Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi: 10.1111/cei.13472

Abnormal processing of IL-1β in *NLRP7*-mutated monocytes in hydatidiform mole patients

P. Zhang,* X. Zhu,* X. Yu,* B. Huang,* T. Jiang,* X. Zhang,† H. Yang‡ and J. Qian *

*Department of Gynecology, The First Affiliated Hospital, Zhejiang University of Medicine School, Zhejiang University, †Department of Pathology, Women's Hospital, Zhejiang University School of Medicine, and ‡Department of Pathology, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Accepted for publication 26 May 2020
Correspondence: J. Qian, Department of Gynecology The First Affiliated Hospital, Zhejiang University of Medicine School, Zhejiang University, Hangzhou, 310003, China.
E-mail: qianjianhua@zju.edu.cn

These authors contributed equally to this work and should be considered co-first authors.

Summary

NOD-like receptor pyrin 7 (*NLRP7*) has been identified as the major gene responsible for the recurrent hydatidiform mole (RHM). The immunological role of *NLRP7* mutation in HM patients has not been conclusively demonstrated. Hence, we aim to demonstrate this role in our study. We followed 12 new patients with *NLRP7* non-synonymous variations (NSVs) from date to date. Peripheral blood mononuclear cells (PBMCs) were collected separately from patients with and without *NLRP7* mutation. Supernatant interleukin (IL)-1β secretion, intracellular pro-IL-1β and mature IL-1β expressions were measured after 24 h lipopolysaccharide (LPS) stimulation. Plasmids with corresponding NSVs were generated to evaluate the ability of processing pro-IL-1β into mature IL-1β *in vitro*. Homozygous or compound heterozygous *NLRP7* mutations secreted less IL-1β in roots of abnormal intracellular pro-IL-1β or mature IL-1β, according to different domains. Plasmids with NSVs could also affect processing or/and trafficking together with caspase-1 and apoptosis-associated speck-like protein (ASC). Inflammasome-related *NLRP7* mutation is a potential mechanism of RHM.

Keywords: hydatidiform mole, IL-1β, *NLRP7*

Introduction

Hydatidiform mole (HM) is one of the most common abnormal pregnancy outcomes, with an incidence of sporadic HM higher than 1 in 600–1000 in developing countries [1]. Patients with a third HM are likely to carry a rare maternal-effect autosomal recessive condition. *NLRP7* and *KHDC3L* have been identified from familial recurrent HMs (RHM) as two main pathogenic genes. *NLRP7* (NACHT, leucine-rich repeating and PYD containing 7) has been identified in 48–80% of RHM patients among different populations [2–4] and *KHDC3L* (also known as *c6orf221*) is mutated in 10–14% of RHM patients with no *NLRP7* mutation [5,6].

The theory of abnormal methylation and abnormal maternal inflammation are two etiologies of HM. First, *NLRP7*, as the first identified maternal effect gene [7], has been found to be associated with multi-locus imprinting disturbance in offspring [8,9]. However, the underlying mechanism of abnormal methylation is unknown.

Secondly, oligomerized *NLRP7* was believed to function as a multi-protein recruiting the adaptor protein, apoptosis-associated speck-like protein (ASC), through PYD–PYD interaction. Besides the PYD domain on the N terminus, ASC contains a CARD domain on the C terminus, which can activate caspase-1 through the CARD–CARD interaction. The *NLRP7*, ASC and caspase-1 complex is believed to process and traffic interleukin (IL)-1β [10]. Messaed *et al.* point out that peripheral blood mononuclear cells (PBMCs) from patients with *NLRP7* NSVs secrete lower IL-1β [11,12]. However, it is difficult to obtain fresh blood cells. In this study, only 11 patients with *NLRP7* mutations and rare variants were accessed to evaluate IL-1β secretion and intracellular IL-1β expression.

Considering the abundant *NLRP7* NSVs, the abnormal maternal inflammation hypothesis needs to be further confirmed. Therefore, we enrolled *NLRP7*- associated HM patients to analyze IL-1β expression of PBMCs to estimate the immunological response to lipopolysaccharide (LPS) stimulation. Further, we generated corresponding plasmids for *in-vitro* study to understand the pathogenicity of *NLRP7* mutations with HM.

Materials and methods

Patients and controls

RHM out-patients were clinically evaluated at the First Affiliated Hospital, Zhejiang University School of Medicine. The study was approved by the Institutional Review Board of the First Affiliated Hospital, Zhejiang University School of Medicine. All the participants in this study provided a written consent. The enrolled HMs are based on clinical features, especially on hematoxylin and eosin (H&E) tissue sections reviewed independently by two gynecological pathologists. They were followed-up with a telephone interview to track their reproductive outcomes. The controls were unrelated out-patient subjects (aged between 20 and 40 years) with no family histories of inflammatory condition or recurrent fetal loss and carrying no *NLRP* variants.

