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ARTICLE

Live births in women with recurrent hydatidiform mole and two *NLRP7* mutations

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Abstract Hydatidiform mole (HM) is an aberrant human pregnancy with abnormal embryonic development and excessive proliferation of the trophoblast. Recessive mutations in *NLRP7* are responsible for recurrent HM (RHM). Women with recessive *NLRP7* mutations fail to have normal pregnancies from spontaneous conceptions with the exception of three out of 131 reported patients. Because there is no treatment for RHM and maternal-effect genes are needed in the oocytes to sustain normal embryonic development until the activation of the embryonic genome, one patient with recessive *NLRP7* mutations tried ovum donation and achieved a successful pregnancy. This study reports three additional live births from donated ova to two patients with recessive *NLRP7* mutations. The occurrence of two live births from spontaneous conceptions to two other patients is also reported. The reproductive outcomes and mutations of all reported patients were reviewed and it was found that live births are associated with some missense mutations expected to have mild functional consequences on the protein. The data support a previous observation that ovum donation appears the best management option for these patients to achieve normal pregnancies and provide an explanation for the rare occurrence of live births from natural spontaneous conceptions in patients with two *NLRP7* mutations.

KEYWORDS: live birth, NLRP7, ovum donation, recurrent hydatidiform mole

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Introduction

Hydatidiform mole (HM) is an aberrant human pregnancy characterized by abnormal embryonic development and hyperproliferation of the trophoblast. HM affects 1 in every 600 to 1000 pregnancies in western countries and most of them are not recurrent (Grimes, 1984; Savage et al., 2010). Recurrence of moles affect 1 to 9% of patients with a prior mole (Berkowitz et al., 1998; Boufettal et al., 2011; Horn et al., 2006; Kim et al., 1998; Kronfol et al., 1969; Sebire et al., 2003) and few of these patients have a family history of moles (for reviews see Fisher et al., 2004; Slim and Mehio, 2007). Two maternal-effect genes responsible for recurrent hydatidiform moles (RHM), *NLRP7* and *KHDC3L*, have been identified (Murdoch et al., 2006; Parry et al., 2011). *NLRP7* codes for a nucleotide oligomerization domain-like receptor pyrin containing protein and is mutated in 48 to 80% of patients with RHM (Estrada et al., 2013; Hayward et al., 2009; Qian et al., 2011; Sebire et al., 2013; Slim et al., 2009). *KHDC3L* codes for a KH domain containing 3-like, a member of the subcortical maternal protein complex, and is mutated in 10 to 14% of *NLRP7*-negative patients (Parry et al., 2011; Reddy et al., 2013).

To date, all reported women with RHM and two defective alleles in either of the two known genes failed to have live births from spontaneous conceptions, with the exception of three patients with *NLRP7* mutations, two cousins from one family (MoLb1) (Moglabey et al., 1999) with a homozygous splice mutation, c.352 + 1G>A, p.G118Dfs*2 that leads to the inclusion of 4-bp of intron 3 in the mRNA and consequently creates a premature stop codon two amino acids after exon 3 (Murdoch et al., 2006), and a recently reported patient with a homozygous missense mutation, c.2248C>G, p.Leu750Val (Mahadevan et al., 2013). Three patients with two defective alleles in *NLRP7* have so far tried ovum donation (Deveault et al., 2009; Sensi et al., 2000), and only one successful pregnancy leading to a live birth has been reported (Fisher et al., 2011).

This study reports the occurrence of five live births, three from donated ova and two from spontaneous conceptions, in four patients with two *NLRP7* defective alleles.

Materials and methods

NLRP7 mutation analysis

This study was approved by the Institutional Review Board of the McGill University, (reference: A01-M07–03A) on 24 January 2003 and is renewed every year. Mutation analysis was performed as previously described by polymerase chain reaction (PCR) amplification of genomic DNA of the 11 exons of *NLRP7* followed by DNA sequencing in the two directions (Murdoch et al., 2006). Sequences were analysed using DNASTAR. DNA mutations are numbered according to cDNA sequence reference NM_001127255.1 and UniProt reference sequences Q8WX94 for *NLRP7* (Murdoch et al., 2006).

DNA cloning and phase establishment

For patients with compound heterozygous mutations, the phase was established based on the analysis of other family members with the exception of patient 678 with one live birth and for whom no other family members were available for analysis. For this patient, the following PCR primers, N7Ex5–9ChF1: 5′-GAACTGGGCTCGGCAGGATCTTCGCTCTC and N7Ex5–9ChR1: 5′-AAACCAGCCCGGGAAAGATGACAAGACCTC, were designed to amplify a 7955-bp genomic DNA fragment that encompasses her two mutations, R693P and N913S. The 7955-bp PCR fragment was cloned into the pCR4-TOPO vector and DNA from five insert-containing vectors were sequenced and analysed.

