RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

NLRP12-associated autoinflammatory disease in Chinese adult patients: a single-centre study

Junke Miao ,[1] Jingyuan Zhang,[1] Xin Huang ,[1] Na Wu ,[1] Di Wu,[2] Min Shen[1]

To cite: Miao J, Zhang J, Huang X, *et al*. *NLRP12*-associated autoinflammatory disease in Chinese adult patients: a single-centre study. *RMD Open* 2023;**9**:e003598. doi:10.1136/rmdopen-2023-003598

Received 10 August 2023
Accepted 27 November 2023

Check for updates

© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to
Dr Min Shen;
shenmpumch@163.com

Dr Di Wu; med_wudi@sina.com

ABSTRACT

Background *NLRP12*-associated autoinflammatory disease (*NLRP12*-AID) is an autosomal dominant autoinflammatory disorder caused by variants of *NLRP12* gene. We aimed to report a cohort of Chinese adult patients with *NLRP12*-AID and summarised phenotypes and genotypes.

Methods Twenty patients were diagnosed with *NLRP12*-AID after performing whole-exome sequencing and were included in our cohort. Demographic information, clinical data and treatment response were collected and evaluated. A literature review of *NLRP12*-AID was performed, and the clinical features and mutated sites were summarised and compared with our cohort.

Results Among the 20 *NLRP12*-AID patients, the main clinical features of *NLRP12*-AID included fever, cutaneous rash, arthralgia/arthritis, pharyngitis/tonsillitis, lymphadenopathy, myalgia and abdominal pain/diarrhoea. Thirteen *NLRP12* variants were detected as F402L, G39V, R1030X, R7G, E24A, Q90X, A218V, A259V, W581X, G729R, R859W, c.-150T>C and c.*126G>C. Glucocorticoids were used in 14 patients, immunosuppressive agents in 13, and tocilizumab in 2. Seventeen patients had good responses to therapy. When compared with 50 *NLRP12*-AID patients from other countries, Chinese patients had fewer variants in exon 3, higher incidences of cutaneous rash, pharyngitis/tonsillitis and lymphadenopathy. Among all these 70 *NLRP12*-AID patients, patients carrying non-exon-3 variants had higher frequencies of ocular involvement, pharyngitis/tonsillitis, headache and lymphadenopathy than those with exon-3 variants.

Conclusion This is the largest cohort of *NLRP12*-AID in the world and seven novel variants of *NLRP12* were identified. Chinese adult patients of *NLRP12*-AID had more non-specific symptoms such as pharyngitis/tonsillitis and lymphadenopathy when compared with patients from other countries, for which the less occurrence of exon-3 variants might be one possible reason.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

⇒ *NLRP12*-associated autoinflammatory disease (*NLRP12*-AID) is an autosomal dominant autoinflammatory disorder caused by the variants of *NLRP12* gene. The role of NLRP12 played in *NLRP12*-AID is not clear and only a few cases of *NLRP12*-AID patients have been reported mainly focused on the Caucasic and paediatric populations with clinical heterogeneities and incomplete penetrance of the variants.

WHAT DOES THIS STUDY ADD

⇒ The phenotype, genotype and treatment responses of Chinese adult patients with *NLRP12*-AID were described and seven novel variants were identified. Compared with patients from other countries, Chinese adult patients of *NLRP12*-AID had more nonspecific symptoms such as pharyngitis/tonsillitis and lymphadenopathy and the less occurrence of exon-3 variants. The distinction of the clinical manifestations in our cohort might be related to the mutation sites.

HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE

⇒ We provided the clinical experiences on the treatment of *NLRP12*-AID adult patients in China. Our study also indicated many *NLRP12*-AID patients have autoimmune manifestations and a higher frequency of definite allergic histories. More clinical attentions might be needed on *NLRP12*-AID patients with exon 3 variants considering the phenotype–genotype interaction we found.

INTRODUCTION

Systemic autoinflammatory diseases (SAIDs) are a group of rare diseases caused by dysregulation of innate immune system, manifested as episodes of recurrent sterile inflammation and the lack of pathogenic autoantibodies. Monogenic SAIDs are prototypical SAID conditions, caused by the loss-of-function or gain-of-function mutations on the SAID-associated genes.[1 2] *NLRP12*-associated autoinflammatory disease (*NLRP12*-AID) is an autosomal dominant SAID caused by variants of the *NLRP12* gene, which encodes the NLRP12 protein, a member of nod-like receptor families as an intracellular pattern recognition receptor (PRR).[3]

The main symptoms of *NLRP12*-AID include recurrent fever, arthralgia/arthritis, cutaneous rash and abdominal pain. It is also known as familial cold-induced autoinflammatory syndrome 2 (FCAS2) because of the similar phenotype to FCAS, the mild type of *NLRP3*-associated autoinflammatory disease (*NLRP3*-AID). The nucleotide sequence of *NLRP12* also bears high similarity to *NLRP3*. However, the severity of *NLRP12*-AID is milder than *NLRP3*-AID in view of the less occurrence of serious complications and the less use of biological agents.[4 5]

Since the first definition in 2008, only a few cases of *NLRP12*-AID have been reported mainly in the Caucasic and paediatric populations.[6 7] There have been no established diagnostic criteria up till now, possibly because of the considerable clinical heterogeneity and the incomplete penetrance of the variants. Phenotypes and genotypes of Chinese adult-onset *NLRP12*-AID patients were poorly studied. Here, we reported the largest cohort of Chinese adult patients with *NLRP12*-AID and performed a literature review on *NLRP12*-AID patients from other countries that have been reported so far.

