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Original Article

Evaluation of common NLRP3 Q703K variant in pediatric patients with autoinflammatory disease: CAPS and PFAPA

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ABSTRACT

Background. Gain-of-function mutations of the NLR family pyrin domain containing 3 (*NLRP3*) gene have been implicated in autoinflammatory diseases. The *NLRP3* Q703K variant is a common variant associated with Cryopyrin-associated periodic syndromes (CAPS) and periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome. However, the genotype-phenotype correlation between *NLRP3* Q703K variant, CAPS and PFAPA is unclear. In this study, we aimed to investigate the frequency of the *NLRP3* Q703K variant in patients with and without autoinflammatory disease and characterize the phenotype in only Q703K variant positive patients.

Methods. A retrospective analysis of 639 patients with autoinflammatory symptoms was conducted. Patients underwent next-generation sequencing (NGS) panel analysis of 16 genes, including *NLRP3*. For the 68 patients carrying the only Q703K variant, their clinical and demographic information was evaluated. Genetic data from 1461 patients without autoinflammatory symptoms were used as the control group.

Results. Of our 639 autoinflammatory symptomatic patients, the Q703K mutation was detected in 68 (5.3% allele frequency). Heterozygous mutations were detected in 141 patients without autoinflammatory symptoms (4.8% allele frequency, p=0.4887). Of the patients with variant in Q703K, 10 patients were diagnosed with CAPS , 7 patients were diagnosed with PFAPA and the remaining 39 were diagnosed with undefined systemic autoinflammatory disease (uSAID)

Conclusions. The Q703K variant, which is seen with similar frequency in the control and autoinflammatory groups, is also of higher prevalence in patients with mild CAPS symptoms and PFAPA syndrome. This variant, together with other undetected genetic variants or epigenetic modifications, may be responsible for the corresponding phenotype. As such, it is essential for clinicians to evaluate their patients using both genetic and clinical evaluations.

Key words: cryopyrin-associated periodic syndromes; periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis; NLRP3 gene; Q703K variant.

Systemic autoinflammatory diseases (SAIDs) are clinically defined by the recurrence of multisystemic inflammatory attacks without infection or autoantibody formation.[1] The term undefined systemic autoinflammatory diseases (uSAIDs) is used with increasing frequency in patients with a deficient phenotype, although there is no defined diagnostic criterion.[2,3]

Cryopyrin-associated periodic syndromes (CAPS) includes 3 clinically overlapping entities, namely, from familial cold-induced autoinflammatory syndrome 1 (FCAS, OMIM #120100), Muckle-Wells syndrome (MWS, OMIM #191900) and chronic infantile neurological, cutaneous, and articular syndrome (CINCA, OMIM #607115) with a broad clinical spectrum of severity. Patients

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with FCAS have a mildest phenotype.[1,4-6] CAPS are caused by gain-of-function mutations in the NLR family pyrin domain containing 3 (*NLRP3)* gene, which is a key component of the interleukin-1 (IL-1) inflammasome.[7] Recurrent episodes of fever, myalgia, abdominal pain, arthralgia, cutaneous inflammation, ocular and central nervous system involvement are common symptoms in cases with pathogenic mutations.[8-10] One low-penetrance variant in particular, Q703K (rs35829419, c 2107C>A, p.Gln703Lys) (also known in the literature as Q705K), is seen with a similar frequency in both pathogenic patients and healthy individuals, calling its functional significance into question.[11-13] Due to Q703K noted as high frequency in the general population (5-11%), some studies have accepted it as a clinically unremarkable polymorphism.[14,15]

Other studies, however, report patients carrying the Q703K variant as pathogenic, given that they have notably high levels of IL-1β.[13,16] This hypothesis was supported by the detection of the Q703K mutation in 7 patients with CAPS-like symptoms.[17]

Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome was first described in 1987 and is characterized by fever, oral aphthosis pharyngitis and cervical lymphadenopathy. Though its symptoms are well defined, its etiology is not as clearly understood.[18,19] When screening for PFAPA, detection of positive family history in pedigree suggests a similar genetic background.[20] *NLRP3* variants have been detected in approximately 20% of patients with PFAPA syndrome, and IL-1β monocyte production has been shown to be irregular, suggesting that inflammatory genes may be involved in this autoinflammatory syndrome.[7] The Q703K variant appears common in healthy populations and its pathogenic significance is unclear. It has been found to be associated with PFAPA and CAPS syndrome.[21]

In this study, we aimed to investigate the frequency of the Q703K variant in patients with autoinflammatory disease and in the control group without autoinflammatory symptoms. Furthermore, we aimed to characterize the phenotype in only Q703K variant positive patients.

