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ORIGINAL ARTICLE

Clinical and molecular evaluation of *MEFV* gene variants in the Turkish population: a study by the National Genetics Consortium

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Abstract
Familial Mediterranean fever (FMF) is a monogenic autoinflammatory disorder with recurrent fever, abdominal pain, serositis, articular manifestations, erysipelas-like erythema, and renal complications as its main features. Caused by the mutations in the MEditerranean FeVer (*MEFV*) gene, it mainly affects people of Mediterranean descent with a higher incidence in the Turkish, Jewish, Arabic, and Armenian populations. As our understanding of FMF improves, it becomes clearer that we are facing with a more complex picture of FMF with respect to its pathogenesis, penetrance, variant type (gain-of-function vs. loss-of-function), and inheritance. In this study, *MEFV* gene analysis results and clinical findings of 27,504 patients from 35 universities and institutions in Turkey and Northern Cyprus are combined in an effort to provide a better insight into the genotype-phenotype correlation and how a specific variant contributes to certain clinical findings in FMF patients. Our results may help better understand this complex disease and how the genotype may sometimes contribute to phenotype. Unlike many studies in the literature, our study investigated a broader symptomatic spectrum and the relationship between the genotype and phenotype data. In this sense, we aimed to guide all clinicians and academicians who work in this field to better establish a comprehensive data set for the patients. One of the biggest messages of our study is that lack of uniformity in some clinical and demographic data of participants may become an obstacle in approaching FMF patients and understanding this complex disease.

Keywords Familial Mediterranean fever · Genotype-phenotype correlations · *MEFV* · National Genetics Consortium

Introduction

Familial Mediterranean Fever (FMF) is an autoinflammatory disorder presenting with recurrent attacks of fever, serositis, articular manifestations, erysipelas-like erythema, and renal complications. It is a disorder with autosomal recessive inheritance caused by the mutations in the MEditerranean FeVer (*MEFV*) gene. FMF has first been accurately described in the literature in 1945 by Siegal. It mainly affects people of Mediterranean descent with a strong distribution in the Turkish, Jewish, Arabic, and Armenian populations. It can also be found in Spain, Italy, and Greece but to a lesser extent. However, with increased immigration in the last few decades, FMF could now be seen worldwide (Siegal 1945; Soriano and Manna 2012; Alghamdi 2017). It is a fact that, with the recent advances in molecular biology and genetics, we may be looking at a more complex picture of FMF with respect to its pathogenesis, penetrance, mutation type (gain-of-function vs. loss-of-function), and inheritance (Ozen and Batu 2015; Alghamdi 2017).

Having been cloned in 1997 by both a French group and an international consortium, the *MEFV* gene is located on chromosome 16p13.3. The protein produced by the gene is called "marenostrin/pyrin" and contains 781 amino acids (Bernot et al. 1997; Consortium 1997). The protein pyrin is part of the innate immune system and is part of the inflammasome. As a result, it can lead to too much inflammation through the unregulated production of interleukin-1 (IL-1) (Alghamdi 2017). The gene contains 10 exons, and nearly 85% of the patients from the Mediterranean basin have variants in exons 2 and 10. Mutations can also be found in other exons (Soriano and Manna 2012; Alghamdi 2017). Currently, 385 variants have been listed on the "Infevers" database. The database includes common, rare, and even unclassified variants that are still under investigation (Infevers: an online database for autoinflammatory mutations. Copyright. Available at https://infevers.umai-montpellier.fr/ Accessed (June 3rd). Some of the variants on the database may also be clinically inconsequential (Alghamdi 2017). It is known that the majority of variants leading to FMF are clustered in the C-terminal B30.2 domain of the pyrin protein. Direct interaction of the B30.2 domain with the catalytic subunits of caspase 1, without needing an adaptor protein like ASC, was shown in the literature. Although contradictory studies were published, it is demonstrated that FMF occurs via gain-of-function mutations that ultimately increase IL-1β activation (Chae et al. 2006, 2011; Özen et al. 2017). These findings are also in accordance with the presence of new treatment options targeting IL-1 and IL-1β.

