The Phenotype and genotype of Chinese adult patients with NLRP3-associated autoinflammatory disease

Na Wu  
Peking Union Medical College Hospital

Di Wu  
Peking Union Medical College Hospital

Junke Miao  
Peking Union Medical College Hospital

Mengzhu Zhao  
Peking Union Medical College Hospital

Yi Wang  
Peking Union Medical College Hospital

Weihong Yu  
Peking Union Medical College Hospital

Min Shen  \(\text{shenmpumch@163.com}\)  
Peking Union Medical College Hospital

Research Article

Keywords: autoinflammatory diseases, NLRP3 gene, NLRP3-associated autoinflammatory diseases

Posted Date: August 29th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1992350/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: NLRP3-associated autoinflammatory disease (NLRP3-AID) is a spectrum of autosomal dominant inherited diseases associated with NLRP3 gene mutations. Reports of Chinese NLRP3-AID cases are limited to date. In the present study, we aim to describe the phenotype and genotype of a cohort of Chinese adult NLRP3-AID patients.

Methods: This single-center study included sixteen adult patients diagnosed with NLRP3-AID at Department of Rheumatology, Peking Union Medical College Hospital between July 2015 to September 2021. Whole-exome sequencing using next-generation sequencing was performed in each patient. Clinical data and mutational information were compared with a European cohort.

Results: The median age of disease onset was 16 (0-46) years old, and adult-onset was observed in 4 patients (25%). The median time of diagnosis delay was 20 (0–39) years. Five patients (31.3%) had family history of similar symptoms. The most common clinical manifestations were recurrent fever (93.8%), arthralgia/arthritis (81.3%), skin rash (75%), myalgia (62.5%), and central nervous system manifestations (50%). Heterozygous NLRP3 variants detected in these patients were p.T348M (n=4, 25%), Q703K, V70M, K131R, M116I, P38S, V442I, D303G, G328E, A439V, K829T, L632F and V198M (n=1, separately). All the variants were missense mutations.

Conclusions: We reported the largest case series of Chinese adult NLRP3-AID patients. The distinct symptoms of NLRP3-AID patients suggest the heterogeneity of disease. P38S, M116I, K131R, V442I and K829T were identified as novel NLRP3 variants. These data expand the clinical phenotypic and genotypic profiles of NLRP3-AID.

Background

Systemic autoinflammatory diseases (SAIDs) are a group of genetically heterogeneous disorders characterized by febrile attacks and unspecific systemic inflammation resulting from dysregulation of the innate immune system [1–2]. Since the initial research in the late 1990s, an increasing number of SAIDs have been classified. At present, more than 40 monogenic SAIDs were defined [3]. NLRP3-associated autoinflammatory disease (NLRP3-AID, OMIM 606416), previously called cryopyrin-associated periodic syndrome (CAPS), is a continuum of monogenetic autoinflammatory diseases caused by gain-of-function NLRP3 mutations [4]. NLRP3 mutations result in the aberrant activation of NLRP3 inflammasome, which is a crucial immune sensor in the innate immunity, triggering the maturation of pro-caspase-1 and the overproduction of proinflammatory cytokines interleukin-1β (IL-1β) and IL-18, consequently leading to systemic inflammation [5].

Hal Hoffman et al. reported the first NLRP3-AID case in 2001 [6]. NLRP3-AID encompasses three conditions of increasing severity: familial cold-induced autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile neurological cutaneous articular syndrome (CINCA). FCAS is characterized by cold-induced fever, arthralgia and urticaria-like rashes. The main clinical features of MWS are recurrent febrile attacks, urticaria-like rash, arthritis, conjunctivitis, sensorineural hearing loss and amyloidosis. CINCA is the most severe phenotype of NLRP3-AID, manifesting as childhood onset of fever, rash, conjunctivitis, central nervous system (CNS) inflammation and osteoarthropathy. To prevent long-term organ damages, the early diagnosis and swift initiation of effective treatments are mandatory [7]. Anti-IL-1 biologics (anakinra, canakinumab and rilonacept) are recommended to treat NLRP3-AID. They have sustained efficacy in controlling symptoms and improving quality of life [8].