Material and Methods

Patient selection

A retrospective analysis was carried out by surveying pediatric rheumatology and pediatric genetic patients' data between the years of 2016 and 2020 at the University of Health Sciences Ümraniye Education and Research Hospital, İstanbul, Türkiye. This study was approved by the local Ethics Committee of the same hospital (Approval Number: B.10.1.TKH.4.34.H.GP.0.01/07/26.01.2023). Consent was obtained from the patients' legal guardians according to the Declaration of Helsinki. The 639 Turkish origin patients with autoinflammatory symptoms (including but not limited to recurrent and periodic fever, mouth ulcer, rash, abdominal pain, and arthralgia) and no history of familial mediterranean fever (FMF) who applied to the pediatric rheumatology clinic between 2016 and 2020 underwent a next-generation sequencing (NGS) autoinflammatory panel containing 16 genes. Of these patients, 68 had heterozygous NLRP3 Q703K variant. After a 1-year follow-up, 56 with complete data were evaluated. The preliminary diagnosis was made on The Eurofever clinical diagnostic/classification criteria- Feredici score and Modified Marshall's diagnostic criteria for SAIDs and PFAPA syndrome.[22,23] Patients who were excluded for any of monogenic SAID or PFAPA syndrome were classified as uSAIDs. The final diagnosis of the patients was made using the New Eurofever/PRINTO classification and Modified Marshall's diagnostic criteria, with the results of the autoinflammatory panel and clinical symptom and attack followed-up for at least one year.[23,24] Clinical, demographic and laboratory information were obtained from the information recorded by pediatric rheumatology for all patients whose genetic

panel testing was only positive for the Q703K variant. None of these patients had heterozygous or homozygous pathogenic/likely pathogenic FMF variants. The cutoff values determined by our laboratory were 0.5 mg/dl for C-reactive protein (CRP) and 20 mm/h for the erythrocyte sedimentation rate (ESR).

Data from 1461 patients who did not have any history of autoinflammatory symptoms but who had clinical exome analysis for other reasons (i.e., skeletal dysplasia, dysmorphic features, development delay, etc.) and a high average age with adult patients were included as the control group.

Genetic testing and analysis

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood using a semi-automated robot, as recommended by the manufacturer (Qiagen). The concentration and quality-control (260/280 nm and 260/230 nm absorbance ratios) of the DNA samples were determined by spectrophotometrically (Nanodrop 2000, Thermo Scientific, USA) and fluorometrically (Qubit v3.0, Thermo Fisher, USA). The library preparation for NGS was performed using Fever &Autoinflammatory Diseases Kit by Sophia-Genetics, a custom panel using a capture-based method. panel containing 16 genes (*MEFV, MVK, NLRP3, NLRP12, TNFRSF1A, TNFRSF11A, LPIN2, PSTPIP1, IL1RN, CECR1, ELANE, CARD14, IL10RA, IL10RB, NOD2, and PSMB*8). NextSeq-500-(Illumina) was used as the sequencing platform. In the control group, the library preparation for NGS was performed using a capture-based Clinical Exome Solution Kit by Sophia Genetics, that included 4900 genes. Data quality control, alignment, variant calling and variant annotations were performed using the Sophia DDM analysis tool (version5.2). NCBI Build37 (hg19) version of the human genome was used as a reference. As a primary variant filtering strategy, variants located within the ± 10 base pair boundary of targeted exons with minimum read depth 50× were selected. Any variants outside these regions, variants in homopolymer regions and exonic variants with a variant fraction of less than 20% were considered false positives and were not analyzed. All variants were manually inspected by using IGV visualization tool. We confirmed NLRP3 Q703K mutation in 10 samples by Sanger sequencing and omitted the remaining ones that had well coverage and mappability on manual IGV inspection.

Statistical analysis

Categorical data are presented as numbers and percentages. Statistical package for the social sciences (SPSS) (version 230, SPSS-Inc., Chicago, IL, USA) was used for statistical analysis. Categorical data are presented as numbers and percentages. Numerical data with asymmetrical distribution are presented as the median and interquartile range (IQR). Fisher's exact test was used to compare quantitative data. A p-value less than 0.05 was considered statistically significant.

Results

Within the pool of 639 recruited pediatric rheumatology patients, 68 carried the heterozygous Q703K mutation in the *NLRP3* gene. The overall allelic frequency was 5.3%. In order to evaluate the allele frequency of the Q703K mutation in the general population, the data and clinical files of 1461 patients who were evaluated at the genetic diagnosis center for other reasons (multiple congenital anomalies, severe growth retardation, etc.) were examined. Heterozygous mutations were detected in 141 patients without autoinflammatory symptoms. The allele frequency in individuals without autoinflammatory symptom complaints was found to be 4.8% (p=0.4882).