FMF is the most common of the monogenic periodic fever syndromes, characterized by self-limited recurrent episodes of fever and serositis. Other complications include arthritis, dermal manifestations, long-term renal problems, and pain in the muscles and abdomen. The sporadic attacks of fever and pain usually last between 12 and 72 h (Soriano and Manna 2012; Alghamdi 2017). Functional gastrointestinal disorders may also accompany and cause attacks of diarrhea, constipation, or nausea/vomiting (Beşer et al. 2013; Ekinci et al. 2019; Saito et al. 2020). Canpolat et al. (2017) had also studied the presence of neurological symptoms in FMF patients in a study, where several neurological symptoms were more frequent than the general population. It is possible to establish the diagnosis of FMF in different ways. The most preferred one is using Tel-Hashomer clinical criteria (Livneh et al. 1997; Shohat 2020). Another method physicians can benefit from is starting a colchicine treatment regimen and observing the response. Additionally, detecting biallelic pathogenic

MEFV gene variants can also be used to verify the diagnosis when clinical criteria are not met. Performing solely a genetic test would only have a 70-80% predictive value because a quarter of FMF patients carry either no variant or only one variant in the *MEFV* gene (Soriano and Manna 2012; Alghamdi 2017; Ozdogan and Ugurlu 2019).

Shinar et al. (2012, 2020) have recently published the *Best Practice Guidelines for the Genetic Diagnosis of Monogenic Autoinflammatory Diseases in the Next-Generation Sequencing Era*, which includes FMF. These guidelines are an update of the 2012 guidelines and bring the field more up to par with the next-generation sequencing era.

After sequencing became more widely available, it was thought that sequencing the whole *MEFV* gene would be more beneficial in detecting variants. Current practice guidelines recommend testing exon 10 and optionally exons 2, 3, and 5 of the *MEFV* gene using Sanger sequencing. A next-generation sequencing analysis including all exons of *MEFV* may also be preferred (Shinar et al. 2020). In the previous guideline published in 2012, 14 selected variants were the recommended targets for *MEFV* gene screening (Shinar et al. 2012). We had designed our study based on these recommendations, as the newer guideline was not yet available. Among these 14 variants, 9 are classified as known pathogenic (M680I, M694V, M694I, V726A, A744S, R761H, I692del, E167D, T267I) and 5 are classified as unknown significance (K695R, E148Q, P369S, F479L, and I591T) (Ozen and Batu 2015; Alghamdi 2017; Ozdogan and Ugurlu 2019). The variants and how they are situated concerning the different domains of the pyrin protein have been visualized in Fig. 1. Among these variants, M694V is the most pathogenic and the most prevalent, especially in patients of Mediterranean descent. Its strong pathogenic outlook means earlier onset, higher susceptibility to amyloidosis, and possibly higher doses of colchicine for treatment. M680I, V726A, and M694I are more common in Turks and Armenians, Ashkenazi Jews, and Arabs, respectively (Ozdogan and Ugurlu 2019).

The fact that some patients do not carry biallelic *MEFV* pathogenic variants gave rise to some debates regarding the inheritance pattern of FMF and other possible factors contributing to the disease course. Autosomal dominant transmission of FMF is now recognized as a possibility (Shohat and Halpern 2011; Rowczenio et al. 2017). The presence of possible modifying genes or even environmental factors affecting the clinical phenotype also came under consideration. Serum amyloid A (SAA) and MHC class I polypeptide-related sequence A (MICA) are two genes that are thought to be contributing to the FMF phenotype as modifying genes (Ozdogan and Ugurlu 2019). In a previous study, the effect of variants was investigated in the *TNF-α* and *PAI-1* genes in FMF, where the *PAI-1* 4G/5G genotype showed a possible association with FMF (Dundar et al. 2013).

FMF can be divided into three clinical phenotypes. In phenotype 1, short-term inflammation and serositis, synovitis, and/or erysipelas-like erythema are present. Phenotype 2 is associated with reactive amyloid-associated (AA) amyloidosis, which is the worst complication of FMF in asymptomatic individuals. In phenotype 3, pathogenic variants are present in both copies of the *MEFV* gene, but neither the clinical symptoms nor amyloidosis can be observed in this group (Soriano and Manna 2012).