Due to the rarity, the real-world awareness and practice of NLRP3-AID in China remain limited. Through literature review, we searched only one publication describing a cohort of 15 Chinese pediatric patients with NLRP3-AID [9], and some case reports from our team [10–12]. In this study, we aimed to summarize the clinical and genetic characteristics of Chinese adult NLRP3-AID patients.

Methods

This single-center study included sixteen adult patients diagnosed with NLRP3-AID at Department of Rheumatology, Peking Union Medical College Hospital from April 2015 to September 2021. Demographic information and detailed clinical records including white blood cell (WBC) counts, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum tumor necrosis factor (TNF)-α and interleukin 6 (IL-6) level were carefully documented and analyzed. Whole-exome sequencing using next-generation sequencing was performed in each patient. Clinical data and mutation information were compared with a European cohort. This study was approved by the Institutional Review Board of Peking Union Medical College Hospital. All the participants have given their informed consents.

Results

Clinical characteristics

The demographic information and clinical features of adult NLRP3-AID patients in this study were summarized in Table 1. Sixteen patients were all diagnosed as MWS. Of them, the gender ratio of male to female was 9.7. All patients were of Chinese Han ethnicity. The median age of disease onset was 16 (0–46) years old, and adult-onset was observed in 4 patients (25%). The median time of diagnosis delay was 20 (0–39) years. Five patients (31.3%) had family history of similar symptoms.
Recurrent fever and skin rashes were the most frequent initial symptoms. Among these 16 patients, 4 (25.0%) reported cold-induced disease attacks. Attacks usually lasted several days to weeks (81.2%). The intervals between episodes ranged from several weeks to several months. The most common clinical manifestations were recurrent fever (93.8%), arthralgia/arthritis (81.3%), skin rash (75%), myalgia (62.5%), and CNS manifestations (50.0%) (Fig. 1). The main manifestations of skin involvement were urticaria-like rashes (n = 8, 50.0%), appearing on the trunk, extremities, or face, and erythema nodosa (n = 4, 25%).

Clinical and genetic characteristics of Chinese adult patients with NLRP3-AID

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Age at onset (years old)</td>
<td>46</td>
<td>2</td>
<td>2</td>
<td>46</td>
<td>7</td>
<td>37</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Age at diagnosis (years old)</td>
<td>47</td>
<td>23</td>
<td>31</td>
<td>46</td>
<td>27</td>
<td>42</td>
<td>21</td>
<td>20</td>
<td>22</td>
<td>39</td>
<td>32</td>
<td>45</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>Diagnostic delay (years)</td>
<td>1</td>
<td>21</td>
<td>29</td>
<td>0</td>
<td>20</td>
<td>5</td>
<td>20</td>
<td>12</td>
<td>37</td>
<td>26</td>
<td>39</td>
<td>18</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NLRP3 variants</td>
<td>Q703K</td>
<td>V70M</td>
<td>T348M</td>
<td>K131R</td>
<td>M116I</td>
<td>P38S</td>
<td>V442I</td>
<td>D303G</td>
<td>T348M</td>
<td>T348M</td>
<td>G328E</td>
<td>A439V</td>
<td>K829T</td>
<td>L63:</td>
</tr>
<tr>
<td>Clinical features</td>
<td>Fever</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rash</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Abdominal pain/diarrhea</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Myalgia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Oral ulcers</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Lymphadenopathy</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ophthalmologic involvement</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hearing loss</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CNS involvement</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Treatment</td>
<td>DMARDs</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Glucocorticoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TNF-α inhibitors</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

M: male; F: Female; CNS: central nervous system; DMARDs: disease-modifying antirheumatic drugs.