METHODS AND PATIENTS

There have not been well accepted diagnostic criteria of *NLRP12*-AID so far. In this study, the inclusion criteria were: (1) recurrent inflammatory signs and symptoms frequently seen in SAID patients, such as recurrent fever, arthralgia/arthritis, dermatitis, sensorineural deafness and headache with intervals free of any clinical manifestations; (2) one or more variants of *NLRP12* detected by whole-exome sequencing using next-generation sequencing. Patients diagnosed with malignancies, infectious diseases, autoimmune diseases or other diseases that could explain their recurrent inflammation were excluded. This single-centre study included 20 adult patients diagnosed with *NLRP12*-AID at the Department of Rheumatology, Peking Union Medical College Hospital from April 2015 to June 2023. Demographic information and detailed clinical records were carefully documented and studied. The treatment responses of good, partial or no responses were defined as more than 80%, 20%–80% or less than 20% remission of clinical symptoms after the treatment. The remission of clinical symptoms referred to the decreases in the lasting time or the frequency of each episode and the severity of autoinflammatory symptoms compared with those before treatment. The families of the proband were also performed WES or Sanger sequencing to correct the mutated sites of *NLRP12* if it was possible. This study was approved by the Institutional Review Board of Peking Union Medical College Hospital and was performed in accordance with the Declaration of Helsinki. Informed consents were obtained from all participants.

A PubMed literature search for '*NLRP12*-autoinflammatory disease' and 'familial cold-induced autoinflammatory syndrome 2' up to June 2023 was performed. We collected and analysed detailed information on the phenotypes and genotypes of *NLRP12*-AID patients from other countries reported in the English literature.

Table 1 Summary of the phenotypes of *NLRP12*-AID patients in our cohort

Variables	Total patients N=20
Demographic data	
Ratio of gender (M:F)	11:9
Age at disease onset, median, range, years old	22 (0–61)
Age at diagnosis, median, range, years old	30 (11–63)
Delayed diagnosis, median, range, years	7.8 (0–30)
Child-onset, n (%)	6 (30)
Family history, n (%)	5 (25)
Clinical manifestations, n (%)	
Fever	20 (100)
Fever duration, median, range, days	8.3 (1-28)
Fever episodes/year, median, range, times	7.2 (1-12)
Cold-induced	4 (20.0)
Myalgia	9 (45)
Arthralgia/arthritis	13 (65)
Cutaneous rash	15 (75)
Oral ulcers	6 (30)
Ocular involvement	5 (25)
Sensorineural deafness	2 (10)
Pharyngitis/tonsillitis	12 (60)
Abdominal pain/diarrhoea	8 (40)
Splenomegaly	4 (20)
Headache	6 (30)
Lymphadenopathy	10 (50)

NLRP12-AID, *NLRP12*-associated autoinflammatory disease.

Continuous variables were expressed as median and the range and assessed using Mann–Whiteny test. Categorical variables were described as frequency and compared using the χ^2 test or Fisher's exact test. All the statistical tests were two-sided, and the significant level of p was set as 0.05 with the analyses performed using IBM SPSS Statistics (V.26).

RESULTS

Demographic data and clinical phenotypes

The demographic and clinical features of these 20 patients were summarised in table 1. All the patients were Han Chinese with a median disease-onset age of 22 years old, ranging from several months after birth to 61 years old. The gender ratio of men to women was 11:9. Six patients (30%) had disease-onset during childhood, and 14 (70%) were adult-onset. The median time of diagnosis delay was 7.8 years (ranging from 3 months to 30 years). There were five carriers from the proband's families

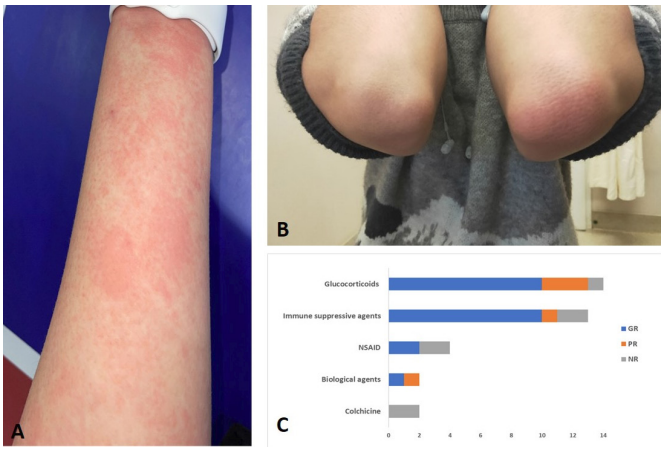

Figure 1 Phenotypes of the *NLRP12*-AID patients in our cohort. (A) Urticaria-like rash; (B) arthritis; (C) summary of the treatment response of *NLRP12*-AID patients (n=20). GR, good response; PR, partial response; *NLRP12*-AID, *NLRP12*-associated autoinflammatory disease; NR, no response; NSAIDs, nonsteroidal anti-inflammatory drugs.

