The Genetics of Recurrent Hydatidiform Moles in China: Correlations between *NLRP7* Mutations, Molar Genotypes, and Reproductive Outcomes

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BACKGROUND Hydatidiform mole is a human pregnancy with abnormal embryonic development. *NLRP7* is a major autosomal recessive gene responsible for recurrent molar pregnancies and associated reproductive wastage in patients from several populations. To date, most patients with recurrent moles have two mutated *NLRP7* alleles and their molar tissues are diploid and biparental. However, so far, seven patients have been shown to have only one defective *NLRP7* allele. In some of these patients, moles of various genotypic types, including diploid androgenetic monospermic and triploid dispermic, have been described.

METHODS We sequenced *NLRP7* in 35 unrelated Chinese patients with recurrent reproductive wastage, including at least one HM. We also determined the parental contribution to some molar tissues from patients with *NLRP7* mutations and describe their reproductive outcomes after the use of assisted reproductive technologies (ART).

RESULTS We report three new protein-truncating mutations in *NLRP7* and show the presence of common ancestral haplotypes carrying identical mutations in unrelated Asian patients. We determined the parental contribution to six molar tissues and show the occurrence of three diploid androgenetic moles in patients with one defective allele while three diploid biparental moles occurred in patients with two defective alleles. We document the failure of pregnancies after ART in three patients with two defective alleles each and a successful pregnancy in one of two patients with one defective allele.

CONCLUSIONS *NLRP7* does not seem to be a major cause of RHMs in China despite the presence of founder effects for three mutations. Our data suggest that patients with a single defective allele have better reproductive outcomes than patients with two defective alleles and some of them may benefit from ART.

Key Words Recurrent Hydatidiform Mole, *NLRP7*, Spontaneous Abortion, Founder Effect, assisted reproductive technologies

Introduction

A hydatidiform mole (OMIM 231090) is a human pregnancy with no embryo, cystic degeneration of chorionic villi, and abnormal proliferation of the trophoblast. The common form of this condition occurs once in every 1000-1500 pregnancies in Western countries, but at higher frequencies in several underdeveloped and developing countries [1]. In China, the reported incidences of HMs vary from 1 to 8.83 in every 1000 pregnancies, with the highest incidence being in the province of Zhejiang [1]. Though the common form of this disorder is sporadic, 1-6% of patients will have a second mole, and about 10-20% will have a second non-molar reproductive wastage, most commonly a spontaneous abortion [2-6]. This indicates a genetic susceptibility of these women for reproductive wastage. The familial form of this condition, known as Familial Recurrent Hydatidiform Moles (FRHM), is rare but its exact frequency is not known.

The diagnosis of moles is made based on histopathological examination which allows the classification of moles as Complete Hydatidiform Mole (CHM) or Partial Hydatidiform Mole (PHM) based on the degree of trophoblast proliferation and the presence or absence of fetal tissues. At the genotype level, most PHMs are triploid with one maternal and two different paternal sets of chromosomes resulting from dispermic fertilization. Most CHMs are androgenetic, containing two sets of paternal chromosomes. The paternal contribution may be monospermic (approximately 80% of moles) or dispermic (20%). In rare cases, CHMs are diploid, with both a maternal and a paternal chromosome complement (BiCHM). These BiCHMs are associated with an autosomal recessive condition, in which affected women have recurrent moles (FRHM). Linkage analysis in affected families initially localized a gene for FRHM to 19q13.4 [7]. Refinement of the region using homozygosity mapping [8] and subsequent screening of candidate genes in the region led to the identification of the first maternal gene, *NLPR7*, as responsible for recurrent moles and reproductive wastage such as spontaneous abortions and stillbirths [9].

For patients with two moles, the quoted risk for a third mole is 10−23% [3, 6]. However, management options for women with recurrent consecutive molar pregnancies are limited, in particular for those who strongly desire to conceive their own biological offspring. These options range from no intervention to the use of assisted reproductive technologies (ART) such as intracytoplasmic sperm injection (ICSI), with or without preimplantation genetic diagnosis (PGD).

