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► To cite this version:

Cm Wang, Ph Dixon, S Decordova, Md Hodges, Nj Sebire, et al.. Identification of 13 novel NLRP7 mutations in 20 families with recurrent hydatidiform mole; missense mutations cluster in the leucine rich region.. Journal of Medical Genetics, 2009, 46 (8), pp.569. 10.1136/jmg.2008.064196 . hal-00552673

HAL Id: hal-00552673

<https://hal.science/hal-00552673>

Submitted on 6 Jan 2011

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Identification of 13 novel *NLRP7* mutations in 20 families with recurrent hydatidiform mole; missense mutations cluster in the leucine rich region.

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ABSTRACT

Background: *NLRP7* (NALP7) has recently been identified as the causative gene for familial recurrent hydatidiform mole (FRHM), a rare autosomal recessive condition in which affected individuals have recurrent molar pregnancies of diploid biparental origin. To date only a small number of affected families have been described. Our objectives were to investigate the diversity of mutations and their localisation to one or both isoforms of *NLRP7*, by screening a large series of women with FRHM, and to examine the normal expression of *NLRP7* in ovarian tissue.

Methods: Fluorescent microsatellite genotyping of molar tissue was used to establish a diagnosis of FRHM. Twenty families were subsequently screened for mutations in *NLRP7* using DNA sequencing. Expression of *NLRP7* in the ovary was examined by immunohistochemical staining.

Results: Sixteen different mutations were identified in the study, thirteen of which were novel. Missense mutations were found to be present in transcript variant 2 of *NLRP7* and cluster in the leucine rich region (LRR). A male with two affected sisters and homozygous for the p.R693P mutation had normal reproductive outcomes. In normal human ovary *NLRP7* expression is confined to the oocytes and present at all stages from primordial to tertiary follicles.

Conclusion: Thirteen novel mutations in *NLRP7* were identified. We confirm that mutations in *NLRP7* affect female, but not male, reproduction and provide evidence that transcript variant 2 of *NLRP7* is the important isoform in this condition. The mutation clustering observed identifies the LRR to be critical for normal functioning of *NLRP7*.

Key words: Recurrent hydatidiform mole, complete hydatidiform mole, *NLRP7*, genomic imprinting, oocyte

INTRODUCTION

Familial recurrent hydatidiform mole (FRHM) is a rare autosomal recessive condition in which affected individuals have a predisposition to the abnormal pregnancy complete hydatidiform mole (CHM). In this condition other pregnancy loss is also common while normal pregnancies are extremely rare [1]. CHM (OMIM 231090) are characterised by hydropic swelling and trophoblastic hyperplasia of the placental villi [2]. Most occur sporadically and are androgenetic in origin [3]. In contrast CHM in women with FRHM appear genetically normal, with a chromosome complement from each parent [4, 5]. These diploid biparental CHM not only have a similar pathology to androgenetic CHM, but also show similar imprinting defects. A paternal methylation pattern on both alleles of maternally transcribed genes has been reported in both types of CHM [6, 7, 8]. Diploid biparental CHM also show aberrant expression of maternally transcribed proteins, such as CDKN1C, similar to that seen in androgenetic CHM [9].

Linkage analysis in families initially localised the gene for FRHM to 19q13.4 [10]. Refinement of the region using homozygosity mapping [11, 12] and subsequent screening of candidate genes in the region led to *NLRP7* (NACHT, leucine rich repeat and PYD containing 7) being identified as the causative gene [13]. To date mutations in *NLRP7* have been reported in only 12 families with CHM of biparental origin [8, 13, 14]. A variety of mutations have been identified including splice site mutations, point mutations and an intragenic duplication [8, 13, 14]. The mechanisms by which mutations in *NLRP7*, a member of a gene family more usually involved in inflammatory and apoptotic pathways [15], result in the characteristic imprinting defects and abnormal development seen in CHM remain elusive.

In order to better understand the role of *NLRP7* in early development, we have performed mutation analysis in a large cohort of families from diverse ethnic backgrounds. We have also examined the expression of *NLRP7* in the ovary and compared this with the expression of other *NLRP* family members shown to be important in early embryonic development.