(25%) who had a family history of recurrent fever or other resembling inflammation symptoms.

Of these 20 *NLRP12*-AID patients, the most frequent symptoms were fever (20/20, 100%), cutaneous rash (15/20, 75%), arthralgia/arthritis (13/20, 65%), pharyngitis/tonsillitis (12/20, 60%), lymphadenopathy (10/20, 50%) and myalgia (9/20, 45%) (figure 1A,B). Abdominal pain/diarrhoea (8/20, 40%), headache (6/20, 30%) and oral ulcers (6/20, 30%) were also described. Four patients had cold-induced onsets of recurrent fever (n=1), arthralgia/arthritis (n=1) and cutaneous rash (n=2). The acute phase reactants including C reactive protein and/or erythrocyte sedimentation rate were increased in 19 patients during the episodes and returned to normal during the intervals. Autoantibodies were negative in most of the patients except for four patients, who had low-titre antinuclear antibodies (ANAs). Ten patients had a definite history of allergic reactions, including six to medicines (macrolides antibiotics, penicillin, acyclovir, ganciclovir and aminophylline), and four to pollen, alcohol, protein foods or animal hair. Four patients had elevated serum levels of immunoglobin (Ig) E.

Genotypes

Thirteen heterozygous *NLRP12* variants were detected including F402L (n=7), G39V (n=3), R1030X (n=2), c.-150T>C, c.*126G>C, R7G, E24A, Q90X, A218V, A259V, W581X, G729R, R859W (n=1, respectively). Among them, two were compound heterozygous (A259V-F402L and R1030X-F402L) and seven were novel variants (R7G, E24A, Q90X, A218V, A259V, G729R, R859W). The minor allele frequency (MAF) and the computational prediction results of these variants are listed in table 2. According to the ACMG guideline, eight variants were categorised as VUS, three as likely benign, and the most common two variants, F402L and G39V, as benign. Among the 20 patients, families of 15 patients also performed WES or Sanger sequencing to correct the mutated sites of *NLRP12* gene and all the variants were found to be inherited from their parents with no de novo variants.

Treatment response

The treatment response was summarised in figure 1C. Glucocorticoids were given to 14 patients with good, partial or no response in 10, 3 or 1, respectively, among

Table 2 Summary of the genotypes of *NLRP12*-AID patients and the functional prediction of the variants

Patients number	*NLRP12* variants	Mutation taster	CADD	Polyphen2	SIFT	PROVEAN	MAF	ACMG
7	c.1206C>G, p.F402L	Polymorphism	Damaging	Probably damaging	Tolerable	Damaging	0.0075	Benign
3	c.116G>T, p.G39V	Polymorphism	Damaging	Benign	Damaging	Damaging	0.1789	Benign
2	c.3088C>T, p.R1030X	Disease causing	Damaging	–	–	–	0	VUS
1	c.19A>G, p.R7G#	Polymorphism	Tolerable	Benign	Damaging	Tolerable	0.0006	Likely benign
1	c.71A>C, p.E24A#	Disease causing	Damaging	Possibly damaging	Damaging	Damaging	–	VUS
1	c.268C>T, p.Q90X#	Disease causing	Damaging	–	–	–	0.0002	VUS
1	c.653C>T, p.A218V#	Disease causing	Damaging	Probably damaging	Damaging	Tolerable	0.00005799	VUS
1	c.776C>T, p.A259V#	Polymorphism	Tolerable	Benign	Tolerable	Tolerable	–	Likely benign
1	c.1742G>A, p.W581X	Disease causing	Damaging	–	–	–	0.00005798	VUS
1	c.2185G>C, p.G729R#	Disease causing	Damaging	Probably damaging	Damaging	Damaging	0.0006	VUS
1	c.2575C>T, p.R859W#	Polymorphism	Damaging	Probably damaging	Damaging	Damaging	0	Likely benign
1	c.-150T>C							VUS
1	c.*126G>C							VUS

#, novel variants; ACMG, American College of Medical Genetics and Genomics; MAF, Minor Allele Frequency from gnomAD_exome_EAS; *NLRP12*-AID, *NLRP12*-associated autoinflammatory disease; VUS, variant of uncertain significance.