Without intervention the likelihood of a successful pregnancy is very low and the risk of malignancy increases with each consecutive CHM [10]. The chance of a successful pregnancy following ICSI is about 40% [11], and about 20% following PGD [12]. On average, ICSI results in a 20% to 25% chance of live birth. Theoretically, ICSI can prevent the dispermic fertilization which causes most PHMs, and, if coupled with PGD to select for male embryos could prevent CHMs that may arise from fertilization by a single diploid sperm carrying an X chromosome. However, IVF is an invasive medical procedure, which carries its own risks. Regardless of the risks, some patients with RHM have attempted ICSI and PGD to fulfill their desire of having their own children. To date, there has been only one report of success using IVF to prevent recurrent molar

pregnancies [13] and more data is required to confirm its general applications and benefits for women with recurrent moles.

In this study, we report *NLRP7* mutation analysis in 35 unrelated Chinese patients with recurrent reproductive wastage, including at least one HM. We identified three new protein-truncating mutations in *NLRP7* and demonstrate the presence of founder effects for three mutations in Asian populations. We also report the use of ART in five of these patients and the results of their conceptions.

Methods

Patients

A total of 35 unrelated Chinese patients with recurrent reproductive wastage, including at least 1 HM and their families, were ascertained. Their reproductive and medical histories are shown in Table 1 and Supplementary Materials. Patients 517, 492, 501, 101, 29, and 77 were previously reported (Table 1) [14, 15]. A medical history of lower reproductive tract infections was noted in five patients, 23, 96, 107, 108 and 492. Two patients, 12 and 781, suffered from endometriosis. Karyotype results were available for patients 29, 492, 517, 529, 765, 781 and 789 and their partners. All karyotypes were normal, except for two patients, 492 and 768, one with a Robertsonian translocation 45XX,rob(13;14)(q10;q10) and another with a reciprocal translocation 46,XX,t(1;13)(q32;q34), respectively. Five patients described in this study sought the use of ART. Patients 29, 492, 765, and 781 used their own oocytes while patient 517 used donated oocytes. Their procedures and results are summarised in Table 3. Semen analyses were performed for the partners of patients 29, 492, 765, and 781 and all met normal criteria. Also, Fluorescent *in situ* Hybridization (FISH) was conducted using a probe for chromosome 8 on a total of 200 sperm nuclei from each partner and revealed absence of diploid cells.

NLRP7 mutation analysis

Mutation analysis was performed by PCR amplification of genomic DNA of the 11 *NLRP7* exons followed by direct sequencing in both directions as previously described [15]. In patient 781 with a homozygous deletion spanning exon 6, the previously described primers to amplify exon 6, did not amplify any PCR product. Additional primers were designed and used to amplify and sequence its flanking sequences: Ex6delfwd: 5'CCACGTAACCCGTAGCACCTGTCA3': Ex6delrev, 5'GCTGCCCATGGGAAGA GGAGACTT3'. All sequences were analyzed using DNASTAR. DNA mutations are numbered according to reference sequence NM_206828.2, with nucleotide 1 being the A of the ATG translation initiation site. Protein numbering is according to reference sequence Q8WX94, beginning with the initiation codon. Our controls are women of Chinese origin from families with at least one child and no abnormal reproductive history.

