertilization was found in 23, one embryo had one PN, one had three PN and 21 had two PN. Pre-implantation genetic diagnosis (PGD) was performed on three fresh embryos using fluorescent in situ hybridization (FISH) with probes specific for chromosomes 13, 15, 16, 18, 21, 22, X and Y chromosomes. One embryo was diploid but could not be transferred because of the patient’s hyperstimulation. All the embryos were frozen, thawed 3 months later, and 16 embryos were biopsied, on day 3. Three embryos displayed a single signal for more than three chromosomes and two signals for the remaining chromosomes and were therefore haploid–diploid. One embryo displayed a single signal for more than three chromosomes, but was chaotic because different blastomeres displayed different chromosome complements and was therefore classified as haploid–aneuploid. The presence of three embryos with haploid blastomeres for at least three chromosomes (12.9%) (Table 4) is higher than what is usually observed in in vitro fertilization (IVF) patients (3%) (28). Three embryos with diploid blastomeres were transferred, two implanted, and the patient delivered at term two dichorionic diamniotic twins in good health. In patient 517, whose sister had diploid biparental moles (19), seven donated oocytes were fertilized, two embryos were of good quality and were transferred 48 h post-insemination (PI). Subsequent failure
was documented by negative β-human chorionic gonadotrophin 14 days PI. Because of the small number of transferred embryos in patient 517, this case does not allow to conclude whether ovum donation fails in patients with NLRP7 mutations.

**DISCUSSION**

Mutations in NLRP7 have been shown to be responsible for recurrent RHMs associated with other forms of reproductive wastage. Here we report 11 new variants in NLRP7 in women with RHMs and various forms of reproductive wastage. Our data expand further the spectrum of reproductive wastage associated with NLRP7 mutations/variants to patients with very early fetal loss, 4 weeks from last menstrual period, and to patients with recurrent spontaneous abortions and no molar pregnancies.

Four autoimmune conditions – Hashimoto disease, lupus antibody, vitiligo and Crohn’s disease, were found in some of our patients. Two of these features, vitiligo and Crohn’s disease are associated with variants in other genes from the CATERPILLER family, NLRP1 and NOD2, respectively. With the exception of an old report of ulcerative colitis, vitiligo, and HM (29), no previous associations between molar pregnancies and autoimmune conditions have so far been reported. However, ulcerative colitis, coeliac and Crohn’s diseases are known risk factors for recurrent spontaneous abortions (30–32). Our data indicate that RHMs, believed so far to be an autoinflammatory condition, has also an autoimmune component and some patients have abnormal autoantibodies and overlapping clinical features with other autoimmune diseases. Our findings indicate that patients with RHMs should be screened for adrenal, thyroid and ovarian autoantibodies and in the presence of such autoantibodies, the patients should be closely followed-up to prevent adrenal, thyroid and ovarian failure. In addition, we note the occurrence of uterine problems in three of the reported patients, which have also been previously observed in patients with HMs (16,33).

In five of the patients included in this study, 647, 636, 501, 565 and 492, we found only a single variant that was not found in controls, by genomic DNA sequencing of the 11 NLRP7 exons in the two directions. The presence of a single NLRP7 variant associated with the disease phenotype in five patients indicates: (i) the presence of undetected mutations, deletions, rearrangements in the exons, introns, or promoter and regulatory regions; (ii) the contribution of one or several other genes as well as environmental factors to the disease phenotype; and (iii) the possibility that a single variant may be associated with the disease phenotype. Furthermore, the presence of a non-synonymous rare variant, p.Gly487Glu, in one patient, that is present in up to 4.6% of women from the general population indicates that this variant could confer susceptibility for sporadic moles and reproductive wastage.

In the current study, we demonstrate that NLRP7 mutations or variants are responsible not only for diploid biparental moles, but also for diploid androgenetic moles, triploid moles and for tetraploid spontaneous abortions. After the demonstration by Kaji and Ohama in 1977 that most sporadic CHMs are diploid and androgenetic, a hypothetical mechanism was postulated to explain their formation (34). This mechanism consisted of the fertilization of an oocyte without nucleus or with an inactivated nucleus followed by the duplication of the paternal chromosomes without cytokinesis (34). Later studies have endorsed this postulate (35) and some investigators raised other possibilities involving the exclusion or the elimination of the egg nucleus before fertilization (36). To date, we do not have any experimental evidence of neither of the above proposed mechanisms and we still do not know how androgenesis occurs and when the maternal nucleus or genome is eliminated. Furthermore, in more than 30 years of assisted reproductive technologies, during which time oocytes from women with various medical conditions have been cultured in vitro and examined, nobody has reported empty oocytes without nucleus that fertilize, cleave and implant. Consequently, scientists have started questioning the concept of the ‘empty oocyte’ and its existence at the origin of androgenetic moles in twin conceptions (37).