Responding well to colchicine treatment is one of the main criteria for FMF diagnosis. First used in 1972, colchicine is still the linchpin in FMF treatment, despite the introduction of new therapies such as the IL-1 inhibitors namely anakinra, canakinumab, and rilonacept. These treatment modalities effectively suppress the FMF attacks in many colchicine-resistant patients. In a systematic review, 76.5% of the patients receiving anakinra showed complete remission of attacks, while 67.5% of the canakinumab recipients had a complete response to treatment (van der Hilst et al. 2016). Rilonacept also reduced the frequency of FMF attacks in a randomized

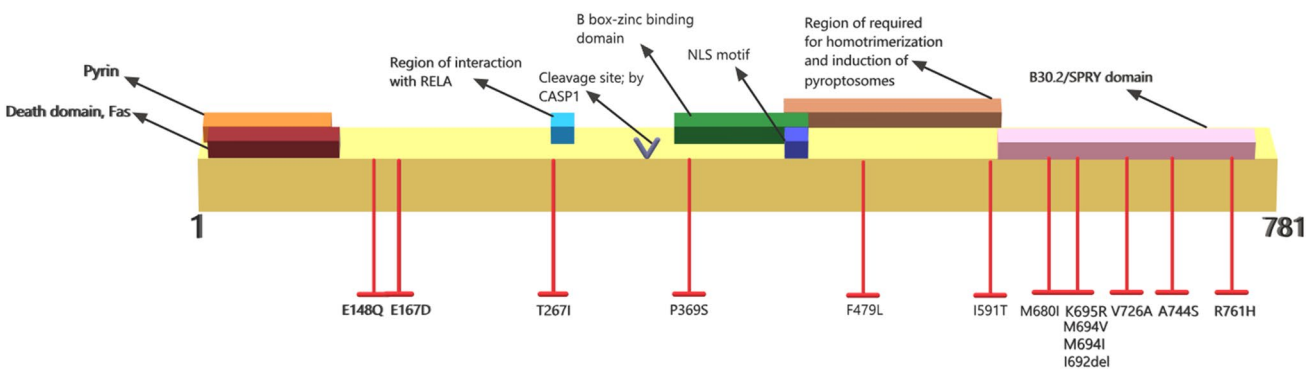

Fig. 1 Location of the "Consensus 14" variants and the different domains on the pyrin protein. The consensus 14 variants have been visualized on the protein with respect to the different domains of the pyrin protein

clinical trial; however, it failed to reduce the duration of attacks (Hashkes et al. 2012). As these drugs need to be administered via injections with varying frequencies due to their half-lives, injection site reaction is one of the most common side effects reported. Pneumonia is another side effect of these drugs. Canakinumab is the first drug to be approved by FDA for colchicine-resistant FMF treatment (Soriano and Manna 2012; Alghamdi 2017; Ozdogan and Ugurlu 2019).

Pyrin protein includes different domains such as the B-box, bZIP basic, and coiled-coil domains and is also known as the triple motif-20 (TRIM20) (Weinert et al. 2015) (Fig. 1). As TRIM20 is part of a larger family of proteins called the triple motif proteins, pyrin was first thought to be a transcription factor; however, it does not have DNA-binding activity. The protein contains two nuclear localization motifs, and an endogenous protein is localized to the nucleus of granulocytes and dendritic cells (Shohat and Halpern 2011). A specific N-terminal pyrin fragment undergoes translocation into the nucleus after being cleaved by caspase-1. N-terminal pyrin appears to activate NF-κB by increased calpain-induced degradation of IKB-alpha and is also detected in the nuclei of leukocytes (Chae et al. 2008).

Although predominantly nuclear, research suggests that native pyrin can also be found in the cytoplasm of monocytes, interacting with microtubules and co-localizing with actin filaments. In this context, the use of a microtubule destabilizing agent, colchicine, provides a highly effective treatment option for FMF (Mansfield et al. 2001). In another study, the N-terminal PYRIN motif of the pyrin protein was shown to interact with an apoptotic speck protein with a caspase recruitment domain (CARD) (ASC), which is a pro-apoptotic protein that induces the formation of large specks in the cytoplasm. Through the homotypic interaction between the pyrin and ASC, caspase-1 activation is initiated, which consequently activates IL-1β production (Richards et al. 2001; Stehlik et al. 2002).

Turkey has a population of approximately 82 million, and the prevalence of FMF is predicted to be 1 in 1000, which makes Turkey one of the most affected countries from FMF in the world. In this study, *MEFV* gene analysis results from 35 universities and institutions in Turkey and North Cyprus are combined in the FMF data bank, aiming to contribute to the Turkish Human Genome Project. This study aims to provide an insight into the genotype-phenotype correlation and how a specific variant contributes to certain clinical findings in FMF patients.

