NLRP12-associated autoinflammatory disease: much more than the FCAS phenotype

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Abstract

Objective

NLRP12-associated autoinflammatory disease (NLRP12-AID) is a rarely seen periodic fever syndrome also known as familial cold autoinflammatory syndrome-2 (FCAS2), caused by autosomal dominant inherited mutations in the NLRP12 gene. We aimed to present our clinical experience constituting one of the largest paediatric NLRP12-AID cohorts.

Methods

The patients with preliminary diagnosis of systemic autoinflammatory disease (SAID) other than familial Mediterranean fever (FMF) and PFAPA syndrome were evaluated with the next-generation-sequence (NGS) genetic-panel analysis between January-2016 and January-2022. Among children carrying NLRP12-variant, patients with recurrent episodes of autoinflammatory disease manifestations were diagnosed with NLRP12-AID. Demographic, clinical and laboratory data, treatments and outcomes of patients were presented.

Results

Seventeen patients were diagnosed with NLRP12-AID. The mean age at diagnosis was 114.7±69.5 months. The most frequently seen clinical manifestations were respectively; fever (100%), arthritis/arthralgia (58.8%), rash (52.9%), abdominal pain (52.9%), diarrhoea (41.2%), myalgia/fatigue (53.2%) and, conjunctivitis (11.7%). Clinical manifestations were triggered by cold exposure in three patients (17.6%). Seven patients had pathogenic, one had likely pathogenic, seven had VUS, and two had novel heterozygous variants. The most common defined variant in the NLRP12 gene was R352C. Complete response was achieved in 5 patients and partial response was in 6 with colchicine treatment. Attacks were prevented with anti-IL-1 treatments in 6 patients unresponsive to colchicine.

Conclusion

In conclusion, the disease can cause effects on various tissues, especially the musculoskeletal and gastrointestinal systems, apart from FCAS symptoms. We think that a patient who can be defined as syndrome of undifferentiated recurrent fever should also be evaluated genetically in terms of NLRP12 previously.

Key words

periodic fever syndrome, systemic autoinflammatory disease, NLRP12, NLRP12-AID, FCAS2

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Introduction

The nucleotide-binding leucine-rich repeat-containing receptor 12 (NLRP12) is an intracellular protein which has been shown to work as both an inflammasome and a negative regulator in the inflammation process. (1-3). It plays a significant role in regulation of innate immunity besides adjusting inflammatory processes via activated with pathogen and damage-associated molecular patterns (4). The multifaceted role of the NLRP12 protein in regulation of immune response can be seen as anti-inflammatory through suppression of the NF-κB pathway and pro-inflammatory by increasing the secretion of interleukin (IL)-1β and IL-18 in response to specific antigens (2, 5)

NLRP12 associated periodic syndrome (NLRP12-AID) is a rarely seen autoinflammatory disease (AID) also known as familial cold autoinflammatory syndrome 2 (FCAS2, OMIM #611762), caused by autosomal dominant inherited mutations in the NLRP12 gene (OMIM *609648). Unfortunately, no classification or definition criteria have been reported so far. It was previously defined as FCAS2 due to disease-related episodes are show similarity with FCAS. Common clinical features of recurrent NLRP12-AID attacks have been described as fever, fatigue and musculoskeletal symptoms that are typically activated by cold exposure and start to appear in the childhood in the majority of patients. Skin manifestations can also be seen during attacks in about half of the patients (6). It has been also shown in different studies that NLRP12-AID can cause distinct clinical manifestations (abdominal pain/diarrhoea, hepatosplenomegaly, lymphadenopathy, aphthous stomatitis, headache, sensorineural hearing loss et.) and be together with different comorbid conditions. (3). The diagnosis of NLRP12-AID is considered in children with disease-causing variant in the NLRP12 gene and exhibit clinical findings consistent with autoinflammatory disease attacks. In recent years, the reporting of patients diagnosed as NLRP12-AID has increased as a result of the widespread use of next-generation sequencing analysis which are followed up with a pre-diagnosis of undifferentiated periodic fever syndrome (7, 8).