PATIENTS AND METHODS

Patients

Seven families, each having two sisters with recurrent CHM, were included in the study (table 1). Five of these have been previously described [11, 12, 16] while cases 9 and 16 were previously unreported families of Asian and Caucasian origin respectively. A further 16 women, with at least 3 CHM but no other family history of molar pregnancies, were identified from the databases of the Trophoblastic Tumour Screening and Treatment Centre, Imperial College Healthcare NHS Trust or referred to the Centre for diagnosis during this study. A single case with 2 CHM and a miscarriage, for which no tissue was available for pathological diagnosis, was also included. These seventeen women were from small families and potentially represent single affected individuals from families with FRHM. Tissue from one or more molar pregnancies was genotyped in both unreported families and all 17 individuals with recurrent CHM. Twenty five unaffected relatives from seven of the families agreed to participate in the study. The study was approved by the Riverside Research Ethics Committee (RREC 2652).

Fluorescent microsatellite genotyping of complete hydatidiform moles

In order to confirm the diagnosis of FRHM tissue from one or more molar pregnancies from each affected individual was characterised using fluorescent microsatellite genotyping of parental blood and molar tissue. DNA was extracted from blood samples using a Qiagen blood mini kit (Qiagen, Sussex, UK). DNA from CHM was prepared from tissue microdissected from formalin-fixed, paraffin-embedded sections as previously described [5]. PCR amplification of DNA was performed with a panel of 12 pairs of primers for microsatellite markers on different chromosomes. PCR products were resolved by capillary electrophoresis using an ABI 310 Genetic Analyser and genotypes determined with ABI 310 PRISM GeneScan software (Applied Biosystems, Warrington, UK). The genotype of the molar tissue was compared with that of the parents to determine whether the CHM was androgenetic or biparental [5].

Mutation screening of *NLRP7*

DNA was extracted from blood samples of both affected and unaffected family members as described above. DNA was amplified using the polymerase chain

reaction and a panel of 15 primers designed to cover the 11 exons and flanking intronic sequence of *NLRP7* (supplementary table 1). The annealing temperature used in the PCR reaction was 58°C for all primer pairs with the exception of those for exons 2 and 7 when annealing temperatures were 60°C and 55°C respectively. PCR products were purified using MicroSpin S-400 columns (GE Healthcare, Buckinghamshire, UK), sequenced in forward and reverse orientation using a 3100 or 3700 Genetic Analyser (Applied Biosystems, Warrington, UK) and analysed with CodonCode aligner software (CodonCode Corporation, MA, USA).

SNP genotyping of missense mutations

To determine whether missense mutations identified in this study were likely to be pathogenic or single nucleotide polymorphisms (SNPs), SNP genotyping was performed for 192 Caucasian and 198 Asian control chromosomes using a Taqman SNP genotyping assay for each mutation. Each assay was designed using File Builder Software (Applied Biosystems, Warrington, UK) and submitted to Applied Biosystems for assay synthesis. Genotyping assays were performed and genotypes of the samples determined using the 7500 FAST Real-Time PCR System and SDS software v1.3 (Applied Biosystems, Warrington, UK). Details of primers and probes are available upon request.

Expression of NLRP7, NLRP5 and NLRP9 in human ovary

Eight formalin-fixed, paraffin-embedded blocks of normal human ovarian tissue, from women with an age range of 30 – 41 years, were provided by the Imperial College Healthcare NHS Trust Human Biomaterials Resource centre. Use of the tissue was approved by COREC of Wales (07/MRE09/54). Immunohistochemistry was performed on 5 µm tissue sections using goat polyclonal antibodies against NLRP7, NLRP5 or NLRP9 (Santa Cruz Biotechnology, CA, USA) at a dilution of 1:100 in phosphate buffered saline. Appropriate epitope blocking controls were performed to confirm the specificity of each antibody. Goat IgG (1:200; Sigma, Poole, UK) staining was also included as a negative control to confirm false positive binding of antibodies was absent. Hydrogen peroxidase conjugated donkey anti-goat secondary antibody (Santa Cruz Biotechnology, CA, USA) was added at a dilution of 1:200, incubated for 1 hour and the slides developed using diaminobenzidine (100 ng/ml;

Sigma, Poole, UK) as the chromogen. Slides were imaged using a brightfield microscope (Nikon Eclipse E600) and a Nikon digital camera (DXM 1200).