Acute phase reactants elevated during flares in all patients (CRP 80.3 ± 51.0 mg/L and ESR 52 ± 28 mm/h), and normalized during intervals in 15 patients (93.7%), while decreased in the other one patient. The baseline levels of white blood cell counts [12.1×10⁹/L, (4.1–50.0)×10⁹/L], IL-6 [10.7 pg/ml, (3.2–91.4) pg/ml], and TNF-α [13.4 pg/ml, (6.5–299.0) pg/ml] were high. Serological autoantibodies of all patients, including anti-nuclear autoantibodies, anti-extractable nuclear antibodies, anti-neutrophil cytoplasmic antibodies, anti-phospholipid antibodies, rheumatoid factor, anti-cyclic citrullinated peptide antibodies, were all negative, except one 38-year-old woman who presented with positive antinuclear antibody (1:160, nucleolar pattern), positive anti-β2GP1 antibody of IgM (54–68 AU/ml, cut-off < 20 AU/ml) and anticoagulant (1.24–1.29, cut-off ≤ 1.2). The mean score of subjective assessment from physicians and patients were relatively high at first visits (PGA 6.5 ± 2.3, VAS 6.1 ± 2.2).

Glucocorticoids were given to 7 patients with partial symptom relief in 6 patients (85.7%). Due to the unavailability of IL-1 inhibitors in China, TNF-α inhibitors (etanercept and adalimumab) were used in 7 patients with improvement of fever, rashes and arthritis (100%). However, severe complications such as brain
atrophy and hearing loss were not obviously relieved.

**Genetic Characteristics**

Thirteen heterozygous *NLRP3* (NM_001243133.1) missense variants detected in these 16 patients were T348M (n = 4, 25%), Q703K, V70M, K131R, M116I, P38S, V442I, D303G, G328E, A439V, K829T, L632F and V198M (n = 1, 6.3%, separately). According to the Infevers database (https://fmf.igh.cnrs.fr/ISSAID/infevers/), T348M, Q703K, D303G, A439V and L632F were described as pathogenic. V70M and V198M were uncertain significant low-penetrance variants, which had been found in some unaffected family members and were present at low frequencies in normal populations [13]. Besides, G328E mutation had been reported by Japanese researchers but not included in the Infevers database yet [14]. K131R, M116I, P38S, V442I and K829T were identified as novel variants.

**Genotype-phenotype Relationship**

In four patients with T348M mutation, the median age of disease onset was 2 (0–10) years old. All of the four patients experienced severe ocular symptoms (100%), referring to papilledema and optic neuritis. In addition, chronic aseptic meningitis and sensorineural hearing loss were found in 3 patients (75%), respectively. Whereas in those with other variants (n = 12), the incidences of abovementioned complications were 6.3%, 33.3% and 33.3% respectively, proving the relationship between the severity of *NLRP3*-AID and *NLRP3* T348M mutation.

Interestingly, two patients in our cohort had symptoms of Behçet's syndrome. *Patient 12* was a 45-year-old woman presenting with recurrent fever, cold-induced urticaria, oral and genital ulcers, conjunctivitis, uveitis, headache and hearing loss. Whole-exome sequencing identified a heterozygous *NLRP3* A439V mutation in the proband and her daughter. This is the firstly recorded Chinese pedigree of *NLRP3*-AID presented with Behçet's syndrome [12]. *Patient 14* was a 33-year-old man who suffered from recurrent oral and genital ulcers, skin erythema, arthritis and sensorineural hearing loss. Genetic testing identified a heterozygous L632F mutation in the *NLRP3* gene. Based on these cases, there may be a potential association between A439V/ L632F mutation and the coexistence of *NLRP3*-AID and Behçet's syndrome. D303N were reportedly associated with severe phenotypes in *NLRP3*-AID [15]. Here, we observed serious sequela such as bilateral sensorineural hearing loss and vision impairment in patient 8 bearing D303G variant. In contrast, patient 11 carrying G328E variant had relatively mild clinical manifestations without obvious organ damage.

**Comparison Between Chinese Cohort And European Cohort**

To date, the largest cohort of *NLRP3*-AID was a study from Eurofever Registry [16], which enrolled 136 patients, including 128 Caucasian and 8 Asian. In the light of this research, R260W was the most frequent mutation (26.5%, n = 36). Other common variants included T348M (n = 20), A349V (n = 14), V198M (n = 13), E311K (n = 9), Q703K (n = 9), and D303N (n = 5). 19.9% (n = 27) patients carried other rare variants and 2.0% (n = 3) did not carry *NLRP3* variants (Fig. 2).