To the best of our knowledge, experience about NLRP12-AID in children seems to be limited to the case reports, in the literature review. In different studies, a total of approximately 80 NLRP12-AID cases, 33 of which were children, have been reported to date (3,9). Here, we aimed to present our clinical experience on this subject, constituting one of the largest paediatric NLRP12-AID patient cohort.

Material and methods

Patients

678 patients with a preliminary diagnosis of systemic autoinflammatory disease (SAID) other than Familial Mediterranean fever (FMF) and PFAPA syndrome were evaluated with the next-generation sequence (NGS) genetic panel analysis including 16 genes, between January 2016 and January 2022 at the Umraniye Training and Research Hospital Department of Paediatric Rheumatology. The data of these patients with a preliminary diagnosis of SAID were evaluated retrospectively and they were followed longitudinally. 23 of these patients carried pathogenic, likely pathogenic, variant of uncertain significance (VUS) or novel variants in at least one allele of the NLRP12 gene. The patients with a follow-up period of less than one year and/or diagnosed with any other aetiological cause (infectious, autoimmune, malignant diseases or other systemic autoinflammatory disease) were excluded (6 patients). The acute phase responses of the patients were recorded during the attack period; normal laboratory ranges were considered <5 mg/L for C-reactive protein (CRP) and <0.5 mg/dl for serum amyloid A (SAA). Demographic, clinical and laboratory data, treatments and outcomes of patients with a diagnosis of NLRP12-AID were presented.

The study protocol was reviewed and approved by the Ethics Committee of the University of Health Sciences, Umraniye Training and Research Hospital with the ethical principles laid down in the Declaration of Helsinki (approval no: B.10.1.TKH.4.34.H.GP.0.01/146). Written informed consent was obtained

from the subjects or their legally authorised representative.

Genetic analysis

Genomic DNA was extracted from peripheral blood samples as recommended by the manufacturer (Qiagen). The concentration and quality control (260/280 nm and 260/230 nm values) of the DNA samples were determined fluorometrically (Qubit-v3.0) and by UV-spectrophotometry. The library preparation for NGS was performed using Fever&Auto-Inflammatory Diseases Kit by Sophia-Genetics, a custom panel using a capture-based method. The gene panel consisted of 16 genes associated with periodic fever syndromes ((MEFV (NM_000243), MVK (NM_000431), NLRP3 (NM_001079821), NLRP12 (NM_144687), TNFRSF1A (NM_001065), TNFRSF11A (NM_003839), LPIN2 (NM_014646), PSTPIP1 (NM_003978), IL1RN (NM_000577), CECR1 (NM_017424), ELANE (NM_001972), CARD14 (NM_001257970), IL10RA (NM_001558), IL10RB (NM_000628), NOD2 (NM_022162), PSMB8 (NM_004159)). NextSeq500-(Illumina) was used as the sequencing platform. Sequence analysis covers coding regions of each gene, including all coding exons, ± 10 base pairs of adjacent intronic sequences, and each nucleotide is read at a depth of at least 50×. Any variants that fall outside these regions and exonic variants with a variant fraction of less than 10% were considered as false positives and not analysed. Variant fractions close to 50% were interpreted as heterozygous, while 100% variant fractions close to 100% were interpreted as homozygous. Deviations from these expected values were considered suspect as potential errors due to incorrect base calls or alignment and were verified by Sanger sequencing. With this analysis, copy number variations (CNV) were not examined. The DNA sequences were aligned to the NCBI Build37 (hg19) version of the human genome. Variant calling and data analysis were performed by the Sophia-DDM-V5.2 bioinformatics analysis programme. The interpretation of the variants was performed according to the 2015 ACMG standards and guidelines (15-19). To collect evidence reported in the ACMG guideline, we use a multistep process. First, we exclude common polymorphic variants with a minor allele frequency of more than 1%. Since there are not enough genome and exome databases for the Turkish population, 1000 genome projects, dbSNP, ExAC, and GnomAD data were used as the control population. In the second step, synonymous variants were filtered out. Then, the effects of the variants on protein function were investigated by using prediction programmes such as SIFT, Polyphen, MutationTaster, and GERP. Variants with a possible pathogenic effect were reevaluated by using databases including ClinVAR, HGMD, and PubMed as well as with the inheritance pattern, clinical findings, and allele frequencies by literature review, based on the patient. In light of acquired evidence, variants were classified into five categories (benign, likely benign, variant of unknown significance (VUS), pathogenic, likely pathogenic) according to the 2015 ACMG standards and guidelines (10-14).