Mutation analysis

DNA were isolated from maternal blood samples by standard protocols. Mutation analysis was performed by polymerase chain reaction (PCR) amplification of genomic DNA of the 11 *NLRP7* exons followed by direct sequencing in both directions, as previously described [15].

Microsatellite genotyping

The chorionic villi region from formalin-fixed, paraffin-embedded molar tissue sections of those RHM patients without *NLRP7* NSV were isolated to extract DNA. The amplified PCR products were determined by capillary electrophoresis using the ABI3130 gene analyzer. The genotypes of the molar tissues were compared with those of the patients and their partners in order to determine the parental origin of the alleles. The average number of amplified loci is 21.

Immunohistochemistry

Patients with *NLRP7* mutations and rare variants as well as biparental CHM (BiCHM) patients without *NLRP7* NSVs provided 4-μm formalin-fixed paraffin-embedded tissue sections for immunohistochemistry with IL-1β antibody (2022; Cell Signaling Technology, Danvers, MA, USA).

Cytokine assay

Blood [with tripotassium ethylenediamine tetraacetic acid (K_3EDTA)] from *NLRP7*-deficient RHM patients and BiCHM patients without *NLRP7* NSVs were analyzed in parallel with blood from controls within 24 h after withdrawal. PBMCs were isolated using Ficoll-Paque Plus; $1{\cdot}5 \times 10^6$ cells were counted, plated in 24-well plates and

Table 1. Patients with defective *NLRP7* alleles

ID	DNA	Protein	Reproductive history	Reference
Ch783	c.1137G>C	p.Lys379Asn*	3 CHM	This study
Ch815	c.3062A>T	p.Asp1021Val	SA, PHM, CHM	This study
Ch838	c.251G>A	p.Cys84Tyr*	HM	This study
Ch843	c.2155G>A	Ala719Thr	CHM PHM	This study
Ch639	c.2078G>A+c:2078G>A	p.Arg693Gln*+p.Arg693Gln*	SA, PHM, CHM, PHM	This study
Ch734	c.1137G>C+ c.1976G>T	p.Lys379Asn*+p.Arg659Leu	2 CHM	This study
Ch737	c.2165A>G +c.2471+1G>A	p.Asp722Gly*+p.Leu825X*	2 CHM, 3 SA, PHM_GTT	This study
Ch772	c.2161C>T+c.2161C>T	p.Arg721Trp+p.Arg721Trp	CHM, CHM_GTT	This study
Ch776	c.2165A>G + c.2760G>A	p.Asp722Gly*+p.Trp920Ter	3 CHM	This study
Ch806	c.1294C>T+c.1294C>T	p.Arg432X *+ p.Arg432X *	1 DA, 2 CHM	This study
Ch823	c.1294C>T+c.2111G>A	p.Arg432X*+p. Cys704Tyr	2 HM	This study
Ch522	c.1719_1720insT + c.2165A>G	p.Asp722Gly*	2 PHM, 1 HM_GTT	This study
MoCh293	c. 1294C>T +c.2156C>T +c.2473T>C	p.Arg432X*+p.Ala719Val+p.Ile858Thr	2 CHM, CHM_GTT	[14]
MoCh492	c. 251G>A	p.Cys84Tyr	CHM, SA, failed ART	[13]
MoCh501	c.1137G>C	p.Lys379Asn *	1 SA/HM, CHM, 1 SA	[13]
MoCh765	c. 2468T>A	p.Leu823X	2 ET, 2 CHM	[14]
Ch29	c.2165A>G+ c.2165A>G	p.Asp722Gly*+p.Asp722Gly*	2 SA, 2 PHM,	[13]
Ch77	c. 1294C>T + c.2471+1G>A	p.Arg432X*+ p.Leu825X*	SA, 3 CHM	[13]
Ch78	c. 1294C>T + c.2471+1G>A	p.Arg432X*+ p.Leu825X*	3 SA, 4 CHM	[13]
Ch101	c. 2101C>T+ 2078G>A	p.Arg701Cys+Arg693Gln	2 HM, SB, SA, CHM	[13]
MoCh517	c.295G>T+ c.1970A>Ta	p.Glu99X+p.Asp657Val	2 CHM, 1 failed ART	[15]
MoCh519	c.295G>T+ c.1970A>T	p.Glu99X+p.Asp657Val	3 CHM, PHM	[15]
MoCh781	c.2130-312_2300+737del1218 +c.2130-312_2300+737del1218		2 CHM	[14]
MoCh791	c.1622_1698del76+C.2471+1G>A	p.Arg541RfsX1+ p.Leu825X*	3 CHM	[14]

New variants are underlined. Asterisks indicate mutations reported in at least two unrelated patients of Chinese origin. HM = hydatidiform mole; CHM = complete HM; PHM = partial HM; SA = spontaneous abortion; DA = voluntary 162 termination using drug; GTT = gestational trophoblastic tumor.

stimulated with lipopolysaccharide (LPS) (1000 ng/ml) (Sigma, St Louis, MO, USA; L6529, from *Escherichia coli* 055:B5) for 24 h.