The gender ratio of male to female in Chinese and European series were similar (9:7 vs. 1:1). The median age at disease onset of our patients was older than the European cohort (16 vs. 0.8 years old), and the average time of diagnosis delay was longer (20 vs. 15 years). In terms of symptoms, recurrent fever (93.8 vs. 84.0%), myalgia (62.5 vs. 44%), and hearing impairment (62.5 vs. 42%) were more commonly seen in our case series than the European cohort. While the incidences of rashes (75 vs. 97%) and eye involvement (43.7 vs. 71%) were relatively lower. The frequencies of CNS and joint involvement of the two cohorts were alike. Four patients (25%) in our cohort reported abdominal pain or diarrhea, which was seldom recorded in the European patients. About 4% patients (5/136) from the European Registry cohort developed amyloidosis, yet no amyloidosis was seen in our patients (Table 2).
with these findings, in Chinese patients, T348M is related to serious CNS manifestations including sensorineural hearing loss, chronic aseptic meningitis, course sensorineural hearing loss and neurological phenotype. Based on previous studies, T348M was the most common mutation in Chinese patients. In our cohort, characteristic symptoms of MWS were recurrent fever, urticaria-like rashes and late-onset organ involvement such as neurological symptoms, hearing and vision impairment. The distinct symptoms in each individual suggested the heterogeneity of disease. The disease severity and complications may vary with the mutation type and treatment process.

In this cohort, 75% MWS patients were child-onset while 25% were adult-onset, implying NLRP3-AID should be considered in both pediatric and adult patients. Most patients had their initial visits at departments of dermatology or infection diseases for the unexplained fever and rashes. A small proportion of patients initially attended departments of ophthalmology or otology for the vision or hearing loss. Clinically, misdiagnosis and delayed diagnosis are common to see due to the rarity and phenotypic variability of this autoinflammatory disorder. Awareness of NLRP3-AID among patients presenting with unknown reason of recurrent fever, rash and CNS manifestations, as well as accurate diagnosis and appropriate treatments would avoid organ damage and prevent disease progression, especially the irreversible sequelae such as chronic aseptic meningitis, hydranencephaly, brain atrophy, optic papiledema, optic neuritis, vision impairment and hearing loss.

Compared with the European cohort, fever, myalgia and hearing impairment were more common, yet rash and eye involvement were less common in Chinese patients. In our cohort, eight patients (50%) presented with the intermittent urticaria-like rashes on the trunk, limbs or face. Four patients had erythema nodosa on the lower limbs, which was seldom seen in the European cohort or other research. Notably, 25% patients in our cohort had abdominal pain/ diarrhea, however, it was not mentioned in European patients. In untreated NLRP3-AID patients, amyloidosis represents a serious long-term complication, and according to previous research, up to a quarter of MWS patients may develop amyloidosis. Meanwhile, in the European Registry cohort, about 4% patients developed amyloidosis. Consistently, in Chinese pediatric patients with NLRP3-AID, only one patient (6.7%) got amyloidosis, whereas none of our adult patients had amyloidosis. We suggest that ethnic difference and different genotypes may partially account for the diversity of clinical phenotype.

To date, a total of 254 NLRP3 variants are listed in the Infevers database. Most variants leading to NLRP3-AID are found in exon 3 of the NLRP3 gene, encoding the NACHT subdomains and NAD domain. Few non-exon 3 variants have been reported before. In the present study, thirteen NLRP3 variants were identified, and five of them (P38S, M116I, K131R, V442I and K829T) were unreported. Based on patients’ typical clinical manifestations and the results of in silico analysis, we preliminarily identify them as novel variants. Intriguingly, four of the novel variants found in our cohort are non-exon 3 variants: K829T in exon 6, K131R, M116I in exon 2, and P38S in exon 1. This illustrates non-exon 3 variants may also lead to clinical symptoms and should be considered as a unique feature of Chinese NLRP3-AID patients. Further functional experiments are required to confirm the causative roles of these variants.