RESULTS

Fluorescent microsatellite genotyping of complete hydatidiform moles

Eleven CHM from the two previously unreported families with two affected sisters were genotyped and all confirmed to be diploid biparental CHM. CHM from a further 16 individuals with three or more pathologically confirmed CHM and no normal pregnancies were also genotyped. In 12 cases all CHM were shown to be diploid biparental CHM, confirming a diagnosis of FRHM and the families included in the study. All CHM in the remaining four cases were shown to be androgenetic and the cases excluded. Finally a woman with two CHM and a miscarriage of unknown pathology was included after both CHM were shown to be of diploid biparental origin.

Mutation screening of *NLRP7*

Twenty families with a confirmed diagnosis of FRHM were available for screening (table 1). Sixteen different mutations were identified in 17 of the families including seven novel mutations, predicted to give rise to premature stop codons and a truncated protein, and 6 novel missense mutations (table 1; fig 1). In all families where two affected sisters were screened, identical mutations were found in both sisters. The parents were screened in four of these cases (cases 4, 6, 9, 11) and shown to be heterozygous for the same mutation, confirming normal Mendelian inheritance of the defective gene.

Only three of the 16 mutations identified in the present study, p.R693W, p.R693P and p.N913S, have been previously described [13]. Case 10 was of interest in that she was homozygous for the p.R693P mutation but also carried a single copy of the p.N913S mutation. Sequencing of DNA from other family members confirmed inheritance of the p.R693P mutation from her mother and both the p.R693P and p.N913S mutation from her father. Both these variants have been found in the same individual in a previous case of Asian origin [13]. This case was described as a compound heterozygote although no evidence was provided to show that the two mutations were inherited independently. One other mutation occurred in more than one case in the present study, a novel 14 base duplication in exon 4 (fig 2), found in three Caucasian

families. This was homozygous in two cases (cases 2, 3) and heterozygous in a third (case 16). In this case both sisters were also heterozygous for a single base deletion, c.2030delT suggesting they were compound heterozygotes. Two further cases (cases 15, 17) were apparent compound heterozygotes. In case 17 screening of other family members demonstrated inheritance of the p.R432X mutation from the father and the novel missense mutation, p.R693Q, from the mother. Parental DNA samples were not available in case 15 and 16. However, screening of DNA from the 3 CHM in case 15, and a CHM in case 16, identified only a single mutation in each CHM confirming the mutations were not carried on the same haplotype and that these cases were compound heterozygotes. To date no mutations have been found in three families investigated (cases 18-20).

Five sisters with normal reproductive outcomes were screened in three families (cases 4, 9, 17). Two were found to be carriers and three to have no mutations. Six male siblings from three families (cases 9, 10, 11) were also screened. One male sibling (case 9) was found to be homozygous for the same p.R693P mutation as his two sisters. While the sisters have a history of nine pregnancies all of which resulted in a CHM, their brother has a normal male child.

SNP genotyping of missense mutations

SNP genotyping of DNA from individuals homozygous or heterozygous for missense mutations (p.L398R, p.P651S, p.R693W, p.R693P, p.R693Q, p.P716A, p.R721W, p.N913S, p.C761Y) confirmed the presence of the expected mutations in affected individuals and unaffected family members. None of these mutations were identified in 192 Caucasian or 198 Asian control chromosomes.

Expression of NLRP7, NLRP5 and NLRP9 in human ovary

Within the ovary, expression of NLRP7, NLRP5 and NLRP9 was confined to the oocytes. Strong cytoplasmic staining was identified in all stages of growing oocytes, including primordial, primary, secondary and tertiary follicles, in all tissue samples examined (fig 3). The three NLRP proteins showed a similar pattern of expression within the oocytes. No expression was observed in the surrounding granulosa or stromal cells. Specific epitope peptides, used to raise the antibodies, successfully inhibited positive staining when added to the primary antibodies confirming the

specificity of the antibodies (fig 3). Goat IgG staining showed no false positive binding of NLRP antibodies to the tissue.