Site-directed mutagenesis of human *NLRP7* plasmid

Human wt-*NLRP7* cDNA was cloned into PCR-Blunt-II-TOPO vector (IMAGE ID 40036028, accession no. BC109125; Open Biosystems). The *NLRP7* vector was verified following instructions from Rima Slim *et al.* [11] FLAG-wt-NLRP7 was inserted into a pcDNA-3.1(–) vector (Invitrogen, Carlsbad, CA, USA) using restriction enzymes *Afl*II and *Kpn*I. Missense mutations in the *NLRP7* gene were produced by site-directed mutagenesis with PfuUltra high-fidelity DNA polymerase AD (Agilent Technologies, Santa Clara, CA, USA) and the QuikChange^{TM} site-directed mutagenesis (Stratagene, San Diego, CA, USA).

Cell culture and transfection

One day prior to the transfection, human embryonic kidney 293 (HEK293) T cells were seeded at a density of 1×10^5 cells per well using 24-well plates. The human FLAG-pro-IL-1β, FLAG-caspase-1 and FLAG-ASC vectors were co-transfected with pcDNA-3.1(–)-FLAG-*NLRP7* for 24 h.

Western blotting

Monoclonal antibody against FLAG (1 : 1000) (F3165; Sigma, St Louis, MO, USA), monoclonal antibodies directed against human IL-1β (1 : 1000) (2022; Cell Signaling Technology), human *NLRP7* (1 : 1000) (ab126979; abcam, Cambridge, MA, USA) and β-actin (1 : 1000) (4970S; Cell Signaling Technology) were used to detect the immunoblots. Protein bands were revealed using NIH ImageJ software.

Statistical analysis

The data were analyzed by SPSS version 17.0 software (SPSS, Inc., Chicago, IL, USA). Enzyme-linked immunosorbent assay (ELISA) measurements were performed using Student's *t*-test. *P*-values < 0·05 were considered statistically significant.

Results

Characteristics of *NLRP7* mutation

A total of 81 RHM patients were diagnosed in our team between 2007 and 2018, among whom 20 *NLRP7* NSVs were detected, and patient 838, although with only one HM history, contained the previously reported mutation [13] (Table 1). Twelve new patients carried six novel variants and all the missense mutations clustered in the leucine-repeat region (LRR). However, the ratio depended upon in our study was 27·2% (22 of 81), which offers new evidence for the theory that *NLRP7*-associated RHM varies among different ethnic groups, and the genetic background underlying Chinese Han people is complex.

Expression of IL-1β in *NLRP7*-associated RHM patients

The genotypical results showed that the POC (product of conception) of patients 691 and Ch791 were BiCHM without any *NLRP7* NSVs (Table 2).

Table 2. Microsatelite DNA genotyping of patients 691 and Ch791

Loci	Patient 691	POC	Father
D1S1627	13/14	12/13	12/14
D1S1677	14/16	14/14	13/14
D19S433	13/16.2	15.2/16.2	14/15.2
D1GTAT113	7/12	7/7	7/12
D10S1435	11/13	12/13	12/14
AMEL	XX	XX	XY
D2S441	11/11	11/12	11/12
D2S1776	11/12	12/13	12/13
D3S4529	15/15	15/15	15/15
D4S2408	9/10	9/10	9/10
D5S2500	14/17	14/20	14/20
D6S474	15/17	15/17	14/15
D6S1017	8/8	8/8	10/13
D9S1122	13/13	13/13	12/13
D10S1048	15/15	13/15	13/15
D11S4463	13/14	9/14	9/14
D12ATA63	16/17	16/17	17/17
D14S1434	11/13	11/13	12/13
D17S1301	12/12	12/12	12/14
D18S853	12/14	12/14	13/14
D20S482	14/14	14/14	13/14
D22S1045	17/17	11/17	11/17

Loci	Patient Ch791	Molar	Father
D18S51	13/13	13/16	15/16
D7S820	11/11	11/12	12/12
Penta D	13/13	11/13	9/11
vWA	16/17	14/16	14/14
Penta E	9/15	5/9	5/11
AMEL	XX	XY	XY
D2S1338	24/24	24/24	19/24
D3S1358	15/17	15/17	15/17
D5S818	11/12	11/13	11/13
D6S1043	11/12	11/12	10/13
D8S1179	12/16	12/16	11/13
D12S391	18/23	18/23	20/23
D13S317	11/12	11/12	8/9
D16S539	9/11	9/11	10/12
D19S433	13/13.2	13/13.2	13/13
D21S11	30/30	30/31.2	30/31.2
CSF1PO	12/13	11/12	11/12
TPOX	9/11	9/11	11/11
TH01	7/9	7/9	9/9
FGA	19/20	19/20	18/22

POC, product of conception.