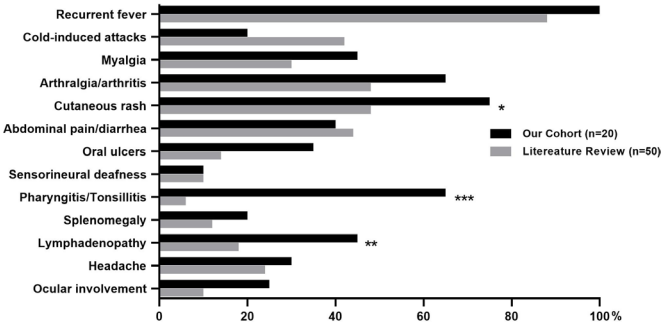

Figure 2 Comparison of the clinical symptoms between *NLRP12*-AID patients in our cohort and those from other countries. *NLRP12*-AID, *NLRP12*-associated autoinflammatory disease. *p-values<0.05; ** p-values<0.01; *** p-values<0.001.

whom two were treated only at the episodes. Immunosuppressive agents were used in 13 patients combined with glucocorticoids. Methotrexate (MTX) was used in nine patients, with no relief in two, partial relief in 1 and good relief in 6. Hydroxychloroquine or thalidomide were given to five and three patients, respectively, with good responses. Tocilizumab was used in two refractory patients manifesting as relapsing arthritis and was effective. Meanwhile, tonsillectomy and colchicine were also treated for 3 and 2 patients with no effects. After a mean follow-up of 55 months, 17 patients showed good response and 2 partial responses to the treatments.

Literature review

Ultimately, 16 articles related to *NLRP12*-AID from other countries containing 50 patients were included, and their phenotypes and genotypes were compared with those from our centre.[3 7–21] In terms of the clinical manifestations, patients from our cohort had higher frequencies of cutaneous rash (75% vs 48%, p=0.04), pharyngitis/tonsillitis (65% vs 6%, p<0.001) and lymphadenopathy (45% vs 18%, p=0.007) than those from other countries (figure 2). A total of 23 variants of *NLRP12* were identified in *NLRP12*-AID patients from other countries, including F402L which was also seen in our patients (figure 3). The exon 3 of *NLRP12* was the main site of the variants in *NLRP12*-AID, and the frequency of exon 3 variants in patients from other countries was higher than that in our cohort (84% vs 40%, p<0.001). Through literature review, 3 out of 50 patients received tumour necrosis factor inhibitors with no response in one and partial responses in two who were also diagnosed with Crohn's disease. Eight out of 50 patients were given interleukin 1 (IL-1) antagonists with good responses in 5, partial response in 1, no response in 1 and another patient had an unknown response.

Phenotype–genotype interaction

Given the significant differences between our cohort and other studies, we speculated the distinction of the clinical manifestations might be related to the mutation sites. We, therefore, performed a comparison between the patients with and without exon 3 variants among all 70 patients from our centre and other countries. Compared with those carrying non-exon 3 variants, the frequencies of pharyngitis/tonsillitis (50% vs 10%, p=0.001), ocular involvement (30% vs 8%, p=0.027), headache (50% vs 16%, p=0.003) and lymphadenopathy (45% vs 20%, p=0.034) were higher in those carrying exon 3 variants (table 3).

DISCUSSION

NLRP12, a member of NLR families as an intracellular PRR, is composed of an N-terminal pyrin domain, a central nucleotide-binding domain and a C-terminal leucine-rich repeat domain with dual functions in innate immune system.[22 23] On the one hand, NLRP12 plays an anti-inflammatory role as a suppressor of canonical and non-canonical NF-κB signalling pathway under the infections of some pathogens such as *Brucella abortus* and *Salmonella typhimurium*, and it also plays a role in attenuating the colon inflammation and tumorigenesis.[24–27] On the other hand, similar to other NLR proteins, NLRP12 is assembled as an inflammasome stimulated by certain conditions, including the viral and bacterial infections

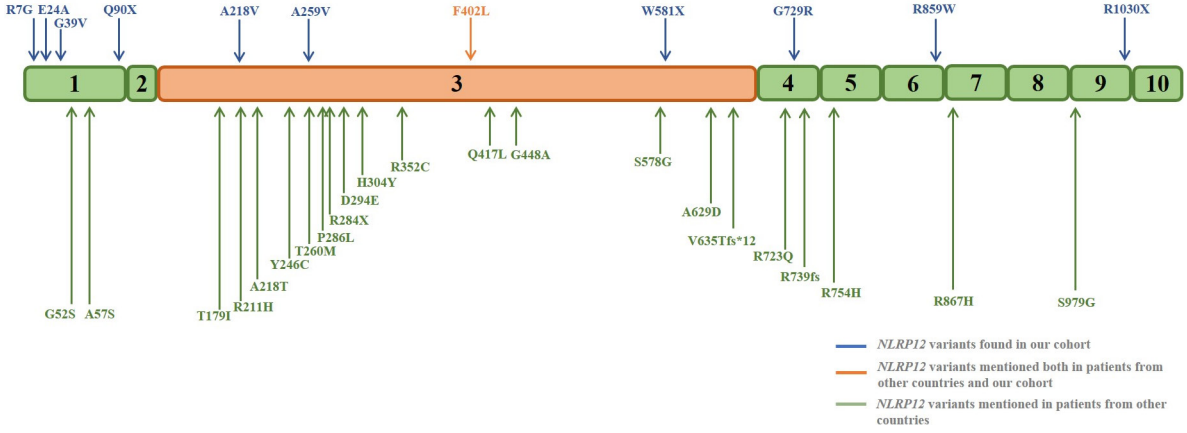

Figure 3 Distribution of the *NLRP12* variants in coding regions mentioned in *NLRP12*-AID patients. *NLRP12*-AID, *NLRP12*-associated autoinflammatory disease.