Table 1 *NLRP7* mutations, reproductive outcomes and ethnic origin of women with FRHM

Case	Affected individuals	Homozygous mutations ^a	Heterozygous mutations	Predicted protein changes	Reproductive / Clinical outcomes		Ethnic origin	Reference
01	Proband	c.183delC	-	p.T61TfsX7	4 CHM; 1 SA	-	Caucasian	
02	Proband	c.939_952dup14	-	p.Y318CfsX7	3 CHM	PTD	Caucasian	
03	Proband	c.939_952dup14	-	p.Y318CfsX7	3 CHM; 1 SA	PTD	Caucasian	
04	2 sisters	c.1193T>G	-	p.L398R	S1: 2 CHM; 1 PHM S2: 3 CHM; 1 SA	PTD -	Asian	[9]
05	Proband	c.1708G>T	-	p.E570X	3 CHM	-	Asian	[5]
06	2 sisters	c.1951C>T	-	p.P651S	S1: 6 CHM; 2 SA S2: 3 CHM; 1 SA	PTD -	Caucasian	[11]
07	Proband	c.2077C>T	-	p.R693W	4 HM; 3 SA	-	Caucasian	[17]
08	Proband	c.2077C>T	-	p.R693W	7 HM	-	Caucasian	
09	2 sisters	c.2078G>C	-	p.R693P	S1: 4 CHM S2: 5 CHM	- -	Asian	
10	Proband	c.2078G>C	c.2738A>G	p.R693P / p.N913S	2 CHM; 1 SA	-	Asian	
11	2 sisters	c.2146C>G	-	p.P716A	S1: 1 CHM S2: 1 CHM; 1 PHM; 1 SB; 6 SA	PTD -	Caucasian	[12]
12	Proband	c.2147delC	-	p.P716LfsX21	4 CHM	2 PTD	Caucasian	
13	Proband	c.2161C>T	-	p.R721W	6 CHM	-	Asian	
14	Proband	c.2282G>A	-	p.C761Y	3 CHM	2 PTD	Chinese	
15	Proband	-	c.337_338insG c.2078G>C	p.E113GfsX7 p.R693P	3 CHM	-	Caucasian	
16	2 sisters	-	c.939_952dup14 c.2030delT	p.Y318CfsX7 p.L677PfsX6	S1: 3 CHM; 2 SA S2: 1 CHM	- -	Caucasian	
17	2 sisters ^b	-	c.1294C>T c.2078G>A	p.R432X p.R693Q	S1: 5 CHM; 7 SA S2: 4 CHM	2 PTD PTD	Chinese	[16]
18	2 sisters	-	-	-	S1: 3 CHM; 1 END S2: 2 CHM; 2 SA; 1 EP	PTD PTD	Chinese	[16]
19	Proband	-	-	-	5 CHM	-	Asian	
20	Proband	-	-	-	4 CHM	PTD	Asian	

^aVariants in coding DNA and protein are annotated with reference to GenBank accession numbers (NM_206828.2) and (NP_996611.2) respectively. ^b Proband only available for screening. S1, proband in families with more than one affected sister; S2, second affected sister; CHM, complete hydatidiform mole; PHM, partial hydatidiform mole; HM, undefined hydatidiform mole SA, spontaneous abortion; SB, stillbirth, END, early neonatal death; EP, ectopic pregnancy; PTD, persistent trophoblastic disease.

DISCUSSION

NLRP7 (NOD12, PYPAF3 and CLR19.4) is a cytoplasmic protein belonging to a group of proteins made up of an N-terminal pyrin (PYD) domain, a NACHT domain and a C-terminal leucine-rich repeat (LRR) domain [18]. Mutations of *NLRP7* have recently been described in a small number of families with FRHM. In the present study a further 20 families have been investigated, seven with two affected sisters and 13 in which a single individual has had recurrent CHM of diploid biparental origin. Seventeen of these families were found to have mutations in *NLRP7*, confirming the importance of *NLRP7* in the aetiology of FRHM. Sixteen different mutations were identified in the present study, demonstrating the wide diversity of mutations in *NLRP7* that give rise to FRHM.