Results

Identification of three new protein-truncating mutations in *NLRP7*

Mutation analysis revealed the presence of four previously reported mutations and three new mutations summarized in Table 1. The three new mutations are c.2468T>A, p.Leu823X (L823X), in patient 765; a 76-bp deletion in exon 4 that is predicted to lead to a frameshift and protein truncation, 1625_1700del76, p.Met542ThrfsX1 (M542fs), in patient 791; and a 1218-bp deletion spanning exon 6, c.2130-312_2300+737del1218, in patient 781. No RNA was available from this patient to investigate the consequences of this deletion on the protein, but the omission of exon 6 from the spliced RNA is predicted to result in a frameshift and protein truncation because exons 5 and 7 are not in frame. Of the 35 analyzed patients, 7 were found to have two mutated alleles (20%), 4 (11.4%) had one mutated allele each and 24 (68.6%) did not have any mutation in *NLRP7* (Table 1 & Supplementary Materials). Where additional family members were available for typing, we analyzed the segregation of the mutations within those families (Fig 1). Of note is the presence of p.Lys379Asn (K379N) in a heterozygous state in patient 774, her mother and sister, both of whom had had two spontaneous abortions each, while the patient had had one HM and one spontaneous abortion. Patient 293 with two previously reported mutations, p.Arg432X (R432X) and p.Ala719Val (A719V), has also another variant, c.2573T>C, p.Ile858Thr (I858T) that was inherited from her mother on the same haplotype carrying A719V (Fig. 1). We also checked for the presence of the two identified missense mutations, A719V and I858T in 50 Chinese control women (each with at least one child) from the general population and did not find them in any control. In addition, A719V was not found in 100 European women (with 5 to 15 children) from the general population. The fact that R432X and A719V were previously reported in other patients indicates that they are probably disease causing while I858T could be either a second mutation or a rare variant on the same haplotype carrying A719V.

Founder effects for three *NLRP7* mutations in China

Among the 12 *NLRP7* mutations found in our patients, three were each found in two unrelated Chinese families of Han origin (Table 1). K379N was found in MoCh73 and MoCh193; R432X in Ch77 and MoCh293; and L825X in Ch77 and MoCh200. To investigate the possibility of a founder effect for these mutations, haplotypes were established by genotyping homozygous patients and additional family members of heterozygous patients at 42 SNPs within, and in the proximity of, *NLRP*7. This analysis revealed the presence of identical mutations on haplotypes common to both carriers indicating their inheritance from common ancestors (Table 2). Interestingly, one of these founder mutations, L825X, was previously identified by our group in a family from Pakistan, MoPa61 [9], who shares the same haplotype with the two unrelated Chinese patients, thus indicating a founder effect in Asian populations (Table 2).

Correlation between *NLRP7* mutations, molar genotype, and ART outcomes

To determine the parental contribution to the moles, available molar tissues from patients 29, 101, and 765 were genotyped along with parental DNAs. The results of this analysis and of previously genotyped molar tissues from all the Chinese patients with *NLRP7* mutations are recapitulated in Table 1 and Supplementary materials. Of the analyzed tissues, three moles were found to be biparental and three were androgenetic. Interestingly, the three biparental moles occurred in women with two *NLRP7* mutations whereas the three androgenetic moles occurred in two patients, each with a single identified defective allele (Table 1).

Among the patients with *NLRP7* mutations included in this study, five underwent IVF. None of the three women carrying two *NLRP7* defective alleles successfully achieved pregnancy despite the fact that these women received a total of 11 embryos. However, of the four embryos transferred to two women who each has a single defective allele, one embryo implanted and the patient is now pregnant (24w) and her pregnancy is normal to date (Table 3).

Discussion

To date, 42 different mutations in *NLRP7* have been reported by different groups in women with RHMs and reproductive wastage from various populations demonstrating that *NLRP7* is a major cause for this condition [9, 14-22]. In this study, we sequenced *NLRP7* in a total of 35 unrelated Chinese patients and found mutations in only 11 of them (31.4%). Of these 11 patients, four have one defective allele and seven have two defective alleles. Among the latter, only two patients were found homozygous for their mutations.