Of the 68 patients with Q703K, 56 (82.3%) had complete clinical data during molecular analysis and at follow-up. Within these 56 patients, they were further classified into CAPS mild phenotype (n=10), PFAPA (n=7) and

Table I. Clinical data of patients with Q703K mutation.

P N	Fever	Abdominal pain	Skin rash	Artralgia /arthritis	Conjunctivitis	Family history	Pharangitis	Tonsillitis	Aphthous stomatitis	Adenitis	Headache	Myalgia	Hearing loss	Elevated acute phase reactans	Preliminary diagnosis	Final diagnosis	Treatment	Genotype
1	+	-	+	-/-	-	-	-	-	-	-	-	-	-	-	CAPS	CAPS	C, IL-1Ra	Q703K/WT
2	+	-	+	+/-	-	-	-	-	-	-	-	-	-	+	CAPS	CAPS	C, IL-1Ra	Q703K/WT
3	+	-	+	-/-	-	-	-	-	-	-	-	-	-	-	CAPS	CAPS	C	Q703K/WT
4	+	-	+	+/-	-	-	-	-	-	-	-	-	-	+	CAPS	CAPS	C	Q703K/WT
5	+	+	-	-/-	+	-	-	-	-	-	-	-	-	-	uSAID	CAPS	C	Q703K/WT
6	+	-	+	-/-	-	-	-	-	-	-	-	+	-	+	CAPS	CAPS	C	Q703K/WT
7	-	-	+	+/-	+	+	-	-	-	-	-	-	-	+	CAPS	CAPS	C	Q703K/WT
8	-	+	-	+/-	+	+	-	-	-	-	+	-	-	+	uSAID	CAPS	C	Q703K/WT
9	+	+	+	-/-	-	-	-	-	-	-	-	+	-	+	uSAID	CAPS	C	Q703K/WT
10	+	-	-	-/-	+	NA	-	+	+	+	+	-	-	+	CAPS	CAPS	C	Q703K/WT
11	+	-	-	+/-	-	-	+	+	-	-	+	-	-	NA	uSAID	PFAPA	C	Q703K/WT
12	+	-	-	-/-	-	-	-	+	+	-	-	-	-	+	uSAID	PFAPA	C	Q703K/WT
13	+	+	-	-/-	-	+	+	+	-	-	-	-	-	+	PFAPA	PFAPA	C	Q703K/WT
14	+	+	-	-/-	-	-	-	-	+	-	-	-	-	+	uSAID	PFAPA	P	Q703K/WT
15	+	-	-	-/-	-	-	-	+	+	+	-	-	-	-	PFAPA	PFAPA	P	Q703K/WT
16	+	-	-	-/-	-	-	-	+	+	-	-	-	-	-	PFAPA	PFAPA	P	Q703K/WT
17	+	-	+	-/-	-	-	+	-	-	-	-	-	-	+	PFAPA	PFAPA	C	Q703K/WT
18	+	+	+	-/-	-	-	-	-	-	-	-	-	-	+	uSAID	uSAID	-	Q703K/WT
19	+	-	+	-/-	-	+	-	-	-	-	-	-	-	+	CAPS	uSAID	C	Q703K/WT
20	+	+	-	-/-	-	-	-	-	+	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
21	+	+	-	-/-	+	-	-	-	-	-	+	-	-	-	uSAID	uSAID	C	Q703K/WT
22	+	+	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
23	+	-	+	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
24	+	-	-	+/-	-	-	-	+	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
25	+	+	-	-/-	-	+	-	-	-	-	-	-	-	-	uSAID	uSAID	-	Q703K/WT
26	+	-	-	-/-	-	-	-	-	-	-	-	-	-	+	HIDS	uSAID	C	Q703K/WT
27	+	+	-	-/-	-	-	-	-	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
28	+	-	-	-/-	-	-	-	+	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
29	-	+	-	-/-	-	-	-	-	-	-	-	-	-	-	TRAPS	uSAID	C	Q703K/WT
30	+	+	-	+/-	-	-	-	-	-	-	-	-	-	+	TRAPS	uSAID	C	Q703K/WT
31	+	+	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
32	+	-	-	-/-	-	-	-	+	-	-	-	-	-	NA	uSAID	uSAID	C	Q703K/WT
33	+	+	-	-/-	-	-	-	+	+	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
34	+	-	-	-/-	-	-	-	-	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT

CAPS:Cryopyrin-associated periodic syndromes, HIDS: Hyperimmunoglobulin D Syndrome, PFAPA: periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis, IVIG: intravenous immunoglobulin, NA: Not available, PN: Patient number, WT: Wild type, uSAID: Undefined systemic autoinflammatory disease, C: colchicine, P: Predinisolone, TRAPS: Tumor necrosis factor receptor-associated periodic syndrome, IL-1Ra: Interleukin-1 receptor antagonist

Table I. Continued.