Segregation analysis for potentially pathogenic mutations was performed by Sanger sequencing. Primer sequences and reaction conditions are available on request. Likely benign and benign variants were not reported.

Statistical analysis

Statistical package for the social sciences (SPSS) (v. 23.0, SPSS-Inc., Chicago, IL, USA) was used for statistical analysis. Categorical data are presented as numbers and percentages. Numerical data with symmetrical distribution are presented as mean ± standard deviation (SD) and with asymmetrical distribution are presented as the median and interquartile range (IQR). The normality of the distribution of numerical variables was assessed by the Shapiro-Wilk test.

Results

Demographical and clinical findings

Seventeen patients with recurrent episodes of autoinflammatory disease (together with high acute phase response) were diagnosed with NLRP12-AID, among children carrying NLRP12 variant. The F/M ratio was found as 11/6. The mean age of onset of the symptoms was 59.7±53 months, and the mean age at diagnosis was 114.7±69.5 months. The duration between disease onset to diagnosis was found 55±45.9 months. The mean follow-up time was found 24.8±13.4 months. The duration of the attack was also found 4.2±3.1 days. There was a family history of an autoinflammatory disease in 4 (23.5%) patients.

The frequency of clinical findings in attack period was found as follow respectively; fever (n/%: 17/100), arthritis/arthralgia (n/%: 10/58.8), rash (n/%: 9/52.9), abdominal pain (n/%: 9/52.9), diarrhoea (n/%: 7/41.2), myalgia/fatigue (n/%: 6/53.2), conjunctivitis (n/%: 2/11.7), oral aphthae (n/%: 1/5.8), erythema nodosum (n/%: 1/5.8), headache (n/%: 1/5.8) and pericarditis (n/%: 1/5.8). These clinical manifestations were triggered by cold exposure in 3 patients (17.6%). C-reactive protein (CRP) and serum amyloid A levels (SAA) were found elevated in all patients during the attack period (Table I).

The disease attacks almost completely regressed in 5 (29.4%) patients after regular colchicine treatment (complete remission). The frequency and severity of attacks decreased significantly after colchicine treatment in 6 patients (partial remission). Anti-IL 1 treatments were started in the other 6 patients who were completely unresponsive to colchicine. 4 patients (%23.5) achieved complete remission after canakinumab and 2 patients (%11.7) anakinra treatments.

Genetic data

In the NGS gene panel, seven patients had pathogenic, one had likely pathogenic, seven had VUS, and two had novel variants. Heterozygous variants were detected in all patients. All seven patients (no: 5, 6, 11, 13, 14, 15, 16) with the pathogenic variant had the same mutation (R352C). Patient 8 with likely pathogenic variant had T260M. From patients with VUS; R343W variant was defined in patients 7 and 9, G448A in patient 1, S117P in patient 3, L943F in patient 4, H304Y in patient

Table I. The frequency of clinical and laboratory manifestations of patients.