When only patients with at least two HMs are considered, 11 out of 23 (48%) were found to have at least one *NLRP7* mutation. Comparing these data with those of Pakistani (11 unrelated patients) [19] and Indian patients (13 unrelated patients) [20] with at least 2 HMs revealed two distinctive features of the Chinese population. First, *NLRP7* is not a major gene for RHM and reproductive wastage in China since only 48% of Chinese patients with at least 2 HMs have *NLRP7* mutations as compared to 81% and 84% of Pakistani and Indian patients, respectively. The absence of *NLRP7* mutations does not exclude the presence of unidentified mutations in the promoter and intronic regions, or the presence of large deletions, duplications, and rearrangements that cannot be detected by conventional DNA sequencing. However, the fact that the same PCR primers and conditions used in this study were also used by our group to analyze Indian patients indicates that the genetic causes of recurrent moles are not the same in China, Pakistan, and India. Another particularity of the Chinese population is the presence of a lower rate of consanguinity in China than in Pakistan and India since only 20% of our Chinese patients with at least 2 HMs have homozygous mutations as compared to 88% among Pakistani and 72% of Indian patients.

We previously suggested that females carrying one mutated *NLRP7* allele are predisposed to pregnancy loss [15]. This conclusion was based on the occurrence of

stillbirths and spontaneous abortions in heterozygous mothers and sisters of women who have had recurrent biparental hydatidiform moles. This was refuted by Hayward *et al.* and Williams *et al.* [19, 23] since there was no reproductive wastage in heterozygous relatives of their patients. In this study, we show a previously reported mutation, K379N, in a new patient with one mole while her mother and sister who carry the same mutation had had two spontaneous abortions each, with other normal pregnancies (Fig.1). These results suggest that some, but not all, *NLRP7* mutations are associated with reproductive wastage when present in a heterozygous state. This also indicates that mild genetic defects due to one defective allele may be modulated by environmental and other genetic factors and consequently are not associated with moles in every patient and do not impede all of their pregnancies.

Founder effects for two *NLRP7* mutations have been demonstrated in the Indian population based on haplotype analysis [20] and in several other populations based on the presence of identical mutations in patients from the same ethnicities [16, 22]. In agreement with these data is the presence of founder effects for three Chinese mutations, K379N, R432X, and L825X with the latter being common to the Pakistani and Chinese populations. The identification of founder mutations in Asian populations is interesting because these populations are known to have higher incidences of moles than European populations.

It has already been reported that in patients with many RHMs, the moles tend to be biparental in origin and most of these patients have two defective *NLRP7* alleles [24]. To date, beside the Chinese patients reported or recapitulated in this study, only one other patient with an androgenetic mole has been reported and in this patient, one *NLRP7* mutation had been identified [14]. In addition, two other patients each with one mutated *NLRP7* allele were also found to have triploid and diandric moles [25]. In support of these observations is our finding that the three biparental moles occurred in patients with two defective *NLRP7* alleles (patients 29, 101, and 519) while the three androgenetic moles occurred in two patients (492 and 765) with one defective allele, each. In addition, there have not been any reports, to date, of patients carrying two defective *NLRP7* mutations who had had androgenetic moles. Altogether, these data indicate an association between *NLRP7* mutation status and molar genotypes in which two defective alleles are associated with biparental moles while a single defective allele is associated with diploid androgenetic and triploid moles. Characterizing more molar tissues from patients with one or two *NLRP7* mutations will be helpful to validate our observations in a larger cohort of patients.