Results

Three live births from donated ova in two patients with recessive *NLRP7* mutations

Case 1

Patient 628 is of Indian origin. She presented at the age of 26 years with a history of four hydatidiform moles, of which one gave a gestational trophoblastic neoplasia (GTN). Because she had RHM, this patient was referred to the present authors' laboratory for mutation analysis in *NLRP7*, which revealed that she is compound heterozygous for two founder mutations c.2078G>C, p.Arg693Pro, and c.2738A>G, p.Asn913Ser, in the Indian population (Slim et al., 2009). After genetic counselling and based on her own desire to have children, the patient sought assisted reproductive technologies, received a donated ovum from an unrelated woman, and had a normal singleton pregnancy that led to a normal live birth of a boy delivered by Caesarean section (C-section). The boy is now 5.2 years old and in good health.

Case 2

Patient 748 is also of Indian origin. She presented at the age of 27 years with a history of three HM. *NLRP7* mutation analysis revealed that she is homozygous for a missense mutation, c.2078G>C, p.Arg693Pro. After genetic counselling, the patient decided to attempt assisted reproductive technologies with donated ova and in-vitro fertilization in the hope of achieving a normal pregnancy. Three embryos were transferred and resulted originally in a triplet pregnancy, of which one was spontaneously lost in the first trimester, and the pregnancy continued with two male embryos. The patient delivered two healthy boys at term by C-section. The twins are now about 4 years old and in good health.

Two live births from spontaneous conceptions in two patients with recessive *NLRP7* mutations

Case 3

Patient 1077 is a 24-year-old woman of European and Afro-Caribbean origin. She had a history of three pregnancy losses that consisted of one complete mole, which developed into GTN, one partial mole with an embryo and fetal crown rump length compatible with a gestational age of 7 weeks, and another molar pregnancy with a non-viable embryo. The patient was referred to our laboratory for *NLRP7* mutation analysis and was found to carry a previously described missense mutation, c.2738A>G, p.Asn913Ser (N913S), in a homozygous state and this patient was previously described (Brown et al., 2013).

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Eight months later, the patient had a spontaneous natural conception. At 39.5 weeks of gestation, she delivered a healthy boy (APGAR score 9/9) weighing at birth 3487 grams, who is now 1 year old and in good health. Histopathological evaluation of the placenta did not reveal any abnormalities.

Case 4

Patient 678 is of Indian origin. She presented at the age of 30 years with a history of three pregnancy losses that consisted of a blighted ovum followed by two HM that developed into GTN. *NLRP7* mutation analysis revealed that the patient has two mutations, c.2078G>C, p.Arg693Pro, and c.2738A>G, p.Asn913Ser, and this patient was previously described (Slim et al., 2009). Three years later, the patient had a live birth from a spontaneous conception.

During the pregnancy that led to the live birth, the patient was on anti-epileptic medication, but the pregnancy was uncomplicated. At 37.5 weeks of gestation, the patient had prelabour rupture of membranes and the fetus was found to be in transverse position. She underwent an emergency C-section and delivered a baby boy, who is currently healthy, attends nursery school, and has normal growth and development. The placenta of the live birth was normal with no histopathological abnormalities.

Because her two mutations have been shown to be frequent in the Indian population (Slim et al., 2009), and to exclude their coincidental presence by chance on the same parental chromosome, a large genomic DNA fragment from the patient's DNA encompassing her two mutations was PCR-amplified, cloned and sequenced. Sequence analysis of five clones demonstrated the presence of N913S in all them and none had the other mutation, R693P. Also, comparing the polymorphic variations observed in DNA in this patient, after direct PCR amplification of genomic DNA, and those observed in the clones showed the transmission of p.N913S, c.2738A>G, on the reference haplotype, NM_001127255.1:c.[2682T>C (rs269951); 2738A>G (rs104895503); 2775A>G (rs269950); 2810 + 98C>T (rs269949); 2810 + 123G>A (rs647845); 2810 + 126T>C (rs647844)] with the following alleles [T;G;A;C;G;T], which is identical to the previously reported haplotype carrying N913S (Slim et al., 2009). Therefore, these data demonstrate the presence of the two mutations in patient 678 on different parental chromosomes.