	n/%
Gender (M/F)	6:11/35:65
Age at disease onset (months)*	59.7 ± 53
Age at diagnosis (months)*	114.7 ± 69.5
Duration between disease onset to diagnosis (months)*	55 ± 45.9
Duration of attacks (days)*	4.2 ± 3.1
Follow-up (months)*	24.8 ± 13.4
Family history of SAID's	4/23.5
Clinical features	
Fever	17/100
Arthritis/arthralgia	10/58.8
Rash	9/52.9
Abdominal pain	9/52.9
Diarrhoea	7/41.2
Myalgia/Fatigue	6/35.2
Conjunctivitis	2/11.7
Lymphadenomegaly	2/11.7
Oral aphthae	1/5.8
Erythema nodosum	1/5.8
Tonsillitis	1/5.8
Headache	1/5.8
Pericarditis	1/5.8
Vomiting	1/5.8
Cold trigger	3/17.6
Increased APRs in attacks	17/100
Complete response to colchicine	5/29.4
Partial response to colchicine	6/35.2
Unresponsive to colchicine	6/35.2
Anakinra	2/11.7
Canakinumab	4/23.5

*(mean ± SD).
APR: acute phase reactant; SAID: systemic autoinflammatory disease.

12, and F402L in patient 17. Novel variants were determined in patients 2 and 10. Genetic data for the respective patients is listed in Table II.

Discussion

Here we present data from one of the largest childhood NLRP12-AID cohorts. In 2008 and 2011, Jeru *et al*. reported 5 cases with FCAS -like clinical manifestations together with mutations in the NLRP12 gene (15, 16). Since then, NLRP12-AID has also been defined as FCAS2 due to the increasing number of patient reports with cold trigger symptoms such as fever, urticaria, conjunctivitis, myalgia and arthralgia.

The clinical findings of the majority of our patients are similar to those of NLRP12-AID paediatric cases in the literature. It has been reported in the literature that the spectrum of clinical findings of NLRP12-AID may vary, such as arthritis-arthralgia (55%), diarrhoea-abdominal pain (48%), lymphadenopathy/splenomegaly (33%), headache (24%), oral ulcer, chest pain, dyspnoea, as well as FCAS symptoms. The age of diagnosis was between 2 months and 17 years and cold trigger was present in 30% of the patients (3, 9, 17, 18). Despite the limited paediatric reports on this topic, it is known that the duration of attacks can last up to 10 days (15). In the association of refractory arthritis and C3 glomerulopathy, there has also been described a case of NLRP12-AID with an atypical course (19). The most common clinical findings accompanying fever in our patients were musculoskeletal symptoms, gastrointestinal complaints, and urticarial rash, in similar to the literature. Our patient with recurrent pericarditis is the first NLRP12-AID case in the literature to the best of our knowledge. While the clinical findings were compatible with FCAS in our four patients, attacks were observed accompanied with varied clinical findings including arthritis, headache, diarrhoea, erythema nodosum and/or pericarditis and lasting 3 days or longer in the other patients. We observed attack periods extending up to 7 days in the patient with diarrhoea and up to 14 days in the patient with pericarditis and that were not compatible with the FCAS. Also, no triggering role of cold was observed in most of our patients. This may be mainly due to genotypic differences. The clinical findings may differ depending on environmental and epigenetic factors in patients from different geographies and ethnicities, even if they are genotypically the same. Variability in genetic load has been reported to be the main reason for phenotypic differences in FMF, another monogenic SAID. It has also been reported that the disease course may persist with varying clinical findings due to epigenetic variants, microbiome influence and additional environmental factors in patients who live in different countries with the same ethnicity and genotype (20). It is our belief that NLRP12-AID may have a diverse clinical presentation, and its clinical characteristics in the Middle East and Turkey may differ from those in Europe, as in FMF.