P N	Fever	Abdominal pain	Skin rash	Artralgia /arthritis	Conjunctivitis	Family history	Pharangitis	Tonsillitis	Aphthous stomatitis	Adenitis	Headache	Myalgia	Hearing loss	Elevated acute phase reactans	Preliminary diagnosis	Final diagnosis	Treatment	Genotype
35	+	+	-	-/-	-	-	-	-	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
36	+	+	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
37	+	+	-	+/-	-	+	-	-	-	-	+	+	-	+	uSAID	uSAID	C	Q703K/WT
38	+	-	+	+/-	+	-	-	-	-	-	-	+	-	+	uSAID	uSAID	-	Q703K/WT
39	+	+	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
40	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
41	+	+	-	-/-	-	-	-	-	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
42	+	-	-	+/-	+	-	-	-	+	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
43	+	+	+	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
44	+	-	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
45	+	+	-	+/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	-	Q703K/WT
46	+	-	-	+/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
47	+	+	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	-	Q703K/WT
48	+	+	-	-/-	-	+	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
49	-	+	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
50	+	+	-	-/-	+	+	-	+	-	-	+	-	-	-	uSAID	uSAID	C	Q703K/WT
51	+	+	-	+/-	-	+	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
52	+	+	-	-/-	-	-	-	+	+	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
53	+	+	-	-/-	-	-	-	-	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
54	+	-	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT
55	+	-	-	-/-	-	-	-	-	-	-	-	-	-	+	uSAID	uSAID	C	Q703K/WT
56	+	-	-	-/-	-	-	-	-	-	-	-	-	-	-	uSAID	uSAID	C	Q703K/WT

CAPS:Cryopyrin-associated periodic syndromes, HIDS: Hyperimmunoglobulin D Syndrome, PFAPA: periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis, IVIG: intravenous immunoglobulin, NA: Not available, PN: Patient number, WT: Wild type, uSAID: Undefined systemic autoinflammatory disease, C: colchicine, P: Predinisolone, TRAPS: Tumor necrosis factor receptor-associated periodic syndrome, IL-1Ra: Interleukin-1 receptor antagonist

the remainder as uSAID (n=39). A flow chart illustrating the inclusion/exclusion process for patients is summarized in Supplementary Fig. 1. The main clinical findings, preliminary and final diagnosis of 56 patients with the Q703K mutation are summarized in Table I.

Among the 56 patients carrying the Q703K mutation, 18 were female (32.14%) and 38 (67.85 %) were male. The median age of the cohort was 10.3 (interquartile range (IQR): 7.06-12-98) years old. The median age of symptom onset was 36 (IQR:18-72) months old.

The common clinical findings are fever, abdominal pain, skin rash and musculoskeletal involvement (92.85%, 53.57%, 25 % and 25% respectively). All patients with musculoskeletal findings had only arthralgia and therefore they were classified as mild. Neurological findings (headaches only) were present in 6 (10.71%) patients. Ophthalmic findings (conjunctivitis)

were observed in 8 (14.28%) patients. None of the participants had hearing loss or amyloidosis. Lastly, 46.42% patients had elevated acute phase reactions.

After one year follow-up with their clinical findings and molecular genetic analysis results 10 patients were evaluated as CAPS mild phenotype. Of these patients two were treated with IL-1 inhibitors and responded well to treatment. An additional 7 patients were evaluated for their history of PFAPA syndrome. All 7 patients responded very well to colchicine treatment. The remaining 39 patients were classified with an uSAID (Supplementary Fig. 1).

Discussion

In this study, we evaluated the clinical findings of 56 patients with the confirmed Q703K heterozygous variant. This patient cohort was identified through the pediatric rheumatology outpatient clinic, specifically for patients with autoinflammatory symptoms who underwent a PFS panel.

We detected the allele frequency of Q703K similar to Exome Aggregation Consortium (ExAC) data, and 1000 Genomes project in both the PFS and control groups (4.1, 5.1, 5.3, 4.8%, respectively). The ExAC database referred to the European/white population and the 1000G project referred to a mixed group of 60,706 subjects. The data from the Genome Aggregation Database (gnomAD) reported an allelic frequency of 3.8% and 5.1% in the general and European populations, respectively. The pathogenic effect and penetrance of the Q703K variant were not detected. The frequent detection of the Q703K variant in various cohorts has led to the investigation of the pathogenicity of this variant. The impact of the Q703K variant on NLRP3 protein function as well as the resulting phenotypic spectrum continues to be debated.

Theodoropoulou et al.[21] found the *NLRP3* Q703K variant to be significantly higher in patients with autoinflammatory disease compared to the gnomAD data, suggesting an association between this variant and CAPS, PFAFA and uSAID. However, they did not show the functional effect of this mutation on basal inflammatory activity. This study suggested that the risk of developing autoinflammatory disease is likely high in patients carrying this variant, but further studies are needed to obtain detailed information about severity and prognosis.