The IL-1β immunohistochemistry of patients 772, 815, 823, 843 and 691 Ch791 are shown in Fig. 1. It was diagnosed that all six POC were HM. Meanwhile, the expression of IL-1β of patients 691 and Ch791 was negative, while the other four *NLRP7*-mutated patients showed that IL-1β was positive only between decidua.

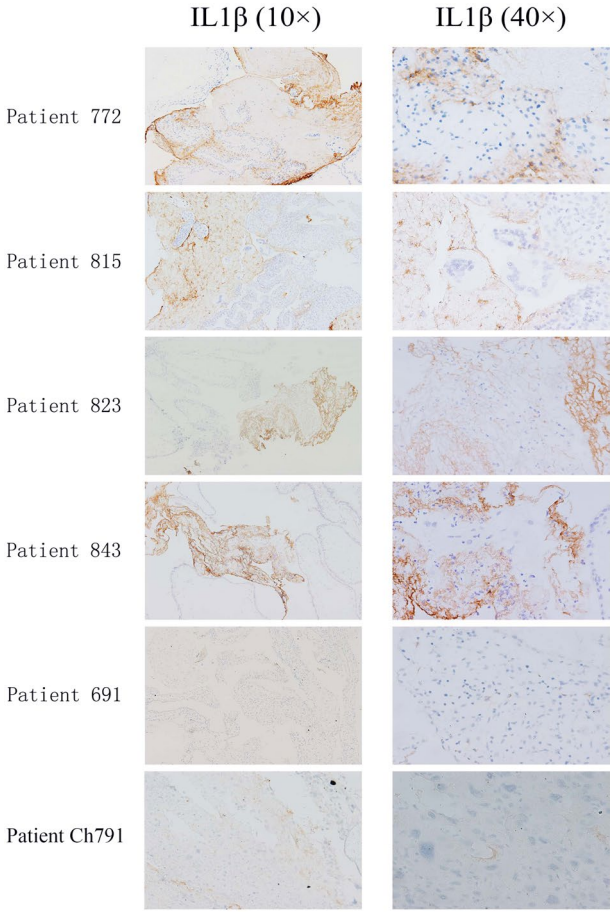

Fig. 1. Expression of interleukin (IL)-1β in recurrent hydatidiform mole (RHM) patients. IL-1β staining (×100, ×400) of patients 772, 815, 823, 843, 691 and Ch791.

Low IL-1β and tumor necrosis factor (TNF)-α secretion by PBMCs from patients with homozygous and compound heterozygous mutation

Twelve patients were analyzed for the first time and PBMCs were assessed.

Our data demonstrate that *NLRP7*-mutated patients tended to secrete less TNF-α except patients 737 and 293, who were diagnosed later with gestational trophoblastic tumor (GTT) (Fig. 2b). However, four patients with only one defective allele each did not secrete less IL-1β (Fig. 2a). Meanwhile, patients with one homozygous *NLRP7* mutation or compound heterozygous-defective alleles, except patient 734, containing two NSVs, tended to secrete lower levels of IL-1β than controls after 24-h LPS stimulation. Additionally, the two RHM patients without *NLRP7* NSVs, patients 691 and Ch 791, were not proved to secrete less IL-1β or TNF-α, and patient 691 was diagnosed later with GTT.

TNF-α of culture supernatant can be affected by *NLRP7* mutation, while only homozygous and compound heterozygous mutations secreted less IL-1β compared with controls after 24-h LPS stimulation.

Pro-IL-1β and mature IL-1β expression *in-vitro*-stimulated patient PBMCs

The ratios of patients' intracellular pro-IL-1β and mature IL-1β (patients 838, 843, 815, 806, 639, 772, 734, 776, 823 and 293) that change with controls after and before LPS stimulation were measured in 10 patients (patient 737 could not offer enough blood). The results showed that

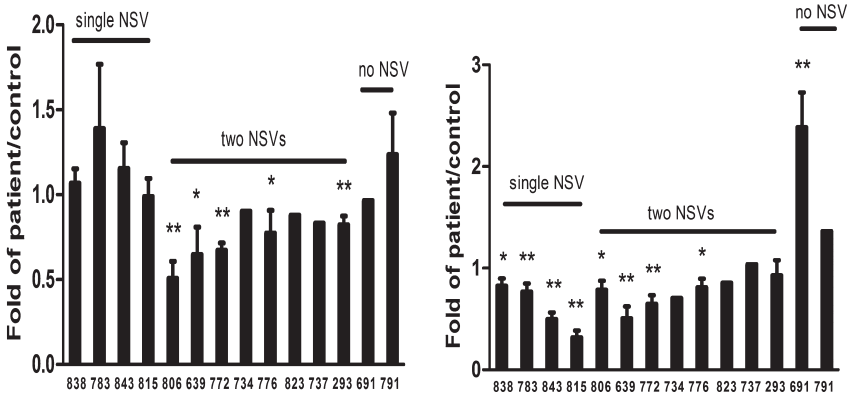

Fig. 2. Low interleukin (IL)-1β and tumor necrosis factor (TNF)-α secretion of peripheral blood mononuclear cells (PBMCs) from patients with homozygous and compound heterozygous mutations. Relative amounts of each cytokine refer to the secreted amounts by patients' cells divided by those secreted by control cells (Δpatient/Δcontrol). The averages and standard deviation (s.d.) were calculated on two to three different enzyme-linked immunosorbent assays (ELISA) on supernatants from the same lipopolysaccharide (LPS) stimulation. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$. (a) IL-1β; (b) TNF-α.

the processing of pro-IL-1β and the trafficking of mature IL-1β are affected by the NSVs.