Despite the diversity of mutations, and that consanguinity was reported in only one family (case 4), affected members in fourteen of the seventeen families were found to be homozygous for the mutation identified. Two of these families, from the same region of Turkey (cases 7, 8), were not only homozygous for the same p.R693W mutation but shared an identical haplotype across the 19q13.4 region flanking *NLRP7* (unpublished observation) suggesting that, even in non-consanguineous families, mutations within families might be inherited from a common ancestor. Affected members of three families were found to be compound heterozygotes. One individual surprisingly had three *NLRP7* mutations, being homozygous for the p.R693P mutation and heterozygous for the p.N913S mutation. Genotyping of other family members showed that all three mutations were inherited from her parents and did not arise *de novo*. While most mutations described in the present study were novel, both these mutations had been previously reported in women from the same ethnic background suggesting that these particular mutations are more common in some populations.

No mutations were identified in three families in the present study. However, mutations in *NLRP7* cannot be completely excluded. Deletions or intragenic duplications, involving whole exons, might not be identified by conventional sequencing. In a previous study three Egyptian families, with no mutations in the coding sequence of *NLRP7*, were subsequently found to have an intragenic

duplication involving several exons [8]. It is also possible that the mutations might be in unidentified regulatory elements of the gene since only exonic sequences were screened in the present study. A more intriguing possibility is that a second gene might be associated with this condition in some families. In case 18, in which no *NLRP7* mutations were identified, the two sisters inherited only one haplotype in common from their parents for the 19q13.4 region suggesting that the causative gene in this family is unlikely to map to this region [16]. The two other cases, without identifiable mutations, are also heterozygous across the 19q13.4 region and for a number of SNPs within *NLRP7*. Although the possibility that the women are compound heterozygotes for mutations missed using the current sequencing strategy, heterozygosity across the region would argue in favour of a mutation in a second locus outside the region in these cases.

Three mutations in the present study have been previously described [8, 13, 14]. Two of these, p.R693W and p.R693P, occurred in five families in the present study and three out of six previously reported families with missense mutations [8, 13, 14]. A sixth case in the present series had a novel mutation R693Q affecting the same codon. This codon, found in the LRR, would thus appear to be a hot spot for mutations in *NLRP7*. All other missense mutations in the present study, with the exception of the p.L398R mutation, were also located within the LRR of *NLRP7*. None of these mutations were found in 390 unrelated control chromosomes or described in the Infevers autoinflammatory Mutation Online Registry [19] which includes known variants of *NLRP7*. Two previously reported missense mutations A657V and L750V [8, 13, 14], not seen in this study, are also located in the LRR. In addition to missense mutations, the present study revealed six novel nonsense mutations occurring at intervals throughout the gene. However, none of these mutated genes would, if translated, give rise to a protein with a functional LRR. This provides very strong evidence that the LRR, which is involved in protein-protein interactions [20], is important for normal functioning of *NLRP7* in reproduction.

NLRP7 has two major transcript variants. These are similar except that only variant 1 contains exon 10 while variant 2 has an additional 84 bases at the 5' end of exon 5. In one family (case 6) the missense mutation, p.P651S, occurred in the 5' end of exon 5 found only in transcript 2. Similarly the A657V mutation previously described [14]

would only affect functioning of transcript 2. Thus it would appear to be the product of variant 2 that is important in normal reproduction. Identification of the ligand to which the LRR of this protein binds will be important to understanding the role of NLRP7.

Most pregnancies in affected women were CHM although other reproductive losses including miscarriages, partial hydatidiform mole and a stillbirth were reported. Some pregnancies, reported as miscarriage, may not have been examined pathologically and may represent missed CHM. However, we have confirmed at least one miscarriage as a hydropic abortion on the basis of pathology. p57^{KIP2} immunostaining correlated with the pathology in this case with the villous tissue showing positive staining. This together with the observation that the methylation status of imprinted genes in the live offspring of one affected individual was normal [7] suggests that the variable phenotypes reflect different degrees of aberrant imprinting. This may in turn be influenced by the specific mutation present in the family. In the present series none of the pregnancies in affected women resulted in normal live births. However, none of the families had the unique splice site mutation reported in the family in which affected individuals achieved occasional normal pregnancies [13]. Due to the large number of different mutations and the relatively small numbers of non-CHM pregnancies it was not possible to identify specific correlations between different mutations and reproductive outcome from the present series.