Vitale et al.[17] showed that patients carrying the *NLRP3* Q703K mutation may present with FCAS-like findings. However, they suggested that caution should be used in the interpretation of the mutation alone, in order to avoid overtreatment in the high frequency of healthy carriers.

While the Q703K variant is detected in a high frequency of healthy individuals, the carrier rate differs significantly across various ethnic groups, as seen in local studies. In particular, Aksentijevich et al.[12] found a 5% allele frequency in a healthy Caucasian group. In another study, the Q703K allele frequency was 8.4% in 130 healthy individuals of various ethnic backgrounds.[25] A third study randomly selected 806 individuals from Sweden and found the allele frequency to be 6.5%.[13] In our study, the allele frequency was 4.8% from the pool of 1341 healthy individuals.

In a 10-year multi-center study, 580 patients were examined for the *NLRP3* gene, following clinical suspicion of CAPS or other PFSs. Of these patients, 57 were found to carry the Q703K variant. The final diagnosis of 13 out of the 36 patients, who had both complete clinical data and genetic confirmation of the Q703K variant, was a PFS separate from CAPS. Additionally, 2 of the 36 patients carrying a Q703K mutation along with another *NLRP3* variant were diagnosed with CINCA and MWS. At the follow-up visit, the remaining 21 patients were reported to have mild clinical findings. Severe CAPS phenotype findings were not observed in any of the 36 patients. Moreover, most of the patients carrying the Q703K variant received

an alternative final diagnosis. As a result of this study, the authors considered the *NLRP3* Q703K variant to be a polymorphism without an evident functional or clinical effects.[26]

Lidar et al.[25] detected the *NLRP3* Q703K variant in 14 of 90 individuals who presented with autoinflammatory symptoms. Only 1 of these patients met the criteria for CAPS and responded well to treatment with IL-1 inhibitors. The Q703K allele frequency was similar in the patient and control groups (7.7% and 8.4%, respectively; p=0.85). The conclusion of the study supports that Q703K is a polymorphism rather than a disease-related mutation. Aksentijevich et al.[12] found a similar allele frequency of Q703K in their patient and control groups (4% and 5%, respectively; p=0.84). Based on these values, the authors concluded that the Q703K variant may in fact not be pathogenic.

When examining the plethora of available studies, we suspect that the Q703K variant is causing the inflammatory effect. Rieber et al.[27] reported that the secretion of NLRP3 inflammatory products (IL-1β, IL-18 and caspase 1) has similar activity in Q703K variant and healthy control groups. Based on this findings, Rieber et al.[27] concluded that symptomatic individuals with the Q703K variant may be experiencing such symptoms due to an underlying pathophysiology other than caspase-1 hyperactivation. Blomgran et al.[28] recently showed that delayed neutrophil apoptosis was not due to caspase-1 or IL-1 β activity, further demonstrating that there must be an alternative cause for the hyperinflammatory responses in the Q703K variants. In contrast, the Q703K variant has a low rate of complete response to anti-IL-1 treatment.

Verma et al.[13] describe a patient with CAPS phenotype and Q703K genotype who responded well to anti-IL-1 therapy. This patient's monocytes, as compared to that of 5 healthy controls, revealed high active IL-1 beta secretion and caspase 1 overactivation after lipopolysaccharide stimulation. This same group showed that the Q703K variant resulted in a gain-of-function mutation that subsequently led to an overactive NLRP3 inflammasome. They also reported that this variant has milder clinical findings.[16] After this study, with a note added to guidelines for genetic diagnosis and inherited recurrent fevers, it was recommended that this variant be considered a variant of uncertain significance (VUS) and that it should be reported by clinicians moving forward.[14]

In our study, we included 56 patients who came in for a clinical follow-up carrying Q703K allele. Of these 10 patients' final diagnosis were CAPS mild phenotype. Seven of these patients' preliminary diagnosis was CAPS according to the Federici score.[22] All patients had mild clinical symptoms and only 2 patients were using IL-1Ra (*interleukin-1 receptor antagonist)* therapy. Only one patient, whose preliminary diagnosis was CAPS according to Federici score was finally diagnosed as uSAID according to clinical follow-up. Although we found a high rate of Q703K positivity in the group in which CAPS was considered, we also found the Q703K variant in those who were not compatible with clinical CAPS. Specifically, the Q703K carrier rate within the control cohort (n=1461) which was evaluated at the genetic diagnosis center for other reasons (multiple congenital anomalies, severe growth retardation, etc.) was 4.8%. Although this situation was similar to previous studies, high Q703K positivity in patients with clinically suspected CAPS was remarkable.