In vitro development of embryos from patient 565 showed a higher rate of mosaic, haploid–diploid and haploid–aneuploid embryos when compared with patients undergoing IVF for various medical reasons (28). In patient 492, all the embryos were degenerated by the 6-cell stage. Our data on these two patients demonstrate in vitro early cleavage abnormalities in patients with NLRP7 variants. To date, three other cases of IVF in women with RHMs have been documented and no pregnancies were achieved in any of them (Table 4) (38–40). In the first case, higher rates of triploid and haploid embryos were observed based on the number of pronuclei 13–21 h PI (40); in the second, higher rates of triploid embryos were noted and one embryo was tetraploid, again based on the number of pronuclei several hours PI (39); while in the third, no aneuploidies were documented based on PGD and FISH with probes that detect three chromosomes (38). Unfortunately, these three cases were studied before the current knowledge about moles and consequently the parental contribution to the different moles and the presence of NLRP7 mutations in the patients were not investigated. Our current study confirms previous observations but in two patients with NLRP7 variants and documented parental contribution to their moles from natural conceptions. Interestingly, embryonic arrest during early cleavage has also been reported in a mouse knockout for Nlrp5, where embryos from null females start to degenerate from the 2-cell stage and none reaches the blastocyst stage (41).

Data on patient 428 demonstrate, in a natural conception, the occurrence of an androgenetic mole and a fetus deriving from the same zygote. This demonstrates in vivo post-zygotic abnormalities and their causal role in the diploid androgenetic mole in this patient. Five similar cases of androgenetic moles and fetuses deriving from the same zygotes have so far been reported (42–44), but our finding is the first in a patient with NLRP7 variants. Our data on POC from five patients with different NLRP7 variants (Fig. 1) demonstrate the alternation, in the same patient, of several types of moles/conceptions, diploid biparental, triploid (or 3n + 2n) and tetraploid (or 4n + 2n), which somehow mimic the spectrum
of in vitro abnormalities observed in patient 565 and in previously reported women with recurrent moles undergoing ART services (38–40). Analyses of large cohorts of normally in vitro-fertilized embryos with two pronuclei have demonstrated that post-zygotic errors leading to mosaic embryos is the major abnormality observed during in vitro cleavage. These studies have shown that the rate of mosaic embryos increases significantly at sequential stages of pre-implantation development and reaches 90.9% at the blastocyst stage (28). Among these embryos, those with haploid blastomeres are the least frequent (0.5%), and when present, they are in the mosaic state with other diploid blastomeres (28). Altogether, our data lead us to propose a two-hit mechanism that may explain androgenetic mole manifestation. NLRP7 mutations lead, directly or indirectly, to an increased rate of stochastic and mosaic aneuploidies during early embryo cleavage with all possible combinations of haploid, diploid, triploid and tetraploid blastomeres. The resulting embryos may, or may not, survive until implantation depending on the severity of the aneuploidies for embryonic development and on the percentage of diploid cells in the chimeric embryos. After implantation, surviving embryonic cells are subject to an additional type of selection that depends on the maternal immune system and its tolerance to the abnormal conception. Recently, we found that patients with NLRP7 mutations, including 428 and 636, are not able to mount an appropriate inflammatory response against various antigens (Deveault et al., manuscript in preparation). Consequently, in such patients, androgenetic blastomeres, which are complete allograft, may thrive, grow and proliferate without being rejected by the patients. It is also possible that androgenetic blastomeres occur, de novo, in an important number of women in the general population. However, in women with active immune system, androgenetic cells most likely die or stay confined, and undetected, to a small portion of the placenta. A similar mechanism may underlie triploid diandric moles, which have a higher antigenicity than diploid biparental embryos; such cells may only survive in women with some forms of immune deficiencies. Our suggestion is corroborated by the high degree of mosaic aneuploidies and chromosomal abnormalities, 50–100%, observed in pre-implantation embryos (45,46).