In the course of the study parental DNA was examined in 6 cases (cases 4, 6, 9, 10, 11 and 17) and both parents shown to be heterozygotes. Two sisters of affected individuals were also heterozygous for one of the mutations in their affected sister. These heterozygous females had normal live births. There was no history of molar pregnancies and the only reproductive loss reported was a single miscarriage in a couple with 8 pregnancies. Although we cannot be sure that all miscarriages were reported, in the present study there was no history of molar pregnancy or any evidence of increased reproductive wastage associated with being a heterozygote for any of the mutations identified.

Functionally, little is known about NLRP7. Some members of the NLRP family have been implicated in the assembly of inflammasomes and maturation of

proinflammatory cytokines [21]. Mutations in these genes, in particular gain of function mutations in the NACHT domain of *NLRP3*, have been shown to cause a number of autoinflammatory disorders [21]. However, FRHM is an autosomal recessive disorder in which mutations occur predominantly in the LRR. Loss of *NLRP7* does not appear to directly affect systemic immunity but instead results in aberrant imprinting similar to that seen in androgenetic conceptuses. Both androgenetic CHM and the diploid biparental CHM found in women with FRHM are characterised by abnormal expression of imprinted genes [9]. In addition, a number of imprinted genes that are normally maternally imprinted have a paternal methylation pattern, and remain unmethylated, in both androgenetic and diploid biparental CHM [6, 7, 8]. These observations fit the hypothesis that the normal role of the defective gene in women with FRHM is in establishing or maintaining the maternal imprint. In both males and females methylation marks are removed in the primordial germ cells and re-established during gametogenesis. In female mice this is achieved by sequential methylation of imprinted genes during oocyte growth [22]. Factors important in differential methylation should therefore be present at all stages of oocyte development.

Although there is no mouse orthologue of *NLRP7*, several other members of the NLRP family have been shown to be specifically expressed in the gonads and play a role in reproduction in mice [23, 24, 25]. A number of these, including *NLRP5* (MATER) and *NLRP9*, are also expressed in the oocytes of both rhesus macaque monkeys and humans [26, 27]. Although *NLRP7* is not itself oocyte-specific in primates [15, 27], transcripts of *NLRP7* have been reported in ovary and in denuded oocytes at the germinal vesicle and metaphase I stages [13, 15, 27, 28]. However, the localisation of the protein has not previously been described. In the present study immunohistochemical staining of human ovarian tissue demonstrated that *NLRP7* expression is confined to the oocytes, is expressed at all stages from primary to tertiary follicles, and is similar to that of *NLRP5* and *NLRP9*. This pattern of expression is consistent with a role for *NLRP7* in the developing oocyte. In mice *NLRP* transcripts are reportedly downregulated immediately after fertilisation [23, 24, 25]. However, in primates *NLRP7* might also play a role in establishment or maintenance of imprints since *NLRP* transcripts, including *NLRP7*, appear to persist in the early cleavage embryos of both rhesus macaque monkeys and humans [13, 27,

28]. Further studies are needed to determine the exact mechanisms by which *NLRP7* functions to control imprinting.

At present counselling of families with FRHM remains difficult. If the major role of *NLRP7* is in setting the maternal imprint in the oocyte, egg donation should provide a strategy to avoid further CHM. However, if *NLRP7* also plays a role in the establishment and maintenance of pregnancy, egg donation may still result in pregnancy failure in women with FRHM. Women with this condition need to be aware that the most likely outcome of any subsequent pregnancy is a HM and the risk of persistent trophoblastic disease (PTD) if this proves to be the case. In the present series there is an appreciable number of patients who experienced PTD requiring chemotherapy (Table 1). In all cases PTD followed a CHM pregnancy. Of the ninety four CHM reported in the series fifteen (16%) were followed by PTD, an incidence similar to that seen after sporadic CHM [29].