In most studies, patients carrying the Q703K variant had a milder phenotype with no central nervous system (CNS) symptoms.[17,26,29] However, some recent studies have reported severe CNS manifestations and inflammation in individuals with the Q703K variant.[11,30] In our study, neurologic deficits were detected in 6 patients. Mild neurological findings (headache) were detected in 5 patients. Headache was observed in 2 of 10 patients diagnosed with CAPS. As a result of all these studies, it can be concluded that the inflammatory effect caused by Q703K is equivocal. Moreover, from our study as well as those conducted

across numerous other sites, the Q703K variant requires a special evaluation.

Because of a high family history, PFAFA syndrome seems to have a genetic background with a low penetrance.[20] In previous studies, MEFV, MVK, CARD15 genes, as well as NLRP3 gene, have been analyzed in PFAPA patients.[31-33] The effect of these genetic variants in PFAPA syndrome is not clearly known. Perko et al.[31] evaluated 62 PPAFA patients and detected a significant variant in the *NLRP3* gene 13 patients (21%) and the Q703K variant was detected in 9 of the PPAFA patients (14.5%) and was detected in 12 of 100 healthy individuals (12%). Thus, no significant difference was found between allele frequencies ($p>0.05$). They hypothesized that PFAPA may result from a combination of low-penetration variants with epigenetic and environmental factors. It has been reported that this variant may play a role in PFAPA pathogenesis by causing excessive NLRP3 inflammasome with a gain-of-function effect.[16] The role of the Q703K variant in the pathogenesis of PFAPA cannot be excluded due to a possible functional effect on inflammasome.

Our study is limited as no further genetic studies have been performed for whole exome sequencing and exclusion of somatic mutations. The small number of patients examined in our study limited the establishment of a genetic causal relationship. In our study, we found similar allele frequencies in the healthy group (141/1451, 4.8%) and the group with autoinflammatory symptoms (68/639, 5.3%) in line with previous studies.

Our study analyzed a cohort of patients to identify those carrying the Q703K variant. They were evaluated by the Eurofever/PRINTO classification criteria, which identified all carriers to have CAPS, within which, 10 patients met the CAPS diagnostic criteria. The final diagnoses were CAPS in 10 of 56 patients with Q703K variant (%17.85), a notably high percentage. Additionally, 7 patients' final diagnosis with this variant was PFAPA by evaluation of the Modified Marshall criteria. However, in the evaluation of a large number of control groups, the allele frequency in the Turkish population was found to be 4.8%. In the evaluation of the patients with autoinflammatory symptoms, the allele frequency was found to be 5.3%. The similar frequency across both groups (control and autoinflammatory groups) supports the hypothesis that Q703K is a polymorphism, however, its high incidence in patients with mild CAPS symptoms leads us to believe that this variant cannot be ignored. Furthermore, this mutation may be responsible for the phenotype when combined with other genetic variants or epigenetic alterations. As clinicians continue to see patients with autoinflammatory symptoms and/or the presence of the Q703K variant on genetic panels, we recommend reporting both the mutation and clinical evaluation of the patient to ensure proper diagnosis. This data will ultimately help solve the causative relationship between *NLRP3* Q703K variant and autoinflammatory diseases.

Ethical approval

This study was approved by the local Ethics Committee of Health Science University, Education and Research Hospital (Approval Number: B.10.1.TKH.4.34.H.GP.0.01/07/26.01.2023). Informed consent was obtained from all individual participants' legal guardians included in the study.