Discussion

To date and including this report, three patients with two *NLRP7* defective alleles had a total of four live births from donated ova. The first case was previously described (Fisher et al., 2011) and three are included in this report. The occurrence of three live births from donated ova in two additional patients confirms the idea that the primary role of *NLRP7* in the pathology of RHM lies in the oocyte and that ovum donation from unaffected individuals rescued the defects of these patients and allowed them to have successful pregnancies. Because it is not known how many patients with two *NLRP7* defective alleles have tried ovum donation, it is impossible to provide the frequency of live births with donated ova. However, given the complexity and high cost of such a procedure, we believe that few patients with two defective alleles in *NLRP7* have tried donated ova.

So far, including the patients described in this report, there have been 48 distinct mutations (**Figure 1**) reported, of which 17 missense and 31 led to premature protein truncation (nonsense, splicing, small/gross deletion or insertion and complex rearrangement) in a total of 131 patients with two defective alleles in *NLRP7* (fmf.igh.cnrs.fr/ISSAID/infevers/) (Milhavet et al., 2008). Among these patients, five (3.8%) had six live births from spontaneous conceptions (**Figure 1**, **Table 1**) in a total of 612 pregnancies (Helwani et al., 1999; Mahadevan et al., 2013; Sunde et al., 1993). Therefore, patients with two defective alleles in *NLRP7* may have live births from spontaneous conceptions from their own oocytes in 1% of their pregnancies. Two of the patients with live births have a homozygous invariant splice mutation, c.352 + 1G>A, that affects the splicing of the main *NLRP7* isoform and results in the insertion of four bases between exons 3 and 4 and is expected to lead to a frameshift and premature protein truncation two amino acids after exon 3 (p.Gly118Aspfs*2). However, this mutation leaves another minor isoform without exon 3 intact (Murdoch et al., 2006). The remaining three patients have one or two of the following missense mutations, p.Leu750Val (Mahadevan et al., 2013), p.Arg693Pro, and p.Asn913Ser, either in homozygous (Brown et al., 2013) or compound heterozygous state (Slim et al., 2009) (**Figure 1**).

The exact role of *NLRP7* in the pathology of molar pregnancies is not fully understood, but available data implicate its protein in the inflammatory response (Khare et al., 2012; Kinoshita et al., 2005; Messaed et al., 2011) and trophoblastic lineage differentiation (Mahadevan et al., 2014). This latter role is in agreement with a recent study from the present authors' group demonstrating that protein-truncating mutations, which are expected to have severe functional consequences on the protein, are associated with the absence of embryonic tissues in the conceptions of these patients, while missense mutations, which are expected to have milder functional consequences on the protein, are associated with the differentiation of some embryonic tissues (Nguyen et al., 2014).

Similarly, three of the six live births observed in patients with two *NLRP7* defective alleles occurred in patients with missense mutations and the remaining three in patients with the splice mutation, c.352 + 1G>A, that leaves a minor isoform intact, which may have compensated for the loss of the other isoforms and attenuated the functional impact of the mutation. The same applies to one early neonatal death, six stillbirths, and five molar pregnancies with well-formed fetuses that occurred either in the same patients who had the live births or in other patients with only missense mutations (**Figure 1**).

In summary, the data corroborate a previous observation on the benefit of ovum donation to overcome the defects of these patients and provide an explanation of the possible, but very rare, occurrence of live births from spontaneous conceptions in patients with two *NLRP7* defective alleles. Based on this study's observations and data described by various groups that are recapitulated in this report, we believe that perhaps a more optimistic genetic counselling can be provided to patients with two defective alleles in *NLRP7* by explaining to them the following: first, ovum donation is the best management option for patients with two defective alleles in *NLRP7* who desire to have children based on three patients, of which two are included in this report; second, exceptionally, some of these patients may have live births from