It is still not known exactly how *NLRP7* mutations cause molar pregnancies. We previously showed the occurrence of postzygotic abnormalities during *in vivo* and *in vitro* development of embryos from two patients carrying one defective *NLRP7* allele each [14]. We proposed that the rate of embryo cleavage abnormalities may be mutation-dependent. In line with our proposal is the fact that among the five patients with at least one *NLRP7* mutation who tried ART, only one patient had postzygotic abnormalities after IVF and this is the same patient in which we previously reported postzygotic abnormalities after ICSI. In this study, none of the three patients with two defective

alleles achieved a successful pregnancy after ART, while one of the two patients with one defective allele in currently five months pregnant and her pregnancy is going well. Also, the data reported in this study indicate, first that not all patients with *NLRP7* mutations have postzygotic abnormalities; and second that patients with one mutated *NLRP7* allele have better reproductive outcomes than patients with two mutated alleles and have higher chances of having normal pregnancies. These observations are in agreement with the association of two *NLRP7* mutations with highly recurrent biparental moles and low chances of successful pregnancies while one mutated allele is associated with androgenetic or triploid moles, less recurrent moles, and more live births. These data are also in line with a recent report by Ogilvie *et al.* documenting the successful use of ICSI and preimplantation genotyping in a patient with recurrent androgenetic complete moles [13]. Validating our observations in a larger cohort of patients will provide more insight about management options for patients with recurrent moles and one or two *NLRP7* mutations.

Among the 35 analyzed patients, two 492 and 768, had abnormal karyotypes 45,XX,rob(13;14)(q10;q10) and 46,XX,t(1;13)(q32;q34), respectively. While the latter did not have any mutation in *NLRP7*, the former is heterozygous for one mutation. Among previously reported patients with recurrent moles and *NLRP7* mutations, one patient homozygous for mutation R693W and carrying an abnormal karyotype 46,XXinv(14)(q21q23~31) [17] has been reported. Although, some of these chromosomal abnormalities are present in the general population and in women with normal pregnancies, their presence in the small number of reported patients with recurrent moles and the fact that they all involve acrocentric chromosomes is intriguing and could reflect some genetic instability that could be related to the genetic defect of the patients. Analyzing the karyotype of the parents of these patients will be important to determine whether these mutations are inherited in their respective families or whether they occured *de novo*.

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Table I. Chinese patients with recurrent reproductive wastage including at least 1 HM and one defective *NLRP7* allele

Family ID	Patient ID	Family Member	Exon	Mutations DNA	Mutations Protein	Genotype of Analyzed Moles	Reproductive history	Other gynecological morbidities, relevant medical and family histories	References
Patients with two *NLRP7* defective alleles									
MoCh76	517		3, 5	c. [295G>T];[1970A>T]	p.[Glu99X];[Asp657Val]		2 CHM, 1 failed ART with a donated ovum		Qian et al., 2007
	519	sister	3, 5	c.[295G>T];[1970A>T]	p.[Glu99X];[Asp657Val]	BiCHM	3 CHM, PHM		
Ch29	29		6	c.[2165A>G];[2165A>G]	p.[Asp722Gly];[Asp722Gly]	**BiCHM***	2 SA, 2 PHM, BiCHM, CHM, SA, PHM, 3 failed ART		Deveault et al., 2009 & this study
Ch77	77		4, 7	c. [1294C>T]; [2471+1G>A]	p.[Arg432X];[Leu825X]		SA, 3 CHM		Deveault et al., 2009
	78	sister	4, 7	c.[1294C>T]; [2471+1G>A]	p.[Arg432X];[Leu825X]		3 SA, 4 CHM		
Ch101	101		5	c. [2101C>T];[2078G>A]	p.[Arg701Cys];[Arg693Gln]	**BiCHM***	2 HM, SB, 2 SA, CHM		Deveault et al., 2009
MoCh195	781		6	c.[**2130-312_2300+737del1218**]; [**2130-312_2300+737del1218**]			2 CHM, failed ART, CHM ^{PTD}	Endometriosis & laparoscopy after the first 2 CHM	This study
MoCh200	791		4, 7	c.[**1625_1700del76**]; [2471+1G>A]	**p.[Met542ThrfsX2]** ;[Leu825X]		3 HM		This study
MoCh293	293		4, 6	c.[1294C>T]; [2156C>T]	p.[Arg432X];[Ala719Val]		2 HM		This study
Patients with one *NLRP7* defective allele									
MoCh73	501		4	c. [1137G>C];[=]	p.[Lys379Asn];[=]		1 SA/HM, 1 CHM, 1 SA		Deveault et al., 2009
MoCh71	492		2	c. [251G>A];[=]	p.[Cys84Tyr];[=]	AnCHM	CHM, SA, failed ART	Blood karyotype of the patient is 45,XX,rob(13;14)(q10;q10) Chlamydia	Deveault et al., 2009
MoCh193	774		4	c. [1137G>C];[=]	p.[Lys379Asn];[=]		ET, HM, SA^{PTD}		This study
MoCh190	765		7	**c. [2468T>A];[=]**	**p.[Leu823X];[=]**	**2 AnCHM***	2 ET, 2 AnCHM	G6PD deficiency	This study