Table 3 Comparison of the clinical manifestations between *NLRP12*-AID patients with and without exon 3 variants

Clinical manifestations, n (%)	Patients with exon 3 variants N=50	Patients with non-exon 3 variants N=20	P value
Fever	44 (88)	20 (100)	0.173
Cold-induced	18 (36)	7 (35)	0.937
Myalgia	18 (36)	6 (30)	0.633
Arthralgia/arthritis	26 (52)	11 (55)	0.82
Cutaneous rash	28 (56)	11 (55)	0.939
Oral ulcers	9 (18)	5 (25)	0.522
Ocular involvement	4 (8)	6 (30)	**0.027**
Sensorineural deafness	5 (10)	2 (10)	1
Pharyngitis/tonsillitis	5 (10)	10 (50)	**0.001**
Abdominal pain/ diarrhoea	21 (42)	9 (45)	0.819
Splenomegaly	7 (14)	3 (15)	1
Headache	8 (16)	10 (50)	**0.003**
Lymphadenopathy	10 (20)	9 (45)	**0.034**

Boldface: p-value<0.05
NLRP12-AID, *NLRP12*-associated autoinflammatory disease.

like herpes simplex virus type 1, *Yersinia pestis*, *Plasmodium vivax* or *P. falciparum* and the hypoxia-inducible factor-1α in microglial cells, leading to the formation of apoptosis-associated speck-like protein containing a CARD (ASC) speck and the cleavage of pro-caspase-1 and pro-IL-1β to control the inflammation.[28–31] One recent study also indicated NLRP12 could form a PANoptosome and drive the lytic cell death called PANoptosis after sensing heme and other pathogen-associated molecular patterns (PAMPs).[32] Despite its multiple biological effects, the role of NLRP12 in the pathogenesis of *NLRP12*-AID is still controversial. For example, *NLRP12* T284X and R352C were reported to be loss-of-function variants amplifying the inflammation because of the impaired suppressive effects on NF-κB.[3 9] However, *NLRP12* D294E could promote the formation of ASC specks and the cleavage of pro-caspase-1, indicating a gain-of-function variant as an inflammasome.[8] Considering the role of NLRP12 might be determined by the types of PAMPs, damage-associated molecular patterns and infectious pathogens, we inferred both the genetic backgrounds and environmental factors might be the risk factors of the pathogenesis of *NLRP12*-AID, where both the proinflammatory and anti-inflammatory effects of NLRP12 might participate simultaneously.

In order to better understand *NLRP12*-AID, we reported the largest cohort of Chinese adult patients with *NLRP12*-AID, performed a literature review and summarised the clinical records in order to identify the difference between our cohort and patients from other countries. Apart from the common symptoms of *NLRP12*-AID such as cold-induced recurrent fever, cutaneous rash, arthralgia/arthritis and abdominal pain, patients in our cohort reported more uncommon symptoms like pharyngitis/tonsillitis, lymphadenopathy, oral ulcer and ocular involvement, indicating the diverse manifestations of Chinese adult *NLRP12*-AID patients. In addition to autoinflammation, a significant proportion of patients in our centre and others had low titers of autoantibodies such as ANAs.[14 15 18 33] Intriguingly, autoimmune symptoms such as Raynaud's phenomenon, malar erythema, morning stiffness, autoimmune haemolytic anaemia, hypothyroidism and Crohn's disease were also reported in the literature.[7 14 20 34] So, the coexistence of the dysregulations of both innate and adaptive immune systems was not rare in *NLRP12*-AID. The regulatory effects of NLRP12 on T cells had been well documented, such as the inhibitory effects on Th17 cell differentiation and the suppression of Th1 response by reducing interferon-γ and IL-2 production. However, the abnormalities of T cell subsets and the specific role they play in *NLRP12*-AID are still unknown.[35 36] We hypothesised that these autoimmune manifestations were caused by the abnormal T cell responses due to genetic variants. Furthermore, we noted that half of the patients in our cohort had definite allergic histories of food or drugs or an elevation of IgE, which has also been described in other studies.[7] Since the strong allergen was reported to downregulate the expression of NLRP12 and to induce the excessive maturation of IL-18, the high proportion of allergic histories in *NLRP12*-AID patients might be not only a causal event but also the consequence of genetic variants.[37]