The rate of embryo cleavage abnormalities in patients with NLRP7 mutations seems to be mutation-dependent with some alleles being more severe than others. For instance, patient 492, who had an androgenetic mole from a natural conception, all her in vitro-fertilized embryos fragmented before the morula stage while in patient 565, who had had a normal pregnancy and several spontaneous abortions from natural conceptions, 35% of her in vitro-fertilized embryos were of good quality, diploid and the patient achieved a second normal pregnancy. Among described patients with NLRP7 mutations, 492 and 428, are the first with reported androgenetic moles and the first with cysteine-to-tyrosine changes. Mutations affecting cysteine residues have been reported in another inflammatory disease, the TNF receptor-associated periodic syndrome (TRAPS), to be more penetrant than non-cysteine mutations and are associated with different clinical features (47). Analyzing more molar tissues from patients with different NLRP7 mutations may or may not allow validating our observation.

In the case of patients with NLRP7 mutations, the use of PGD does not cure their genetic defect, but it helps selecting diploid embryos, if any, for transfer and therefore increasing the likelihood of the patients to have normal pregnancies. Taking into consideration our PGD data and those of other groups (38–40), we believe that the selection of diploid embryos by PGD is currently the best strategy for patients with NLRP7 mutations who wish to conceive.

MATERIALS AND METHODS

Patients, histopathology analysis and mutation analysis

The reproductive outcomes of 13 new patients are summarized in Table 1 and Figure 1. Five patients are from familial cases and eight have no family history of moles. Each patient has had either RHMs or recurrent reproductive wastage including at least one mole with the exception of patient 565, who did not have any molar pregnancy. Patient 565 has had two normal pregnancies, and seven spontaneous abortions; only one of them required dilatation, suction and curettage. Patient 636 has had three very early fetal losses (EFL) that manifested as heavy vaginal bleeding at the time of next menstruation. Patient 428 had a molar pregnancy that was part of a twin pregnancy originating from a natural conception. At 8 weeks, a normal fetus with heart activity and measurements was detected and a small region of 8 mm of placental detachment was noted. Echogenic structures in the placenta started to manifest on ultrasound around week 10, but the fetus continued to have normal growth parameters. The death of the fetus was noted at 15 weeks. Dilatation and suction revealed a female fetus with a supra-umbilical defect attached to a normal placenta and a molar placenta. The fetal foot-length was normal for gestational age. The normal placenta was karyotyped and had normal female karyotype. Histopathology revealed a CHM with abundant fibrin and focal necrosis. An admixture of non-hydatidiform villous structures with dysmature features was noted in some regions. Bowel tissues were identified among the normal placenta. The histopathology diagnosis was established according to Shulman’s criteria (48) (Supplementary Material). Three patients had other immunological manifestations, Hashimoto disease, autoimmune thyroiditis, lupus antibodies, Crohn’s disease and vitiligo (Table 1), two patients had uterine myomas, 428 and 662, and one, 647, was operated for a uterine septum. Patient 492 has an abnormal blood karyotype, 45,XX,rob (13,14)(q10;q10), carrying one of the most frequent Robertsonian translocations.

Patient 517 is from a previously reported family, MoCh76, and is compound heterozygote for p.Glu99X and p.Asp657Val (19). Mutation analysis in the new patients was performed as previously described by genomic DNA sequencing of the 11 exons of NLRP7 in the two directions (19,24). Other approaches were used in patients where a single mutation was found and appropriate material was available. These approaches were: (i) long range PCR amplification and sequencing of the entire genomic region from the start to the stop codons (17.9 kb) in five overlapping fragments using the TAKARA kit (Fisher Scientific); (ii) haplotype establishment at NLRP7 variants on available parents, siblings and
products of conception; and (iii) cDNA sequencing, which was performed only on one patient, 428, from which an EBV-lymphoblastoid cell line was available.

**Parental contribution to the moles**

Ploidy was determined either by karyotype analysis on fresh chorionic villi or by flow cytometry on one paraffin-embedded tissue containing several chorionic villi from each available POC. P57KIP2 immunohistochemistry was performed on one paraffin-embedded tissue from each POC and was used to distinguish between CHM, PHM and hydropic abortions. P57KIP2 is an imprinted, maternally expressed gene that is used as a complementary diagnostic marker; its expression in the cytotrophoblast and the villous mesenchyme indicates the presence of the maternal genome (49) and consequently the non-androgenetic nature of the POC, which could be in this case, triploid, tetraploid, or diploid biparental (50). However, the absence of p57KIP2 expression does not allow concluding that the maternal genome is absent since several diploid biparental moles do not express p57KIP2. P57KIP2 immunohistochemistry slides were screened independently by two observers and the conclusion was based on the majority of chemistry slides were screened independently by two observers and the conclusion was based on the majority of

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

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**Conflict of Interest statement.** The authors declare that they have no conflict of interest.

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**REFERENCES**


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