New mutations are underlined and in bold character. ET, stands for elective termination; SA, for spontaneous abortion; NP, normal pregnancy; HM, hydatidiform mole; PTD, persistent trophoblast disease; PHM, partial HM; BiCHM, indicates a complete mole found biparental; AnCHM, androgenetic complete HM; CHM, complete HM; SB, stillbirth; 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; ART, assisted reproductive technologies. Reproductive outcomes are listed by chonological order starting from the left; the absence of a number indicates one such reproductive outcome. Parental contribution to molar tissues reported in this study are in bold. * indicates tissues for which the parental contribution to the moles is provided in the Supplementary materials.

Table II. Haplotype analysis showing the inheritance of identical mutations on identical haplotypes

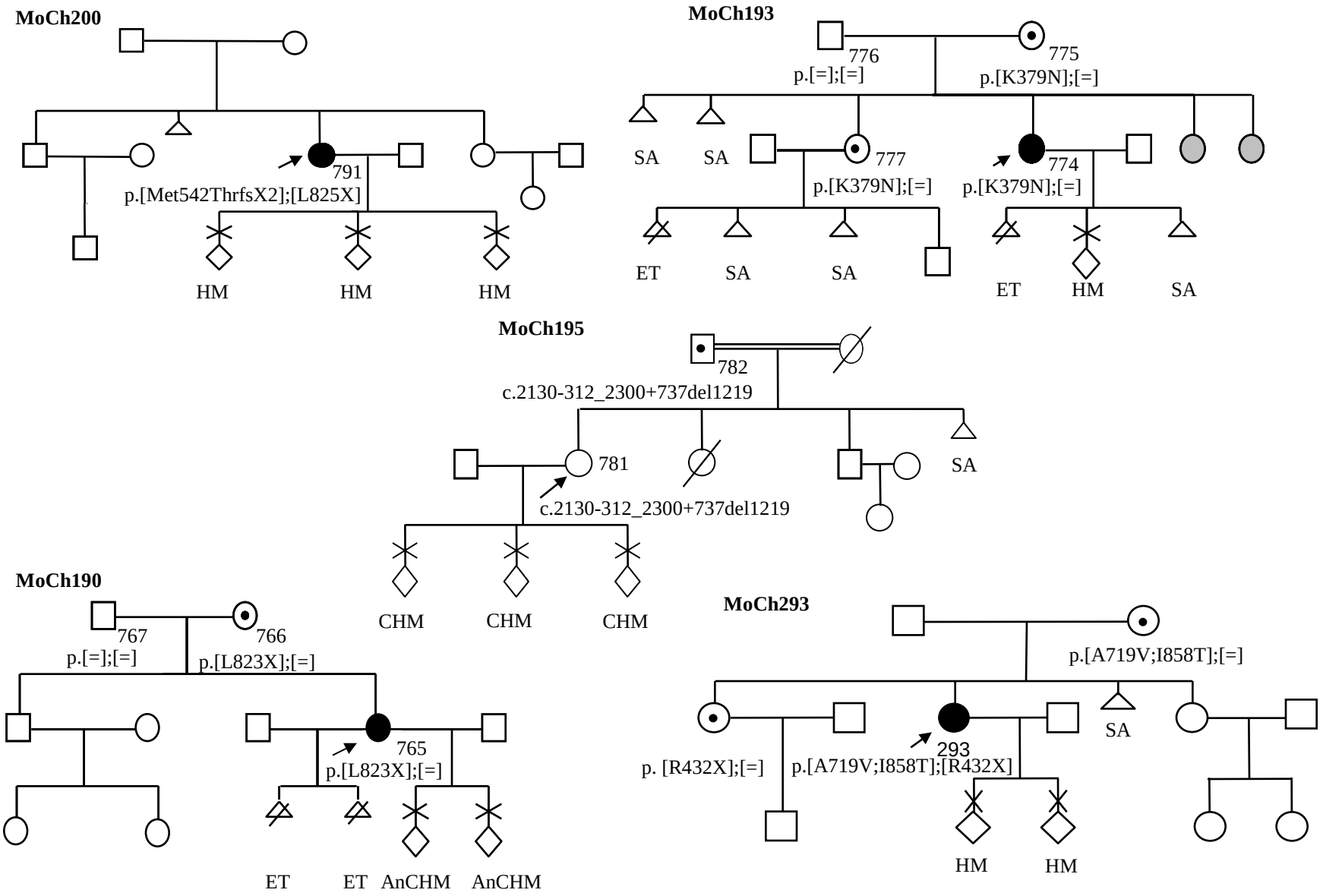
Variants		MoCh73		MoCh93		MoCh293		Ch77		MoCh200		MoPa61	
cDNA	Protein	501		774		293		78		791		443	
c.-40+21 C>T		C	C	C	C	C	C	C	C	C	C	C	C
c.-40+36C>T		C	C	C	C	C	C	C	C	C	C	C	C
c.-40+121G>A		G	G	G	G	G	G	G	G	G	G	G	G
c.-39-90G>C		G	G	G	G	G	G	G	G	G	G	G	G
c.-39-16C>T		C	C	C	C	C	C	C	C	C	C	C	C
c.353-56A>G		A	A	A	A	A	A	A	A	A	A	A	A
c.390G>A	Gln130Gln	G	G	G	G	G	G	G	G	G	G	G	G
c.831A>C	Lys277Gln	A	A	A	A	A	A	A	A	A	A	C	C
c.955G>A	Val319Ile	G	G	G	G	G	G	G	G	G	G	G	G
c.1137G>C	**Lys379Asn**	G	**C**	**C**	G	G	G	G	G	G	G	G	G