Table 2
Comparison of clinical and genetic features between Chinese and European cohort

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>China (n = 16)</th>
<th>Europe (n = 136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: Female</td>
<td>9.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Age at onset (years old)</td>
<td>16</td>
<td>0.8</td>
</tr>
<tr>
<td>Diagnostic delay (years)</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Family history of NLRP3-AID (%)</td>
<td>31.3</td>
<td>55.8</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>93.8</td>
<td>84</td>
</tr>
<tr>
<td>Rash (%)</td>
<td>75</td>
<td>97</td>
</tr>
<tr>
<td>Arthralgia/Arthritis (%)</td>
<td>81.3</td>
<td>78.7</td>
</tr>
<tr>
<td>Myalgia (%)</td>
<td>62.5</td>
<td>44</td>
</tr>
<tr>
<td>Abdominal pain/ diarrhea (%)</td>
<td>25</td>
<td>ND</td>
</tr>
<tr>
<td>Oral ulcers (%)</td>
<td>37.5</td>
<td>ND</td>
</tr>
<tr>
<td>Hearing impairment (%)</td>
<td>62.5</td>
<td>42</td>
</tr>
<tr>
<td>Eye involvement (%)</td>
<td>43.7</td>
<td>71</td>
</tr>
<tr>
<td>CNS involvement (%)</td>
<td>43.7</td>
<td>40</td>
</tr>
<tr>
<td>Amyloidosis (%)</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Heterozygous germline mutation (%)</td>
<td>100</td>
<td>97.8</td>
</tr>
<tr>
<td>Most frequent NLRP3 variant</td>
<td>T348M</td>
<td>R260W</td>
</tr>
</tbody>
</table>

Discussion

NLRP3-AID is a continuum of autosomal dominant inherited autoinflammatory disorders. This study confirms the clinical features of patients with NLRP3-AID described in previous reports. Sixteen Chinese Han patients were all diagnosed as MWS, the moderate type of NLRP3-AID. The mean time of diagnosis delay was 20 years, implying the lack of awareness of NLRP3-AID among Chinese population. In our study, characteristic symptoms of MWS were recurrent fever, urticaria-like rashes and late-onset organ involvement such as neurological symptoms, hearing and vision impairment. The distinct symptoms in each individual suggested the heterogeneity of disease. The disease severity and complications may vary with the mutation type and treatment process.

In this cohort, 75% MWS patients were child-onset while 25% were adult-onset, implying NLRP3-AID should be considered in both pediatric and adult patients. Most patients had their initial visits at departments of dermatology or infection diseases for the unexplained fever and rashes. A small proportion of patients initially attended departments of ophthalmology or otology for the vision or hearing loss. Clinically, misdiagnosis and delayed diagnosis are common to see due to the rarity and phenotypic variability of this autoinflammatory disorder. Awareness of NLRP3-AID among patients presenting with unknown reason of recurrent fever, rash and CNS manifestations, as well as accurate diagnosis and appropriate treatments would avoid organ damage and prevent disease progression, especially the irreversible sequelae such as chronic aseptic meningitis, hydranencephaly, brain atrophy, optic papiledema, optic neuritis, vision impairment and hearing loss.

Compared with the European cohort, fever, myalgia and hearing impairment were more common, yet rash and eye involvement were less common in Chinese patients. In our cohort, eight patients (50%) presented with the intermittent urticaria-like rashes on the trunk, limbs or face. Four patients had erythema nodosa on the lower limbs, which was seldom seen in the Europe cohort or other research. Notably, 25% patients in our cohort had abdominal pain/ diarrhea, however, it was not mentioned in European patients. In untreated NLRP3-AID patients, amyloidosis represents a serious long-term complication, and according to previous research, up to a quarter of MWS patients may develop amyloidosis. Meanwhile, in the European Registry cohort, about 4% patients developed amyloidosis. Consistently, in Chinese pediatric patients with NLRP3-AID, only one patient (6.7%) got amyloidosis, whereas none of our adult patients had amyloidosis. We suggest that ethnic difference and different genotypes may partially account for the diversity of clinical phenotype.