In conclusion this study has identified 13 novel mutations in *NLRP7*, seven nonsense and six missense mutations. Identification of a male, homozygous for a mutation in *NLRP7* but with normal reproductive outcomes confirms that mutations in *NLRP7* affect only females. The demonstration that mutations occur in transcript variant 2 and cluster in the LRR define the LRR of transcript variant 2 as critical domain for normal reproductive function of *NLRP7*.

ACKNOWLEDGEMENTS

We are grateful to the members of the families who have taken part in this study. This work was supported by grants from the Hammersmith Hospitals Trust Research Committee, Cancer Treatment and Research Trust, and the Wellcome Trust. We would like to thank Professor J Kooner and Dr J Chambers of Imperial College London for making control DNA samples available.

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REFERENCES

- 1 Fisher RA, Hodges MD, Newlands ES. Familial recurrent hydatidiform mole: a review. *J Reprod Med* 2004;**49**(8):595-601.
- 2 Szulman AE, Surti U. The syndromes of hydatidiform mole. I. Cytogenetic and morphologic correlations. *Am J Obstet Gynecol* 1978;**131**(6):665-71.
- 3 Kajii T, Ohama K. Androgenetic origin of hydatidiform mole. *Nature* 1977;**268**(5621):633-4.
- 4 Helwani MN, Seoud M, Zahed L, *et al.* A familial case of recurrent hydatidiform molar pregnancies with biparental genomic contribution. *Hum Genet* 1999;**105**(1-2):112-5.
- 5 Fisher RA, Khatoon R, Paradinas FJ, *et al.* Repetitive complete hydatidiform mole can be biparental in origin and either male or female. *Hum Reprod* 2000;**15**(3):594-8.
- 6 Judson H, Hayward BE, Sheridan E, *et al.* A global disorder of imprinting in the human female germ line. *Nature* 2002;**416**(6880):539-42.
- 7 El-Maarri O, Seoud M, Coullin P, *et al.* Maternal alleles acquiring paternal methylation patterns in biparental complete hydatidiform moles. *Hum Mol Genet* 2003;**12**(12):1405-13.
- 8 Kou YC, Shao L, Peng HH, *et al.* A recurrent intragenic genomic duplication, other novel mutations in NLRP7 and imprinting defects in recurrent biparental hydatidiform moles. *Mol Hum Reprod* 2008;**14**(1):33-40.
- 9 Fisher RA, Hodges MD, Rees HC, *et al.* The maternally transcribed gene p57(KIP2) (CDNK1C) is abnormally expressed in both androgenetic and biparental complete hydatidiform moles. *Hum Mol Genet* 2002;**11**(26):3267-72.
- 10 Moglabey YB, Kircheisen R, Seoud M, *et al.* Genetic mapping of a maternal locus responsible for familial hydatidiform moles. *Hum Mol Genet* 1999;**8**(4):667-71.
- 11 Sensi A, Gualandi F, Pittalis MC, *et al.* Mole maker phenotype: possible narrowing of the candidate region. *Eur J Hum Genet* 2000;**8**(8):641-4.
- 12 Hodges MD, Rees HC, Seckl MJ, *et al.* Genetic refinement and physical mapping of a biparental complete hydatidiform mole locus on chromosome 19q13.4. *J Med Genet* 2003;**40**(8):e95.
- 13 Murdoch S, Djuric U, Mazhar B, *et al.* Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. *Nat Genet* 2006;**38**(3):300-2.