Patient 806, with one homozygous NACHT protein-truncating mutation, expressed less pro-IL-1β and more mature IL-1β. Separately, patients 639 and 772, with one homozygous LRR mutation, expressed more pro-IL-1β and less mature IL-1β (Fig. 3b). These data imply that a different domain of *NLRP7* might play a different role to affect supernatant IL-1β.

Meanwhile, patient 734 (containing two rare variants) showed no significant change of both pro-IL-1β and mature IL-1β (Fig. 3c,d).

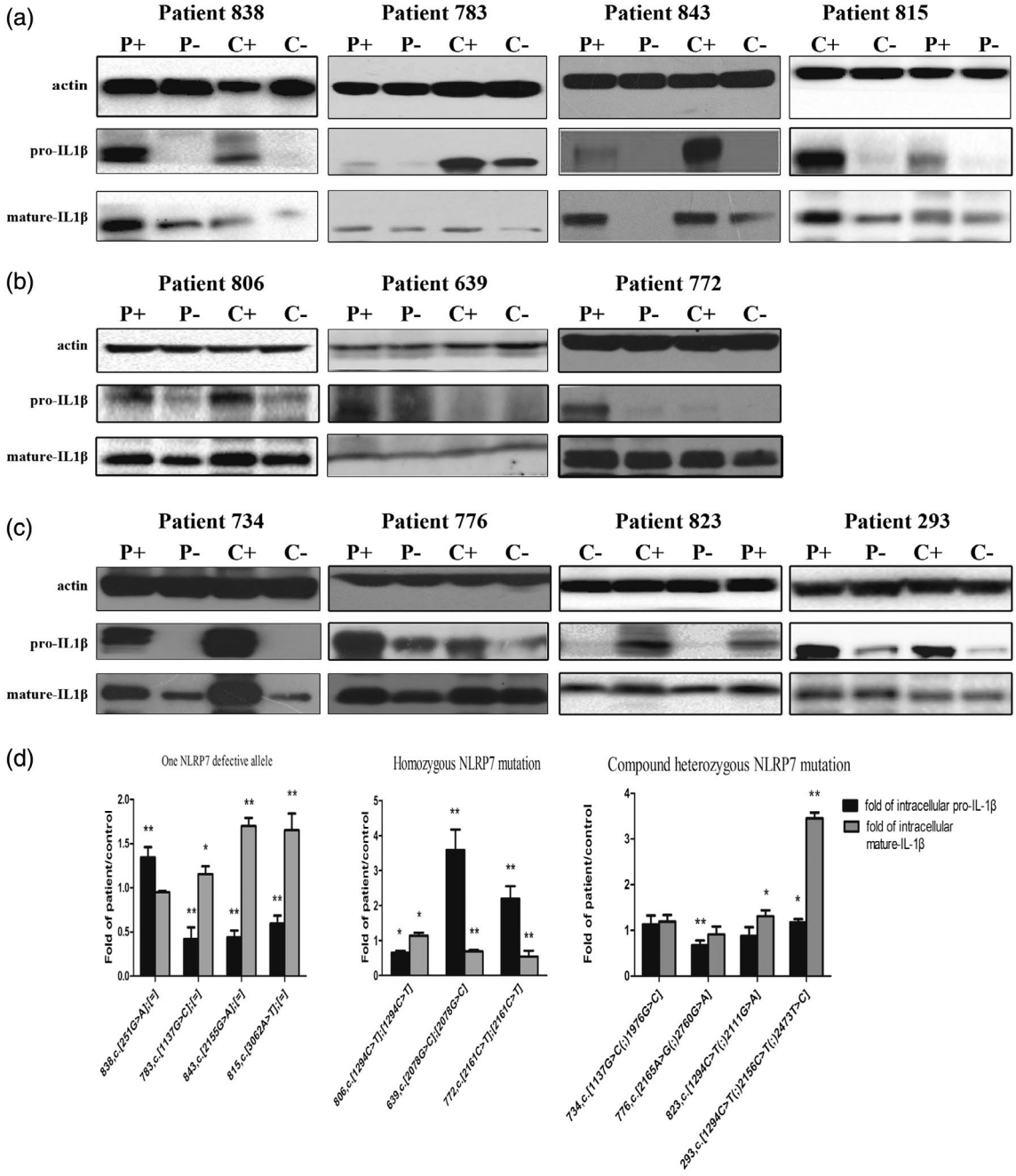

Fig. 3. Immunoblots of whole cell lysates show expressed intracellular pro-interleukin (IL)-1β and mature IL-1β in patients with *NLRP7* mutations in ratio to controls. The ratios of pro-IL-1β and mature IL-1β from the cells of patients divided by control cells (Δpatient/Δcontrol) were presented after signal quantification using Image J software. (a) For one defective allele patients; (b) for one homozygous mutation patients; (c) for compound heterozygous mutation patients. C^-, control without LPS; C^+, control added with LPS; P^-, patient without LPS; P^+, patient with added LPS. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