To date, a total of 254 NLRP3 variants are listed in the Infevers database. Most variants leading to NLRP3-AID are found in exon 3 of the NLRP3 gene, encoding the NACHT subdomains and NAD domain. Few non-exon 3 variants have been reported before. In the present study, thirteen NLRP3 variants were identified, and five of them (P38S, M116I, K131R, V442I and K829T) were unreported. Based on patients’ typical clinical manifestations and the results of in silico analysis, we preliminarily identify them as novel variants. Intriguingly, four of the novel variants found in our cohort are non-exon 3 variants: K829T in exon 6, K131R, M116I in exon 2, and P38S in exon 1. This illustrates non-exon 3 variants may also lead to clinical symptoms and should be considered as a unique feature of Chinese NLRP3-AID patients. Further functional experiments are required to confirm the causative roles of these variants.

Based on previous studies, T348M was the most common mutation in NLRP3-AID patient. This variant is associated with early disease onset, chronic course sensorineural hearing loss and neurological phenotype. Early disease onset was also predictive of a more severe outcome. Consistent with these findings, in Chinese patients, T348M is related to serious CNS manifestations including sensorineural hearing loss, chronic aseptic meningitis,
hydrocephalus and brain atrophy [29]. In addition, we have also reported that T348M was associated with a variety of ocular manifestations, such as conjunctivitis, uveitis, papilledema, optic neuritis and optic atrophy, which led to impaired vision in some cases [30]. In the present research, up to a quarter of Chinese patients carried T348M variant, and they presented with earlier disease onset and more severe sequelae when compared with patients carrying other mutations. These data verified the positive correlation between T348M variant and the severity of clinical genotype.

Autoinflammatory diseases used to be defined in opposition to autoimmune diseases due to the lack of involvement of the adaptive immune system and circulating autoantibodies [31]. Nevertheless, further investigations in this field suggest that most rheumatic and musculoskeletal diseases can be placed along a spectrum of disorders, with autoinflammatory diseases and autoimmune diseases representing the two ends of this spectrum, setting no obvious boundaries [32]. In line with this growing viewpoint, we observed a rare patient with positive ANA and antiphospholipid antibodies, as we have reported before [11]. Moreover, in this study, we also found two NLRP3-AID patients complicated with Behçet's Syndrome, and one of them has been reported by our team [12]. These cases provide clinical evidence of overlapping features between autoinflammatory diseases and autoimmune diseases, in addition, between monogenic and polygenic autoinflammatory diseases.

Conclusions

Herein, we described the largest case series of Chinese adult NLRP3-AID patients. Due to the rarity and complexity of NLRP3-AID, diagnosis delay remains an unsolved problem, resulting in severe complications such as hearing and vision loss. We confirmed thirteen variants in the NLRP3 gene, among which, P38S, M116I, K131R, V442I and K829T were identified as novel variants. We hope these data would expand the phenotypic and genotypic profile of NLRP3-AID.

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Review Board of Peking Union Medical College Hospital. All the participants have given their informed consents.

Consent for publication: All the patients gave their consent for publication.

Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

Funding: This work was supported by the Natural Science Foundation of Beijing (Grant No.7192170); the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (CIFMS) (Grant No. 2017-i2M-3-001); and the National Key Research and Development Program of China (Grant No.2016YFC0901500; 2016YFC0901501).

Authors’ contributions: MS conceived the study and supervised the work. DW, YW, WY and MS collected the clinical data. NW, JM and MZ analyzed and interpreted the data. NW and MS prepared and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements: We would like to acknowledge all the patients for their consents to participate in the study.

References


**Figures**
Clinical manifestations of NLRP3-AID patients. A: Conjunctivitis of patient 9; B: Color fundus photo showed optic papilledema in patient 10; C: Urticaria-like rashes on the trunk of patient 5; D: Computed tomography examination of the head showed brain atrophy in patient 3.

Figure 2

Distribution of NLRP3 gene mutations in European and Chinese cohorts