- 14 Qian J, Deveault C, Bagga R, *et al.* Women heterozygous for NALP7/NLRP7 mutations are at risk for reproductive wastage: report of two novel mutations. *Hum Mutat* 2007;**28**(7):741.
- 15 Kinoshita T, Wang Y, Hasegawa M, *et al.* PYPAF3, a PYRIN-containing APAF-1-like protein, is a feedback regulator of caspase-1-dependent interleukin-1beta secretion. *J Biol Chem* 2005;**280**(23):21720-5.
- 16 Zhao J, Moss J, Sebire NJ, *et al.* Analysis of the chromosomal region 19q13.4 in two Chinese families with recurrent hydatidiform mole. *Hum Reprod* 2006;**21**(2):536-41.
- 17 Ozalp S, Yalcin OT, Tanir HM, *et al.* Recurrent molar pregnancy: report of a case with seven consecutive hydatidiform moles. *Gynecol Obstet Invest* 2001;**52**(3):215-6.
- 18 Martinon F, Tschopp J. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* 2005;**26**(8):447-54.
- 19 Milhavel F, Cuisset L, Hoffman HM, *et al.* The infevers autoinflammatory mutation online registry: update with new genes and functions. *Hum Mutat* 2008;**29**(6):803-8.
- 20 Kobe B, Kajava AV. The leucine-rich repeat as a protein recognition motif. *Curr Opin Struct Biol* 2001;**11**(6):725-32.
- 21 Martinon F, Gaide O, Petrilli V, *et al.* NALP inflammasomes: a central role in innate immunity. *Semin Immunopathol* 2007;**29**(3):213-29.
- 22 Hiura H, Obata Y, Komiyama J, *et al.* Oocyte growth-dependent progression of maternal imprinting in mice. *Genes Cells* 2006;**11**(4):353-61.
- 23 Tong ZB, Gold L, Pfeifer KE, *et al.* Mater, a maternal effect gene required for early embryonic development in mice. *Nat Genet* 2000;**26**(3):267-8.
- 24 Dade S, Callebaut I, Paillisson A, *et al.* In silico identification and structural features of six new genes similar to MATER specifically expressed in the oocyte. *Biochem Biophys Res Commun* 2004;**324**(2):547-53.
- 25 Hamatani T, Falco G, Carter MG, *et al.* Age-associated alteration of gene expression patterns in mouse oocytes. *Hum Mol Genet* 2004;**13**(19):2263-78.
- 26 Tong ZB, Bondy CA, Zhou J, *et al.* A human homologue of mouse Mater, a maternal effect gene essential for early embryonic development. *Hum Reprod* 2002;**17**(4):903-11.
- 27 McDaniel P, Wu X. Identification of oocyte-selective NLRP genes in rhesus macaque monkeys (*Macaca mulatta*). *Mol Reprod Dev* 2008.
- 28 Zhang P, Dixon M, Zucchelli M, *et al.* Expression analysis of the NLRP gene family suggests a role in human preimplantation development. *PLoS ONE* 2008;**3**(7):e2755.

- 29 Seckl MJ, Fisher RA, Salerno G, *et al.* Choriocarcinoma and partial hydatidiform moles. *Lancet* 2000;**356**(9223):36-9.

LEGENDS TO FIGURES

Figure 1 Schematic representation of NLRP7 mutations identified in the present study on a simplified domain model of the protein. The mutations identified in 17 families with FRHM are annotated with reference to NP_996611.2. Novel mutations are shown in bold. (A) Upper panel shows the nine missense mutations identified in the present study. (B) Lower panel shows the seven nonsense mutations identified. (C) Predicted protein domains PYRIN (PYD), NACHT, leucine rich region (LRR) of NLRP7.

Figure 2 Partial sequencing chromatogram of exon 4 from NLRP7. (A) Genomic sequence of control. (B) Genomic sequence in case 3. The protein product of both sequences is represented by the first three letters of the amino acid encoded by the sequence. The affected individual has a homozygous 14 base insertion resulting in a premature stop codon. bps, base pairs.

Figure 3 Comparative expression and localisation of NLRP7, NLRP5 and NLRP9 in human ovary. (A - F') NLRP7 expression is cytoplasmic in all stages of growing oocytes. Protein expression can be observed in primordial oocytes (PrM), primary follicles (PF), secondary follicles (SF) and tertiary follicles (TF). The nucleus (ns) is clearly visible in all oocytes. The oocyte in F' is a higher magnification of the TF in F. (G - I) NLRP5 protein expression in growing follicles. (J - L) NLRP9 protein expression in growing follicles showing similar cytoplasmic expression. (M - O) Negative control sections following epitope peptide competition staining for each antibody. (P) Negative staining with Goat IgG. Scale bars 50 μ m.