NLRP7 NSV affect process and trafficking of IL-1β

To date, 260 mutations of *NLRP7* are listed in *Infevers*; whether these mutations are HM-linked is unclear.

Our data demonstrate that *NLRP7*, together with caspase-1 and ASC, could process the pro-IL-1β into mature IL-1β *in vitro* (Fig. 4).

Added to site-directed mutated plasmids, the intracellular pro-IL-1β expression and intracellular mature IL-1β were affected according to the location of NSVs (Fig. 5). *In vitro*, from plasmids 2078 and 2161, LRR NSVs could affect both pro-IL-1β and mature IL-1β. Plasmid 1137, together with plasmids 1137+1976, as well as plasmid 1294, together with plasmids 1294+2111, show that the NACHT NSVs may play a different role from LRR NSVs.

Discussion

In this study, we report 12 new patients with six new mutations. The data show that one-defective-allele patients only occupy a small proportion (five of 22) and the LRR domain was more frequently involved in other domains (16 of 23). Two rare variants, c.1137G>C and c.1976G>T, were also found in a 300-subject control group (Hu *et al*., under review); that is, the mutation rate of *NLRP7* in RHM is lower than previously reported [3,4]. RHM can also occur in the absence of *NLRP7* mutations, which underlines the multi-factorial nature of HM. Clearly, IL-1β was expressed in the decidua of *NLRP*-associated RHM, while the no *NLRP7*-mutated BiCHM barely expressed IL-1β. Additionally, PBMCs from HM patients with *NLRP7* NSVs were hyporesponsive to LPS stimulation, which was rooted in either processing with, or trafficking of, IL-1β. The abnormally low TNF-α was in line with a previous study showing that IL-1β and TNF-α may share common export pathways to traffick proteins which are affected by *NLRP7* mutations and variants [11]. Furthermore, both the PBMCs and plasmids ascertained that the NACHT domain and the LRR domain might work differently.

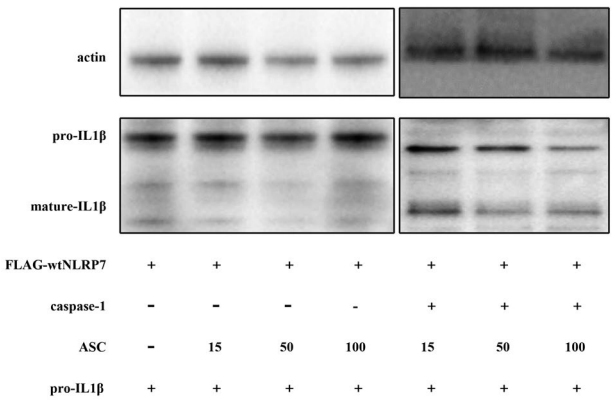

Fig. 4. Apoptosis-associated speck-like protein (ASC) processed pro-interleukin (IL)-1β into mature IL-1β together with FLAG-pro-IL-1β, FLAG-caspase-1 and FLAG-wt-NLRP7 *in vitro*. Immunoblot of whole cell lysates of human embryonic kidney 293 (HEK293) cells that were transfected simultaneously with expression vectors FLAG-wt-*NLRP7* (100 ng), FLAG-pro-IL-1β (150 ng) and different amounts (0, 15, 248 50, 100 ng) of FLAG-ASC with and without FLAG-aspase-1 (15 ng).

Long before the use of epigenetics in the pathology of moles, immunology has been recognized as a reason for various forms of pregnancy loss, including HMs. Currently, the evidence of abnormal maternal inflammation is limiting. It has been verified that *NLRP7* up-regulates intracellular inflammation and increases the secretion of IL-1β in various monocytes, which is consistent with the fact that PBMCs from *NLRP7*-defective-alleles patients secrete less IL-1β [10–12].

Many immune cells have been identified in the endometrium, including uterine NK (uNK) cells, macrophages, mast cells, dendritic cells (DC) and T cells. Together, these endometrial lymphocytes constitute the maternal immune microenvironment. Single-cell transcriptome profiles from the early human maternal–fetal interface show that placental extravillous trophoblast cells are adjacent to macrophages [16]. Androgenetic CHM (AnCHM) is able to introduce maternal immune responses which lead to fetal rejection and recruitment of immune cells to the decidual tissue [17]. Compared with AnCHM, although containing more maternal genetic materials, BiCHM is also characterized by proliferative trophoblast cells. Reduced levels of *NLRP7* accelerate trophoblast differentiation of human embryonic stem cells [18]; however, little is known about the exact function of leukocytes involved in BiCHM.