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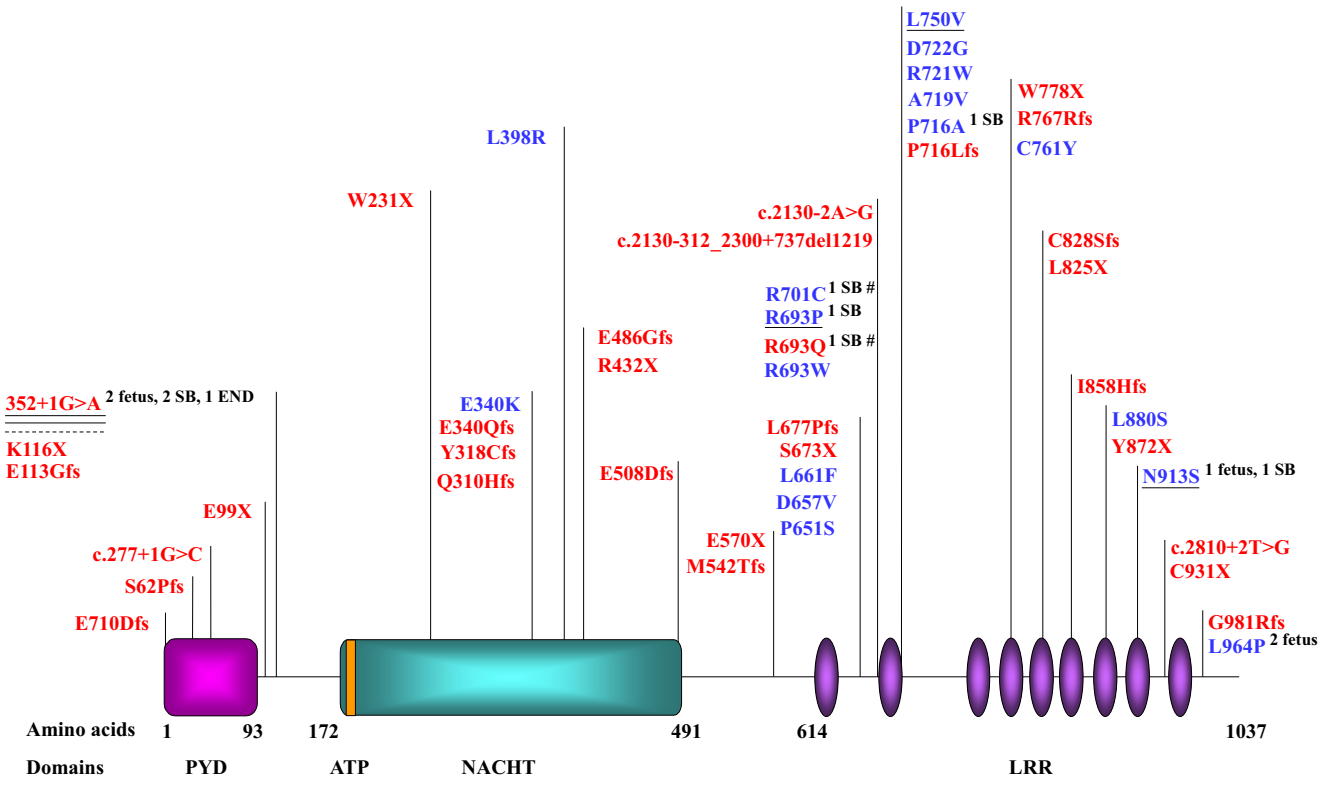

Figure 1 A schematic representation showing the distribution of all recessive *NLRP7* mutations observed in patients with RHM and highlighting those that are associated with live births from spontaneous natural conceptions. Only mutations reported in PubMed are shown. Missense mutations are in blue and mutations (nonsense, splicing, small/gross deletion or insertion and complex rearrangement) leading to premature protein truncation are in red. Mutations in patients who had live births are underlined. Each line corresponds to one normal live birth and the dashed line corresponds to a live birth with several abnormalities. #, refers to one stillbirth that occurred in a patient who is compound heterozygous for the two indicated mutations. ATP = 5′-triphosphate binding motif; END = early neonatal death; LRR = leucine-rich repeats; NACHT = the domain found in NAIP, CIITA, HET-E, and TP1 family of proteins; PYD = pyrin domain; SB = stillbirth. The superscript number before the reproductive outcome (e.g. fetus, SB, etc.) indicates the number of pregnancies with such entity. For instance, "2 fetus" refers to two pregnancies each with a fetus.

Table 1 Summary of the reproductive outcomes of patients with two *NLRP7* defective alleles and the nature of their mutations.

Mutation type	*Live birth*	*END*	*Stillbirth*	*HM+fetus*	*RL (HM+SA)*	*Total pregnancies*
Missense on both alleles	3	0	4	3	313	323
G118Dfs*2 on both alleles	3	1	2	2	24	32
At least one protein truncating[a]	0	0	0	0	257	257
Total	6	1	6	5	594	612

END = early neonatal death; HM = hydatidiform mole; RL = reproductive loss; SA = spontaneous abortion.
[a]Indicates protein truncating mutations with the exception of G118Dfs*2.

their own oocytes and this seems to be associated with some missense mutations. The most important advice to patients is the necessity of close follow-up and monitoring in the event of future pregnancies to prevent eventual complications of moles and the occurrence of stillbirths among their rare conceptions that may reach term.

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