The variants in our patients, which we defined as NLRP12-AID, were evaluated together with the literature data. A study has demonstrated that the R252C variant leads to an increase in CASP1 processing, resulting in an increase in IL-1 beta secretion and a hyperinflammatory state (16). We detected this variant in seven of our patients. We found that patients with the R352C variant had fever, urticaria, arthralgia and myalgia symptoms in general, and gastrointestinal complaints such as diarrhoea and abdominal pain were accompanied in three of them. We observed that our patient with T260M, a likely pathogenic variant, had pericarditis attacks with an atypical presentation. In the literature, there is a reported case with this variant with SAID manifestations (21). In genetic databases; algorithms developed to predict the effect of missense changes on protein structure and function suggest that variants of S117P, L943F and R343W are likely to be disruptive. Although there are no cases reported in the literature; we diagnosed four of our patients with these variants of NLRP12-AID due to the recurrent symptoms associated with SAID. There are articles in the literature reporting that G448A,

Table II. The clinical and genetic characteristics of patients.

Patient Number	NLRP12 variant	Type of variant	Clinical features in episodes	Treatment	Response
1	c.1343G>C (p.Gly448Ala)	Heterozygous, VUS	Fever, abdominal pain, chest pain, myalgia, arthralgia, and urticarial rash	Colchicine	Complete remission
2	c.377dupA	Heterozygous, Novel	Fever, abdominal pain	Colchicine	Partial remission
3	c.349T>C (p.Ser117Pro)	Heterozygous, VUS	Fever, abdominal pain, diarrhoea, arthralgia and oral aphthous	Colchicine	Complete remission
4	c.3000G>C (p.Leu1000Phe)	Heterozygous, VUS	Fever and urticarial rash	Colchicine	Partial remission
5	c.1054C>T (p.Arg352Cys)	Heterozygous, Pathogenic	Fever, abdominal pain, myalgia, arthralgia, and urticarial rash	Colchicine	Partial remission
6	c.1054C>T (p.Arg352Cys)	Heterozygous, Pathogenic	Fever, myalgia, arthralgia, conjunctivitis and urticarial rash	Canakinumab	Complete remission
7	c.1027C>T (p.Arg343Trp)	Heterozygous, VUS	Fever, myalgia, headache, abdominal pain, vomiting and urticarial rash	Colchicine	Complete remission
8	c.779C>T (p.Thr260Met)	Heterozygous, Likely pathogenic	Fever, conjunctivitis, diarrhoea and recurrent pericarditis	Canakinumab	Complete remission
9	c.1027C>T (p.Arg343Trp)	Heterozygous, VUS	Fever, diarrhoea and tonsillitis	Colchicine	Partial remission
10	c.848T>A	Heterozygous, Novel	Fever and irritability	Colchicine	Complete remission
11	c.1054C>T (p.Arg352Cys)	Heterozygous, Pathogenic	Fever, abdominal pain, diarrhoea, myalgia, arthralgia and urticarial rash	Colchicine	Partial remission
12	c.910C>T (p.His304Tyr)	Heterozygous, VUS	Fever, abdominal pain, diarrhoea, arthralgia and arthritis	Colchicine	Partial remission
13	c.1054C>T (p.Arg352Cys)	Heterozygous, Pathogenic	Fever, abdominal pain and arthralgia	Colchicine	Complete remission
14	c.1054C>T (p.Arg352Cys)	Heterozygous, Pathogenic	Fever, diarrhoea and urticarial rash	Anakinra	Complete remission
15	c.1054C>T (p.Arg352Cys)	Heterozygous, Pathogenic	Fever, arthralgia and urticarial rash	Canakinumab	Complete remission
16	c.1054C>T (p.Arg352Cys)	Heterozygous, Pathogenic	Fever, abdominal pain, diarrhoea, arthralgia and urticarial rash	Canakinumab	Complete remission
17	c.1206C>G (p.Phe402Leu)	Heterozygous, VUS	Fever, myalgia, arthralgia, erythema nodosum and urticarial rash	Anakinra	Complete remission

VUS: variant of unknown significance.