According to Singer *et al*., *NLRP7* domains play certain roles in inflammasome activity [19]. In this study, a mutation located in the LRR domain is different from a protein-truncating mutation in the NACHT domain, which suggests that each domain of *NLRP7* might play a different role in either activating or polymerizing the inflammasomes, and lower IL-1β was caused by less processing or with trafficking of IL-1β. Interestingly, the IL-1β of patients whose NSVs were considered to be rare variants did not show differences in pro-IL-1β and mature IL-1β expression or secretion.

The exact mechanism underlying the *NLRP7*mutations and RHM is unclear; whether the NSV is a missense mutation or a rare variant may lead to different therapeutics. Currently we are still far from offering a comprehensive view of the relationship between mutation and pathogenicity due to incomplete data from patients. Whether HM-linked *NLRP7* mutants are gain- or loss-of-function defects impacting inflammasome activity, in

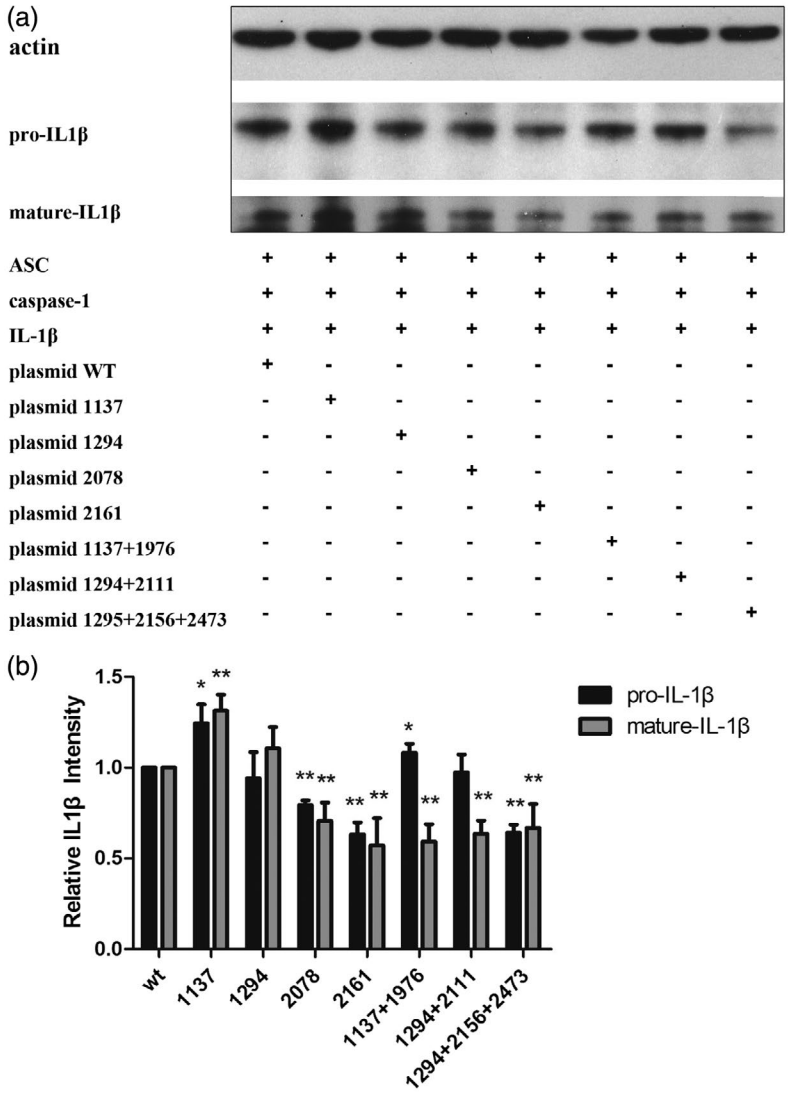

Fig. 5. Missense mutations in NOD-like receptor pyrin 7 (*NLRP7*) affected the interleukin (IL)-1β expression. (a) Immunoblots of human embryonic kidney 293 (HEK293) cells that were transfected simultaneously with expression vectors encoding FLAG-pro-IL-1β (150 ng), FLAG-caspase-1 (15 ng), FLAG-ASC (100 ng) and FLAG-wt-NLRP7 (100 ng) or mutant *NLRP7* (100 ng) expression vectors. (b) Representations of the quantification of pro-IL-1β and, respectively, using ImageJ software. The averages and standard deviation (s.d.) were calculated on three different Western blottings on cellular lysates from different transfection experiments. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

this study we provide more data, especially by adding the essential inflammasome adaptor ASC *in vitro*.