Materials and methods

Data of 27,504 patients that were suspected of FMF and evaluated according to Tel-Hashomer criteria or their first-degree relatives for segregation analysis were collected from 35 centers. Some patients only partially meet the Tel-Hashomer criteria initially and received the definitive FMF diagnosis after genetic testing; these patients were also included in the study. The patient files between the years 2006 and 2018 were examined retrospectively. Clinical features and genetic test results were requested from participating institutions. These features include age, gender, age of onset, arthritis/arthralgia, fever, abdominal pain, back pain, chest pain, constipation/diarrhea/irritable bowel syndrome (IBS), nausea/vomiting, erythema, amyloidosis, FMF history in close relatives, family history of kidney disease, response to colchicine, detected variants, and molecular methods used for variant detection. Patients' data were collected from all regions of Turkey and, also Northern Cyprus. Clinical information, demographic information, and *MEFV* gene mutation results were recorded in a standard form. The ethical permission required for this study was obtained from the Local Ethics Committee of Erciyes University Clinical Studies (date and number of approval: 10.06.2020/278).

The FMF variant information provided by different centers has been generated using different methods such as Sanger sequencing, next-generation sequencing (NGS), pyrosequencing, strip assay, or amplification-refractory mutation system (ARMS)/restriction fragment length polymorphism (RFLP). These methods will be briefly described below. Blood samples were collected using vacutainer tubes with ethylenediaminetetraacetic acid (EDTA) in all methods.

Sanger sequencing: DNA extraction was performed from peripheral blood by using the automated extraction MagNA Pure 32 System according to the manufacturer's instructions. Forward and reverse primers were used for the PCR amplification of the exons 2, 3, 5, and 10 in the *MEFV* gene. Products from this amplification were sequenced with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), as per the manufacturer's instructions. Sequence analysis was carried out through an automated fluorescent-based sequencer system (ABI PRISM 3130, Applied Biosystems) (Gunesacar et al. 2014).

NGS: PCR analysis was performed by using the NEXTflex Mediterranean Fever Amplicon Panel (Bio Scientific, Austin, TX, USA) via BIO.RAD T100 Thermal Cycler (Bio-Rad, Dubai, United Arab Emirates). All coding exons of the *MEFV* gene were amplified and sequenced using the 24 primer pairs of the panel (in two pools). Sequence PCR products were then loaded to MiniSeq (Illumina, San Diego, CA, USA) for NGS analysis, as per the manufacturer's instructions. Genomize bioinformatics tool was used for data analysis (SEQ, Istanbul, Turkey) (Gumus 2018).

Pyrosequencing: PCR was performed using the PyroMark Q24 PCR Kit (Qiagen, Germany). E148Q, H478Y, P369S, F479L, G678E, S675N, M680I (G>C), M680I (G>A), M680L, T681I, I692del, M694I, M694V,

M694L, K695R, K695M, V722M, V726A, A744S, and R761H variants were identified as heterozygous or homozygous with the FMF Pyrosequencing test, PCR was performed using an 8-point PCR strip tube per sample. Amplification of the *MEFV* gene exons 2, 3, 5, and 10 was achieved with PCR. Eight pyrosequencing reactions were implemented per sample after the PCR reaction. Variants are determined by comparing the sequence outputs with the exon region and its sequence given in the reference template. Pyrosequencing reaction was completed by processing with the PyroMark Q24 instrument (Qiagen, Germany).

Strip assay: Strip assay analysis utilizes the polymerase chain reaction (PCR) and reverse hybridization method, which allows the detection of the 12 most common variants. These variants were E148Q, P369S, F479L, M680I (G/A), M680I (G/C), I692del, M694V, M694I, V726A, K695R, A744S, and R761H. Using standard protocols, DNA is extracted from peripheral blood. Following DNA extractions, multiplex PCR was performed to amplify exons 2, 3, 5, and 10 using biotinylated primers. PCR products are then hybridized to a strip containing immobilized wild-type and mutated oligonucleotide probes (FMF StripAssay®, ViennaLab, Vienna, Austria). Hybridizations were visualized using streptavidin-alkaline phosphatase and a color substrate reaction. The results were interpreted according to the manufacturer's instructions (Yilmaz et al. 2016).

ARMS technique: Mutations involving single base changes or small deletions were detected using the ARMS. In this method, allele-specific polymerase chain reaction (ASPCR) or PCR amplification of specific alleles was performed. In the ARMS method, only the target allele was amplified with sequence-specific PCR primers. Following the ARMS reaction, the presence or absence of a PCR product was evaluated based on the presence or absence of the target allele (Little 1995).