Author contribution

The authors confirm contribution to the paper as fallows: study conception and design: YKD, BS; data collection: YKD, FD; analysis and interpretation of results: YKD; draft manuscript preparation: YKD, LAJ. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Stojanov S, Kastner DL. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. Curr Opin Rheumatol 2005; 17: 586-599. https://doi.org/10.1097/bor.0000174210.78449.6b
2. Papa R, Rusmini M, Volpi S, et al. Next generation sequencing panel in undifferentiated autoinflammatory diseases identifies patients with colchicine-responder recurrent fevers. Rheumatology (Oxford) 2020; 59: 344-360. https://doi.org/10.1093/rheumatology/kez270
3. Demir F, Doğan ÖA, Demirkol YK, et al. Genetic panel screening in patients with clinically unclassified systemic autoinflammatory diseases. Clin Rheumatol 2020; 39: 3733-3745. https://doi.org/10.1007/s10067-020-05108-1
4. Hoffman HM, Wanderer AA, Broide DH. Familial cold autoinflammatory syndrome: phenotype and genotype of an autosomal dominant periodic fever. J Allergy Clin Immunol 2001; 108: 615-620. https://doi.org/10.1067/mai.2001.118790
5. Muckle TJ. The 'Muckle-Wells' syndrome. Br J Dermatol 1979; 100: 87-92. https://doi.org/10.1111/j.1365-2133.1979.tb03572.x
6. Kilcline C, Shinkai K, Bree A, Modica R, Von Scheven E, Frieden IJ. Neonatal-onset multisystem inflammatory disorder: the emerging role of pyrin genes in autoinflammatory diseases. Arch Dermatol 2005; 141: 248-253. https://doi.org/10.1001/archderm.141.2.248
7. Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: master switches of inflammation. Cell Death Differ 2007; 14: 10-22. https://doi.org/10.1038/sj.cdd.4402038
8. Kitley JL, Lachmann HJ, Pinto A, Ginsberg L. Neurologic manifestations of the cryopyrin-associated periodic syndrome. Neurology 2010; 74: 1267-1270. https://doi.org/10.1212/WNL.0b013e3181d9ed69
9. Mamoudjy N, Maurey H, Marie I, Koné-Paut I, Deiva K. Neurological outcome of patients with cryopyrin-associated periodic syndrome (CAPS). Orphanet J Rare Dis 2017; 12: 33. https://doi.org/10.1186/s13023-017-0589-1
10. Shinkai K, McCalmont TH, Leslie KS. Cryopyrin-associated periodic syndromes and autoinflammation. Clin Exp Dermatol 2008; 33: 1-9. https://doi.org/10.1111/j.1365-2230.2007.02540.x
11. Schuh E, Lohse P, Ertl-Wagner B, et al. Expanding spectrum of neurologic manifestations in patients with NLRP3 low-penetrance mutations. Neurol Neuroimmunol Neuroinflamm 2015; 2: e109. https://doi.org/10.1212/NXI.0000000000000109
12. Aksentijevich I, Putnam CD, Remmers EF, et al. The clinical continuum of cryopyrinopathies: novel CIAS1 mutations in North American patients and a new cryopyrin model. Arthritis Rheum 2007; 56: 1273-1285. https://doi.org/10.1002/art.22491
13. Verma D, Lerm M, Blomgran Julinder R, Eriksson P, Söderkvist P, Särndahl E. Gene polymorphisms in the NALP3 inflammasome are associated with interleukin-1 production and severe inflammation: relation to common inflammatory diseases? Arthritis Rheum 2008; 58: 888-894. https://doi.org/10.1002/art.23286
14. Shinar Y, Obici L, Aksentijevich I, et al. Guidelines for the genetic diagnosis of hereditary recurrent fevers. Ann Rheum Dis 2012; 71: 1599-1605. https://doi.org/10.1136/annrheumdis-2011-201271
15. Milhavet F, Cuisset L, Hoffman HM, et al. The infevers autoinflammatory mutation online registry: update with new genes and functions. Hum Mutat 2008; 29: 803-808. https://doi.org/10.1002/humu.20720
16. Verma D, Särndahl E, Andersson H, et al. The Q705K polymorphism in NLRP3 is a gain-of-function alteration leading to excessive interleukin-1β and IL-18 production. PLoS One 2012; 7: e34977. https://doi.org/10.1371/journal.pone.0034977
17. Vitale A, Lucherini OM, Galeazzi M, Frediani B, Cantarini L. Long-term clinical course of patients carrying the Q703K mutation in the NLRP3 gene: a case series. Clin Exp Rheumatol 2012; 30: 943-946.
18. Batu ED. Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome: main features and an algorithm for clinical practice. Rheumatol Int 2019; 39: 957-970. https://doi.org/10.1007/s00296-019-04257-0
19. Marshall GS, Edwards KM, Butler J, Lawton AR. Syndrome of periodic fever, pharyngitis, and aphthous stomatitis. J Pediatr 1987; 110: 43-46. https://doi.org/10.1016/s0022-3476(87)80285-8
20. Manthiram K, Nesbitt E, Morgan T, Edwards KM. Family history in Periodic Fever, Aphthous Stomatitis, Pharyngitis, Adenitis (PFAPA) syndrome. Pediatrics 2016; 138: e20154572. https://doi.org/10.1542/peds.2015-4572
21. Theodoropoulou K, Wittkowski H, Busso N, et al. Increased prevalence of NLRP3 Q703K variant among patients with autoinflammatory diseases: an international multicentric study. Front Immunol 2020; 11: 877. https://doi.org/10.3389/fimmu.2020.00877

22. Federici S, Sormani MP, Ozen S, et al. Evidence-based provisional clinical classification criteria for autoinflammatory periodic fevers. Ann Rheum Dis 2015; 74: 799-805. https://doi.org/10.1136/annrheumdis-2014-206580

23. Thomas KT, Feder HM, Lawton AR, Edwards KM. Periodic fever syndrome in children. J Pediatr 1999; 135: 15-21. https://doi.org/10.1016/s0022-3476(99)70321-5

24. Gattorno M, Hofer M, Federici S, et al. Classification criteria for autoinflammatory recurrent fevers. Ann Rheum Dis 2019; 78: 1025-1032. https://doi.org/10.1136/annrheumdis-2019-215048

25. Lidar M, Brantz Y, Shinar Y, et al. A high and equal prevalence of the Q703K variant in NLRP3 patients with autoinflammatory symptoms and ethnically matched controls. Clin Exp Rheumatol 2017; 108: 82-85.