In conclusion, our results directly support the hypothesis of lowering the immunity level in cases of *NLRP7* mutation due to decreased levels of IL-1, and hence the decreased immunological ability to repel HMs. Moreover, plasmid 734, taken from a patient with AnCHM, did not show such a decrease in the *in-vitro* processing of pro-IL-1β. Until now, the first hit of *NLRP7* played during fertilization and/or very early in the zygote is unclear; our observations support that *NLRP7* mutation may help the retention of pregnancy products as the second hit to the HMs. In future, larger trials are needed to more clearly understand this association by assessing other types of interleukins and interferons as being potential cofounders.

Acknowledgements

This work was supported by the National Natural Science Foundation of China and the National Natural Science Foundation of China (81261120569 and 81370729 to J. H. Q.).

Disclosures

The authors declare no conflicts of interest.

References

1 Savage P, Williams J, Wong SL *et al*. The demographics of molar pregnancies in England and Wales from 2000–2009. J Reprod Med 2010; **55**:341–5.

2 Abdalla EM, Hayward BE, Shamseddin A, Nawar MM. Recurrent hydatidiform mole: detection of two novel mutations in the NLRP7 gene in two Egyptian families. Eur J Obstet Gynecol Reprod Biol 2012; **164**:211–5.

3 Hayward BE, De Vos M, Talati N *et al*. Genetic and epigenetic analysis of recurrent hydatidiform mole. Hum Mutat 2009; **30**:E629–E639.

4 Slim R, Bagga R, Chebaro W, Srinivasan R, Agarwal N. A strong founder effect for two NLRP7 mutations in the Indian population: an intriguing observation. Clin Genet 2009; **76**:292–5.

5 Parry DA, Logan CV, Hayward BE *et al*. Mutations causing familial biparental hydatidiform mole implicate c6orf221 as a possible regulator of genomic imprinting in the human oocyte. Am J Hum Genet 2011; **89**:451–8.

6 Fallahian M, Sebire NJ, Savage PM, Seckl MJ, Fisher RA. Mutations in NLRP7 and KHDC3L confer a complete hydatidiform mole phenotype on digynic triploid conceptions. Hum Mutat 2013; **34**:301–8.

7 Murdoch S, Djuric U, Mazhar B *et al*. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. Nat Genet 2006; **38**:300–2.

8 Caliebe A, Richter J, Ammerpohl O *et al*. A familial disorder of altered DNA-methylation. J Med Genet 2014; **51**:407–12.

9 Soellner L, Begemann M, Degenhardt F, Geipel A, Eggermann T, Mangold E. Maternal heterozygous NLRP7 variant results in recurrent reproductive failure and imprinting disturbances in the offspring. Eur J Hum Genet 2017; **25**:924–9.

10 Khare S, Dorfleutner A, Bryan NB *et al*. An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in human macrophages. Immunity 2012; **36**:464–76.

11 Messaed C, Akoury E, Djuric U *et al*. NLRP7, a nucleotide oligomerization domain-like receptor protein, is required for normal cytokine secretion and co-localizes with Golgi and the microtubule-organizing center. J Biol Chem 2011; **286**:43313–23.

12 Messaed C, Chebaro W, Roberto RBD *et al*. NLRP7 in the spectrum of reproductive wastage: rare non-synonymous variants confer genetic susceptibility to recurrent reproductive wastage. J Med Genet 2011; **48**:540–8.

13 Deveault C, Qian JH, Chebaro W *et al*. NLRP7 mutations in women with diploid androgenetic and triploid moles: a proposed mechanism for mole formation. Hum Mol Genet 2009; **18**:888–97.

14 Qian J, Cheng Q, Murdoch S *et al*. The genetics of recurrent hydatidiform moles in China: correlations between NLRP7 mutations, molar genotypes and reproductive outcomes. Mol Hum Reprod 2011; **17**:612–9.

15 Qian J, Deveault C, Bagga R, Xie X, Slim R. Women heterozygous for NALP7/NLRP7 mutations are at risk for reproductive wastage: report of two novel mutations. Hum Mutat 2007; **28**:741.

16 Vento-Tormo R, Efremova M, Botting RA *et al*. Single-cell reconstruction of the early maternal-fetal interface in humans. Nature 2018; **563**:347–53.

17 Hussein MR, Abd-Elwahed AR, Abodeif ES, Abdulwahed SR. Decidual immune cell infiltrate in hydatidiform mole. Cancer Invest 2009; **27**:60–6.

18 Mahadevan S, Wen S, Wan Y-W *et al*. NLRP7 affects trophoblast lineage differentiation, binds to overexpressed YY1 and alters CpG methylation. Hum Mol Genet 2014; **23**:706–16.

19 Singer H, Biswas A, Zimmer N, Messaed C, Oldenburg J, Slim R, El-Maarri O. NLRP7 inter-domain interactions: the NACHT-associated domain is the physical mediator for oligomeric assembly. Mol Hum Reprod 2014; **20**:990–1001.