Molecular diagnostic methods that were used for the diagnosis were available for 21,833 (79.38%) patients. On the other hand, the preferred method was not specified for remaining 5671 (20.62%) patients. Pyrosequencing constitutes the largest portion of the patient, as it was used for 7712 patients (28.04%). It is followed by Sanger sequencing for 6587 patients (23.95%), NGS for 3.942 patients (14.33%), strip assay for 2886 patients (10.49%), and ARMS technique for 572 patients (2.08%). Apart from these methods, there are several combined techniques which were also involved in the study. These are consisted of Pyrosequencing and NGS with 101 patients (0.37%); strip assay and NGS with 20 patients (0.07%); Sanger sequencing and NGS with 13 patients (0.05%).

Bioinformatics

The dataset contains the characteristic information for 27,504 samples (patients) and 18 features. For each patient, demographic features such as the patient's gender and age, information related to the clinical characteristics such as fever, abdominal pain, chest pain, etc., and the medical history of the family are included in the dataset.

Since several institutes provided the data, the data format needed to be restructured. For the data cleaning and preprocessing steps, we have written a series of custom-made python scripts. The pseudo-codes of the custom-made scripts were provided as Supplementary material. Firstly, the data entries provided by different institutes were all merged, and then each data entry regardless of the institute was processed separately. Therefore, we were able to treat each entry the same way, which allowed us to make use of the data in later stages without any bias.

Depending on the nature of the clinical feature, the exact processing technique that we used varied for each feature. Features related to the patients' age were reformatted from their initial format to an integer format and then different ages were grouped into bins. Features that could use binary classification were grouped into two sets, regardless of their input format. Finally, variant changes and zygosity types were processed. The variant entries were indexed based on their order of occurrence on a given entry. These indexes were then used to match the position of the variant change, as well as the zygosity information provided (if any). All the processed data was then stored locally and used to generate graphs using Chart.JS.

Statistical analysis

The qualitative variables being studied were summarized with descriptive statistics using frequency and percentages. To understand the possible associations between the genetic variants and clinical conditions, Chi-square tests were applied. Either the Pearson Chi-square test or Fisher's exact test was performed depending on the expected values. The same methods were also used to see the pairwise associations between clinical conditions, as well. Selected variants which were reported to have significant clinical importance in previous studies were then investigated with multivariate logistic regression analysis. A separate logistic model was conducted for each clinical condition against the selected variants where sex and age group were included for statistical adjustment. Odds ratios with 95% confidence intervals were reported. The level of significance was accepted to be 0.05. All statistical calculations were performed with SPSS software (Demo Version for Mac, 18.0).

Results

Studied groups

Thirty-five centers across different regions of Turkey and Northern Cyprus have contributed to the study. Eskisehir Osmangazi University, Erciyes University, and Afyon Health Sciences University were the top 3 contributors to the study making up nearly 59% of the study population. All 7 geographical regions of Turkey have been represented by the data. Only 3 centers were from the Mediterranean region, and the majority of the data came from the central Anatolian, Aegean, Black Sea, and the Marmara region.

Variant distribution

Eighty-eight different FMF variants were identified in the 27,504 patients in the study; 7236 people had no variants, despite clinical findings. Nearly 86% of the study population had one of the M694V (29.47%), E148Q (18.27%), R202Q (17.90%), M680I (10.61%), and V726A (10.14%) variants, ranging from highest to lowest. Of the 88 variants, 25 variants were only detected in one individual, and they altogether accounted for a mere fraction of all the study population. V469L, S749C, M694K, and D389V are examples of some of the variants that have been reported in only one person in the study population. M694V was the most common variant, which was detected in 8106 patients with different zygosities. It was followed by E148Q, R202Q, M680I, and V726A with frequencies of 5024, 4924, 2919, and 2788, respectively. The top 5 variants and the total number of people with each variant, regardless of the zygosity of the variant, are shown in Fig. 2. All the variants were listed for each clinical category, age group, and gender, together with their statistical significance in Table 1.

Analysis of the selected variants (Consensus 14)

We selected 14 variants according to the practice guidelines proposed by Shinar et al. (2012). These variants consist of 9 pathogenic variants, namely M694V, M694I, M680I, V726A, R761H, A744S, E167D, T267I, and I692del and 5 variants of uncertain significance (VUS) that are K695R, E148Q, P369S, F479L, and I591T. The 14 variants will be mentioned as "Consensus 14 variants" throughout the manuscript. In our study, out of the Consensus 14 variants, only I692del was not represented in the population. These variants altogether account for nearly 80% of the variants in the study population, of which nearly 55% are the variants within the pathogenic group. M694V, M680I, and V726A were the most common, and M694I, E167D, and T267I were the least common variants out of the pathogenic group of the Consensus-14. E148Q and P369S were the most frequent variants out of the "VUS" group. E148Q has been reported as the second most common variant after the M694V out of all the variants reported in the study. The pathogenic group variants were more than double the variants of the unknown consequence group (Fig. 3).