26. Naselli A, Penco F, Cantarini L, et al. Clinical characteristics of patients carrying the Q703K variant of the NLRP3 gene: a 10-year multicentric national study. J Rheumatol 2016; 43: 1093-1100. https://doi.org/10.3899/jrheum.150962

27. Rieber N, Gavrilov A, Hofer L, et al. A functional inflammasome activation assay differentiates patients with pathogenic NLRP3 mutations and symptomatic patients with low penetrance variants. Clin Immunol 2015; 157: 56-64. https://doi.org/10.1016/j.clim.2015.01.003

28. Blomgran R, Patcha Brodin V, Verma D, et al. Common genetic variations in the NALP3 inflammasome are associated with delayed apoptosis of human neutrophils. PLoS One 2012; 7: e31326. https://doi.org/10.1371/journal.pone.0031326

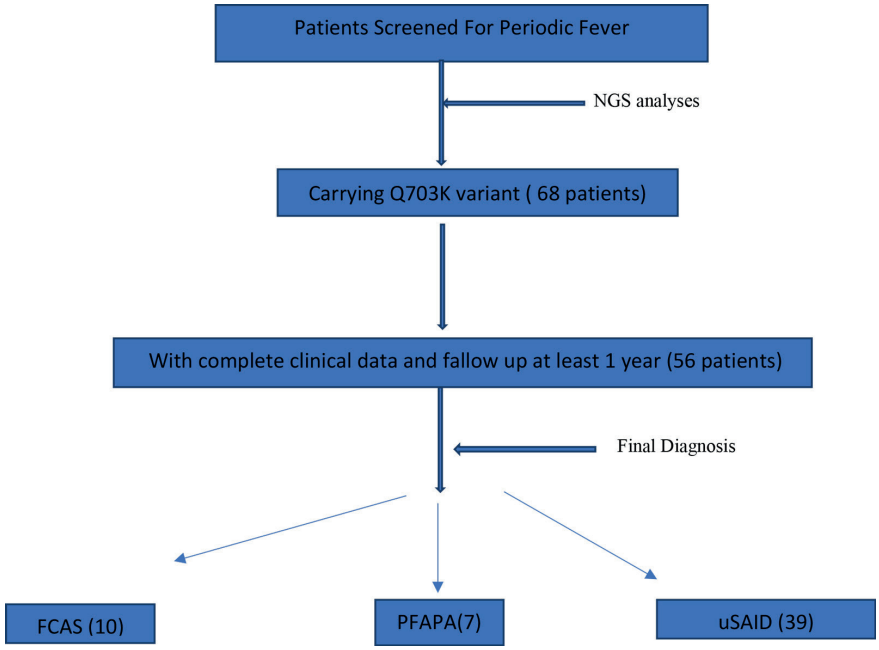
29. Levy R, Gérard L, Kuemmerle-Deschner J, et al. Phenotypic and genotypic characteristics of cryopyrin-associated periodic syndrome: a series of 136 patients from the Eurofever Registry. Ann Rheum Dis 2015; 74: 2043-2049. https://doi.org/10.1136/annrheumdis-2013-204991

30. Höhne C, Schuh E, Kümpfel T, Straube A. Cryopyrin-associated periodic fever syndrome manifesting as Tolosa-Hunt syndrome. Cephalalgia 2016; 36: 1392-1396. https://doi.org/10.1177/0333102416629239

31. Perko D, Debeljak M, Toplak N, Avčin T. Clinical features and genetic background of the periodic Fever syndrome with aphthous stomatitis, pharyngitis, and adenitis: a single center longitudinal study of 81 patients. Mediators Inflamm 2015; 2015: 293417. https://doi.org/10.1155/2015/293417

32. Berkun Y, Levy R, Hurwitz A, et al. The familial Mediterranean fever gene as a modifier of periodic fever, aphthous stomatitis, pharyngitis, and adenopathy syndrome. Semin Arthritis Rheum 2011; 40: 467-472. https://doi.org/10.1016/j.semarthrit.2010.06.009

33. Dagan E, Gershoni-Baruch R, Khatib I, Mori A, Brik R. MEFV, TNF1rA, CARD15 and NLRP3 mutation analysis in PFAPA. Rheumatol Int 2010; 30: 633-636. https://doi.org/10.1007/s00296-009-1037-x



Supplementary Fig. 1. Flow-chart of the patients.