F402L and H304Y variants may present with mild form FCAS-like symptoms (17, 22). NLRP12-AID was also diagnosed in three of our patients with these VUS variants, with recurring episodes of fever, urticaria-erythema nodosum, and musculoskeletal manifestations. Novel variants were determined in the remaining our two patients.

The genetic inheritance of autoinflammatory disorders can vary depending on the specific disease, and there are still some controversial points. It has been shown in the literature that low-penetrance variants can cause clinical manifestations in some SAIDs. Studies have shown that these patients may display milder disease symptoms that are not associated with a chronic course (23-25). It was shown that the levels of secreted IL-1 beta, IL-18 and caspase-1 were higher in patients with a confirmed genetic variant than in patients with the low-penetrance, as a result of *in vitro* inflammasome activation (25). These studies also suggest that interfamilial clinical heterogeneity may be more varied in patients carrying low-penetrance variants. 13 of the 17 patients we diagnosed in our study did not have a family history of autoinflammatory disease. It may be due to the presence of de-novo variants or genetically low-penetrance in these patients, but we cannot accurately determine this due to the lack of familial segregation analysis. We aim to obtain more precise information about genetic inheritance and phenotype-genotype comparison of our patients, with family segregation analysis and functional studies that we plan to conduct in the future.

NLRP12-AID is known to be an NF-κB-mediated autoinflammatory disease (26). It is also known that NLRP12 protein suppresses proliferation and inflammation of fibroblast-like synoviocytes via NF-κB and MAPK signalling pathways (27). Besides, dysfunctions in the NLRP12 protein result in hyperactivation of non-canonical NF-κB and the overexpression of chemokines CXCL12 and CXCL13. It is known that these chemokines have a role in the development of inflammatory bowel disease and colonic inflammation (28, 29). When we evaluate the reports in the literature and our own cases, it can be seen that musculoskeletal and gastrointestinal complaints accompany a significant proportion of patients. Arthralgia was accompanied in 58% of our patients, abdominal pain in 52% and diar-

rhoea in 41%. In this respect, suspected AID patients with gastrointestinal and musculoskeletal complaints may need to be evaluated also for NLRP12-AID.

It was thought that the variants of uncertain significance that have been in our patients may be causal for NLRP12-AD, when our patients autoinflammatory disease compatible clinical and laboratory manifestations and the presence of reported similar patients with the same variant in the literature were evaluated together. As a result, these patients were defined as NLRP12-AID and their treatment were started and the patients benefited partially or completely. The classification of these variants is expected to be done in the future by demonstrating the potential protein changes they cause through functional analysis.

These patient groups, previously known as undefined SAID or undifferentiated SAID, have recently been redefined as syndrome of undifferentiated recurrent fever (SURF). It is recommended to demonstrate that patients do not have one of the common monogenic SAIDs by analysing MEFV, MVK, TNFRSF1A and NLRP3 genes before SURF diagnosis (30). NLRP12-AID should be considered in the differential diagnosis in patients with clinical manifestations of autoinflammatory diseases who do not show typical signs for any of the monogenic SAIDs, or in patients who have typical findings of any monogenic SAID but cannot be genetically identified.

Our study has two major limitations. The first one is that the functional studies to show disease-causing effects on associated variants have not been performed, and the second is that familial segregation analysis were not performed in the families of the patients. Due to financial constraints, these studies have not been completed and will be carried out in the future. In terms of its effects on the disease, as mentioned, predictions were made supporting clinical, laboratory and genetic findings with literature data.

Conclusion

The present study presents one of the largest paediatric NLRP12-AID cohorts. The R352C variant in the NLRP12 gene was found to be the most frequent mutation in our patients. When we take into account all aspects, we can observe that the disease course doesn't always present isolated FCAS symptoms, and it can begin without a cold trigger. The disease can affect various tissues, particularly the musculoskeletal and gastrointestinal systems, in addition to FCAS symptoms. We think that a patient who can be defined as SURF should also be genetically evaluated in terms of NLRP12 previously.

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