c.1294C>T	**Arg432X**	C	C	C	C	C	**T**	**T**	C	C	C	C	C
c.1441G>A	Ala481Thr	G	G	G	G	G	G	G	G	G	G	G	G
c.1460G>A	Gly487Glu	G	G	G	G	G	G	G	G	G	G	G	G
c.1491C>T	Iso497Iso	C	C	C	C	C	C	C	C	C	C	C	C
c.1532A>G	Lys511Arg	A	A	A	A	A	A	A	A	A	A	A	A
c.1625_1700del76	**Met542ThrfsX2**	nl	nl	nl	nl	nl	nl	nl	nl	del	nl	nl	nl
c.2156C>T	**Ala719Val**	C	C	C	C	**T**	C	C	C	C	C	C	C
c.2300+57T>C		T	T	T	C	T	C	C /	T	C	T	T	T
c.2471+1G>A	**Leu825X**	G	G	G	G	G	G	G	**A**	G	**A**	**A**	**A**
c.2472-67A>G		A	G	G	A	A	G	A	A	A	A	A	A
c.2573T>C	Ile858Thr	T	T	T	T	C	T	T	T	T	T	T	T
c.2682T>C	Y894Y	T	C	C	C	T	C	C	C	C	T	T	T
c.2775A>G	A925A	A	G	G	G	A	G	G	G	G	A	A	A
c.2810+98C>T		C	T	T	T	C	T	T	T	T	C	C	C
c.2810+123G>A		G	A	A	A	G	A	A	A	A	G	G	G
c.2810+126T>C		T	C	C	C	T	C	C	C	C	T	T	T
c.2811-523C>T		T	C	C	C	T	C	C	C	C	C	n.a.	
c.2811-496T>C		T	C	C	C	T	C	C	C	C	T	n.a.	
c.2811-402C>T		C	T	T	T	C	T	T	T	T	C	n.a.	
c.2811-399A>G		A	G	G	G	A	G	G	G	G	A	n.a.	
c.2811-394G>T		G	T	T	T	G	T	T	T	T	G	n.a.	
c.2811-329A>G		A	G	G	G	A	G	G	G	G	A	n.a.	
c.2811-312C>A		C	A	A	A	C	A	A	A	A	C	n.a.	
c.2811-228T>C		T	C	C	C	T	C	C	C	C	T	n.a.	
c.2811-178G>A		A	G	G	G	G	G	G	G	G	G	n.a.	
c.2811-54T>G		T	G	G	G	T	G	G	G	G	T	T	T
c.2811-25G>C		C	G	G	G	C	G	G	G	G	G	G	G
c.2811-23A>G		A	G	G	G	A	G	G	G	G	A	A	A
c.2981+29_32del		nl	del	del	del	n.a.		del	del	n.a.		nl	nl
c.2981+123T>C		T	C	C	C	n.a.		n.a.		n.a.		T	T
c.2982-28delG		nl	nl	del/nl		nl	nl	nl	nl	del/nl		nl	nl
c.*290T>C		C	C	C	C	T	C	C	C	n.a.		T	T