The main controversy on *NLRP12*-AID was the pathogenicity of *NLRP12* in view of the uncertain significance and low penetrance of the variants. Different from other monogenic SAIDs, there were no pathogenic variants and only 7.2% (6/83) likely pathogenic variants of *NLRP12* according to the Infevers database (https://infevers.umai-montpellier.fr/web/). Although most of them were VUS or even benign variants according to the ClinVar database considering the high MAF, such as F402L and G39V, they were predicted to be damaging to the protein function assessed by the bioinformatic software in silico as we mentioned in the results. Therefore, whether the variants play a modifying or a pathogenic role are still debated. The low penetrance of *NLRP12* is also another feature of this disease. According to the literature review, only 24% (12/50) patients were reported to have a positive family history, similar with our data for about 25%. Meanwhile, some variants of *NLRP12* could also be seen in asymptomatic relatives of the patients, patients of multiple sclerosis, stroke and psoriatic arthritis with no manifestations of FCAS2, and even healthy people.[38–42] No variants in untranslated regions have been reported in *NLRP12*-AID except for these two patients in our centre, which might cause the disease by affecting the expression of NLRP12. Yet functional experiments should be performed to elucidate

the pathogenic effects of these variants. Although many patients were adulthood onsets, mosaicism was not taken into consideration because we had adjusted the cut-off frequency to 10%–90% for heterozygosity in SAID-related genes during the bioinformatic analysis, which was able to rule out the mosaicism.

Many Chinese adult patients with monogenic SAIDs had different phenotypes from those in other countries. Similarly, our study indicated that patients from our cohort had more infrequent symptoms, including pharyngitis/tonsillitis and lymphadenopathy. We also noticed there is a lower frequency of the variants occurring in exon 3 in our centre, where the majority of the variants in *NLRP12* were reported in literature.[15] Thus, we performed a separate subgroup analysis and compared the patients with and without exon 3 variants to explore whether the differences in genotype could explain the differences in phenotype. The results of our study showed that the symptoms of ocular involvement, pharyngitis/tonsillitis, headache, and lymphadenopathy were more common in patients carrying non-exon 3 variants, which implied its relationship with those aforementioned atypical symptoms. Further studies are needed to exclude the possible errors caused by the small size of the cohort.

Based on our experience, the therapy strategy of *NLRP12*-AID was determined by the severity of disease. The combination of glucocorticoids and MTX was the most common choice. In general, the treatment responses were good with no severe adverse events. Interestingly, 2 out of 3 patients who didn't have good response to treatments carried F402L variant in exon 3. Besides, two refractory patients with severe arthralgia and arthritis who eventually had satisfactory response to tocilizumab had variants in exon 3 as well, F402L and W581X. According to the literature review, 10 out of 11 patients who received biological agents also carried variants in exon 3.[7 10–12 15 16 18 21] Thus, we inferred that patients carrying exon 3 variants seemed to have poor prognosis because of the more use of biological agents and the worse responses to the treatment.

The over-production of proinflammatory cytokines in *NLRP12*-AID patients has been reported, providing the theoretical basis for the application of biological agents, but the choice of biological agents in refractory *NLRP12*-AID patients has not been well defined. IL-1 antagonists were the most common ones because of the elevation of the final concentration of IL-1 or the increased kinetics of IL-1 release in *NLRP12*-AID patients.[43 44] Due to the unavailability of IL-1 antagonist in China, tocilizumab was given to two patients in our centre for refractory arthritis. It has been reported that there is an increased level of IL-6 in the serum of *NLRP12*-AID patients, indicating the possibility of tocilizumab as the treatment for *NLRP12*-AID, especially in those suffering from arthritis.[45]

CONCLUSION

In this study, we reported the largest cohort of Chinese adult patients with *NLRP12*-AID, and described the clinical features and the genetic backgrounds. Seven novel variants of *NLRP12* gene were identified including R7G, E24A, Q90X, A218V, A259V, G729R, R859W. The phenotype of Chinese adult patients with *NLRP12*-AID had more atypical manifestations, which might be related to the fact that more variants occurred in non-exon 3 regions. Our study enlarged the clinical phenotypic and genotypic profiles of *NLRP12*-AID and indicated the possible links between phenotype and genotype. We hope these data could provide the basis for further studies on the pathogenesis of *NLRP12*-AID.

Author affiliations
[1]Department of Rare Diseases, State Key Laboratory of Complex Severe and Rare Diseases, Department of Rheumatology and Clinical Immunology, National Clinical Research Center for Dermatologic and Immunologic Diseases (NCRC-DID), Ministry of Science & Technology, Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Peking Union Medical College Hospital (PUMCH), Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China
[2]Department of Rheumatology and Clinical Immunology, National Clinical Research Center for Dermatologic and Immunologic Diseases (NCRC-DID), Ministry of Science & Technology, Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Peking Union Medical College Hospital (PUMCH), Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

Acknowledgements The authors would like to acknowledge the conference for the prior presentation of this study (https://acrabstracts.org/abstract/nlrp12-associated-autoinflammatory-disease-in-chinese-adult-patients-a-single-center-study/). The authors also want to acknowledge the patients for their consents to participate in the study.

Contributors MS and DW designed the study, contributed to the disease diagnosis and revised the draft. JM was involved in the data collection and data analysis and drafted the manuscript. JZ, XH and NW also participated in the acquisition of the clinical data. All authors were involved in the revision of the draft and approved the final version to be published. MS is responsible for the overall content as the guarantor.

Funding This work was supported by the National High Level Hospital Clinical Research Funding (grant number 2022-PUMCH-D-002, 2022-PUMCH-B-013).

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and was approved by Institutional Review Board of Peking Union Medical College Hospital (ZS-3272) Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer-reviewed.