Mutations found in the different patients are in bold and underlined characters. n.a., indicates not available; nl, inidcates no deletion at this site. Identical haplotypes are indicated by identical border lines.

Table III. Reproductive Outcomes after ART in five patients with at least one *NLRP7* mutation

Patient ID	Oocyte source	*NLRP7* mutation	Number of oocytes		PGD/PGH/ FISH results	Number of embryos transferred	Pregnancy Results	Remarks
			IVF	ICSI				
517	Donor	c.[295G>T];[1970A>T]	7			2	negative	Deveault *et al.* 2009
29	Patient	c.[2165A>G];[2165A>G]		10				Deveault *et al.* 2009
Cycle 1			8			1	negative	This study
Cycle 2						3	negative	This study
Cycle 3						3	negative	This study
781	Patient	c.[2130-312_2300 +737del1218];[2130-312_2300 +737del1218]	4			2	negative	This study
492	Patient	c. [251G>A];[=]						
Cycle 1				6	3 embryos (6c)	0	negative	Deveault et al. 2009
Cycle 2			11		4 embryos: 3 abnormal, 1 normal	1	negative	This study
765	Patient	c.[2468T>A];[=]						This study
Cycle 1				5	Fresh embryo	1	negative	
					Frozen embryos	2	positive	currently 5 months pregnant with a single fetus, no abnormalities detected

IVF, stands for *in vitro* fertilization; ICSI, for intracytoplasmic sperm injection; 6c, indicates that the three embryos arrested at the 6 cell stage.


MoCh200
791
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HM
HM
MoCh193
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775
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SA
SA
777
p.[K379N];[=]
774
p.[K379N];[=]
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SA
SA
ET
HM
SA
MoCh195
782
c.2130-312_2300+737del1219
781
SA
c.2130-312_2300+737del1219
CHM
CHM
CHM
MoCh190
767
p.[=];[=]
766
p.[L823X];[=]
765
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ET
AnCHM
AnCHM
MoCh293
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SA
p. [R432X];[=]
293
p.[A719V;I858T];[R432X]
HM
HM