Data availability statement No data are available. The original data presented in the study are included in this article. Further inquiries can be directed to the corresponding author.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Junke Miao http://orcid.org/0000-0003-3453-6850
Xin Huang http://orcid.org/0000-0002-5447-1129
Na Wu http://orcid.org/0000-0002-1611-3945

REFERENCES

1 Kastner DL, Aksentijevich I, Goldbach-Mansky R. Autoinflammatory disease reloaded: a clinical perspective. *Cell* 2010;140:784–90.
2 Ben-Chetrit E, Gattorno M, Gul A, *et al*. Consensus proposal for taxonomy and definition of the autoinflammatory diseases (AIDS): a Delphi study. *Ann Rheum Dis* 2018;77:1558–65.
3 Jéru I, Duquesnoy P, Fernandes-Alnemri T, *et al*. Mutations in NALP12 cause hereditary periodic fever syndromes . *Proc Natl Acad Sci USA* 2008;105:1614–9.
4 Hoffman HM, Mueller JL, Broide DH, *et al*. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and muckle-wells syndrome. *Nat Genet* 2001;29:301–5.
5 Harton JA, Linhoff MW, Zhang J, *et al*. Cutting edge: CATERPILLER: a large family of mammalian genes containing CARD, pyrin, nucleotide-binding, and leucine-rich repeat domains. *J Immunol* 2002;169:4088–93.
6 De Pieri C, Vuch J, Athanasakis E, *et al*. F402L variant in NLRP12 in subjects with undiagnosed periodic fevers and in healthy controls. *Clin Exp Rheumatol* 2014;32:993–4.
7 Kostik MM, Suspitsin EN, Guseva MN, *et al*. Multigene sequencing reveals heterogeneity of NLRP12-related autoinflammatory disorders. *Rheumatol Int* 2018;38:887–93.
8 Borghini S, Tassi S, Chiesa S, *et al*. Clinical presentation and pathogenesis of cold-induced autoinflammatory disease in a family with recurrence of an NLRP12 mutation. *Arthritis Rheum* 2011;63:830–9.
9 Jéru I, Le Borgne G, Cochet E, *et al*. Identification and functional consequences of a recurrent NLRP12 missense mutation in periodic fever syndromes. *Arthritis Rheum* 2011;63:1459–64.
10 Vitale A, Rigante D, Maggio MC, *et al*. Rare NLRP12 variants associated with the NLRP12-autoinflammatory disorder phenotype: an Italian case series. *Clin Exp Rheumatol* 2013;31(3 Suppl 77):155–6.
11 Borte S, Celiksoy MH, Menzel V, *et al*. Novel NLRP12 mutations associated with intestinal amyloidosis in a patient diagnosed with common variable immunodeficiency. *Clin Immunol* 2014;154:105–11.
12 Başaran Ö, Uncu N, Çakar N, *et al*. C3 glomerulopathy in NLRP12-related autoinflammatory disorder: case-based review. *Rheumatol Int* 2018;38:1571–6.
13 Ghosh K, Mishra K, Shah A, *et al*. Novel deleterious sequence change in the NLRP12 gene in a child with the autoinflammatory syndrome, joint hypermobility and cutis laxa from India. *Mediterr J Hematol Infect Dis* 2019;11:e2019018.
14 Jacob N, Dasharathy SS, Bui V, *et al*. Generalized cytokine increase in the setting of a multisystem clinical disorder and carcinoid syndrome associated with a novel NLRP12 variant. *Dig Dis Sci* 2019;64:2140–6.
15 Del Porto F, Cifani N, Proietta M, *et al*. NLRP12 gene mutations and auto-inflammatory diseases: ever-changing evidence. *Rheumatology (Oxford)* 2020;59:3129–36.
16 Ayla AY, Eren H, Zare J, *et al*. A rare case of an NLRP12-associated autoinflammatory disease. *Eur J Med Genet* 2021;64:104168.
17 Ghosh K. NLRP12 gene mutation in India: case finding and diagnosis made easy in the days of whole exome sequencing. *Ann Rheum Dis* 2021;80:e152.
18 Lévy D, Mariotte A, DeCauwer A, *et al*. Contrasting role of NLRP12 in autoinflammation: evidence from a case report and mouse models. *RMD Open* 2021;7:e001824.
19 Suri D, Rawat A, Jindal AK, *et al*. Spectrum of systemic auto-inflammatory diseases in India: a multi-centric experience. *Front Immunol* 2021;12:630691.
20 Antunes-Duarte S, Marcos-Pinto A, French LE, *et al*. NLRP12 and IL36RN mutations in a Portuguese woman with autoinflammatory syndrome. *JAAD Case Reports* 2022;26:91–4.
21 Mendonça LO, Toledo-Barros MAM, Leal VNC, *et al*. In-vitro NLRP3 functional test assists the diagnosis of Cryopyrin-associated periodic syndrome (CAPS) patients: a Brazilian cooperation. *Clin Immunol* 2022;245:109159.
22 Tuncer S, Fiorillo MT, Sorrentino R. The multifaceted nature of NLRP12. *J Leukoc Biol* 2014;96:991–1000.
23 Tuladhar S, Kanneganti TD. NLRP12 in innate immunity and inflammation. *Mol Aspects Med* 2020;76:100887.
24 Zaki MH, Vogel P, Malireddi RKS, *et al*. The NOD-like receptor NLRP12 attenuates colon inflammation and tumorigenesis. *Cancer Cell* 2011;20:649–60.
25 Allen IC, Wilson JE, Schneider M, *et al*. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-kappaB signaling. *Immunity* 2012;36:742–54.
26 Zaki MH, Man SM, Vogel P, *et al*. Salmonella exploits NLRP12-dependent innate immune signaling to suppress host defenses during infection. *Proc Natl Acad Sci U S A* 2014;111:385–90.
27 Silveira TN, Gomes MTR, Oliveira LS, *et al*. NLRP12 negatively regulates proinflammatory cytokine production and host defense against Brucella abortus. *Eur J Immunol* 2017;47:51–9.
28 Vladimer GI, Weng D, Paquette SWM, *et al*. The NLRP12 inflammasome recognizes Yersinia pestis. *Immunity* 2012;37:96–107.
29 Ataide MA, Andrade WA, Zamboni DS, *et al*. Malaria-induced NLRP12/NLRP3-dependent caspase-1 activation mediates inflammation and hypersensitivity to bacterial superinfection. *PLoS Pathog* 2014;10:e1003885.
30 Coulon P-G, Dhanushkodi N, Prakash S, *et al*. NLRP3, NLRP12, and IFI16 Inflammasomes induction and caspase-1 activation triggered by virulent HSV-1 strains are associated with severe corneal inflammatory herpetic disease. *Front Immunol* 2019;10:1631.
31 Chen H, Deng Y, Gan X, *et al*. NLRP12 collaborates with NLRP3 and NLRC4 to promote pyroptosis inducing ganglion cell death of acute glaucoma. *Mol Neurodegener* 2020;15:26.
32 Sundaram B, Pandian N, Mall R, *et al*. NLRP12-PANoptosome activates PANoptosis and pathology in response to heme and PAMPs. *Cell* 2023;186:2783–801.
33 Wang W, Zhou Y, Zhong L-Q, *et al*. The clinical phenotype and genotype of NLRP12-autoinflammatory disease: a Chinese case series with literature review. *World J Pediatr* 2020;16:514–9.
34 Chen S, Li Z, Hu X, *et al*. Rare mutations in NLRP3 and NLRP12 associated with familial cold autoinflammatory syndrome: two Chinese pedigrees. *Clin Rheumatol* 2022;41:3461–70.
35 Cai S, Batra S, Del Piero F, *et al*. NLRP12 modulates host defense through IL-17A-CXCL1 axis. *Mucosal Immunol* 2016;9:503–14.
36 Gharagozloo M, Mahmoud S, Simard C, *et al*. The dual immunoregulatory function of NLRP12 in T cell-mediated immune response: lessons from experimental autoimmune encephalomyelitis. *Cells* 2018;7:119.
37 Papale A, Kummer E, Galbiati V, *et al*. Understanding chemical allergen potency: role of NLRP12 and Blimp-1 in the induction of IL-18 in human keratinocytes. *Arch Toxicol* 2017;91:1783–94.
38 Vilariño-Güell C, Zimprich A, Martinelli-Boneschi F, *et al*. Exome sequencing in multiple sclerosis families identifies 12 candidate genes and nominates biological pathways for the genesis of disease. *PLoS Genet* 2019;15:e1008180.
39 Alsultan A, Al-Suliman AM, Aleem A, *et al*. Utilizing whole-exome sequencing to characterize the phenotypic variability of sickle cell disease. *Genet Test Mol Biomarkers* 2018;22:561–7.
40 Hidaka Y, Fujimoto K, Matsuo N, *et al*. Clinical phenotypes and genetic analyses for diagnosis of systemic autoinflammatory diseases in adult patients with unexplained fever. *Mod Rheumatol* 2021;31:704–9.
41 Atschekzei F, Dubrowinskaja N, Anim M, *et al*. Identification of variants in genes associated with autoinflammatory disorders in a cohort of patients with psoriatic arthritis. *RMD Open* 2022;8:e002561.
42 Schmenger T, Diwan GD, Singh G, *et al*. Never-homozygous genetic variants in healthy populations are potential recessive disease candidates. *NPJ Genom Med* 2022;7:54.
43 Ter Haar N, Lachmann H, Özen S, *et al*. Treatment of autoinflammatory diseases: results from the Eurofever registry and a literature review. *Ann Rheum Dis* 2013;72:678–85.
44 Demir F, Gürler E, Sözeri B. Efficacy of anakinra treatment in pediatric rheumatic diseases: our single-center experience. *Arch Rheumatol* 2022;37:435–43.
45 Jéru I, Hentgen V, Normand S, *et al*. Role of interleukin-1 beta in NLRP12-associated autoinflammatory disorders and resistance to anti-Interleukin-1 therapy. *Arthritis Rheum* 2011;63:2142–8. 10.1002/art.30378 Available: http://doi.wiley.com/10.1002/art.v63.7