Zygosity

Ten thousand four hundred forty-nine (39%) variants were reported as heterozygous, 6339 (24%) as compound heterozygous, and only 759 (3%) variants were reported as homozygous. However, in nearly 34% of the variants, the

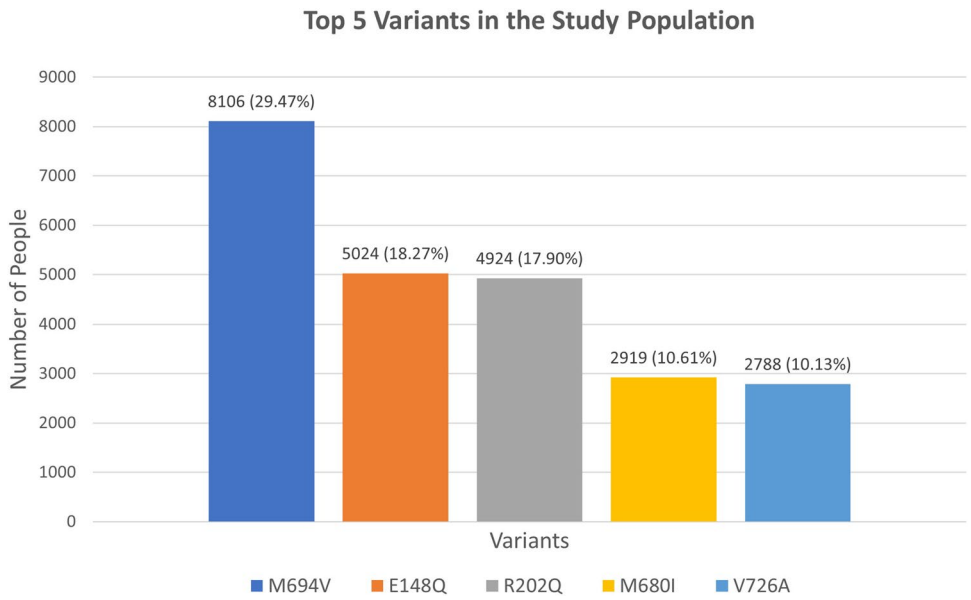

Fig. 2 Top 5 variants in the study population. M694V, E148Q, R202Q, M680I, and V726A were the top 5 variants from highest to lowest in the study population. Together, they make up 86% of the study population

Table 1 Variant and clinical feature relationship. Each variant in the study is provided with respect to the different clinical features, age groups and gender. Degree of statistical significance was indicated for each variant wherever relevant

	M680I	M694V	M694I	R202Q	V726A	R408Q	A744S	R761H	K695R	E148Q	P369S	F479L	E167D	T267I
Gender association														
Female	53%*	52%**	50%	57%*	55%	59%	56%	50%*	58%	57%*	60%*	56%	60%	53%
Male	47%*	48%**	50%	43%*	46%	42%	44%	50%*	42%	43%*	40%*	44%	40%	47%
Zygosity														
Heterozygous	99%**	97%**	99%*	92%**	96%**	100%**	99%**	98%$^{**-}$	98%$^{**-}$	85%**	98%**	96%	100%	100%
Homozygous	1%**	3%**	1%*	8%**	5%**	0%**	1%**	2%$^{**-}$	2%$^{**-}$	15%**	2%**	4%	0%	0%
Compound Het.	52%**	49%**	53%**	52%**	76%**	99%**	38%*	53%**	27%	37%**	64%**	62%**	96%**	55%$^{**-}$
Colchicine response	56%**	40%**	63%*	36%**	36%**	75%**	49%$^{**-}$	27%**	18%**	30%**	30%*	48%*	43%	56%
Fever	58%**	52%**	56%	53%**	52%*	53%	52%	51%	40%**	46%**	46%	56%	47%	60%
Arthralgia	44%*	45%*	42%	54%**	42%	63%**	51%*	41%	34%**	42%*	46%	53%*	54%*	63%*
Back pain	13%	14%	8%	13%	15%	9%	16%	17%	13%	14%	13%	19%	11%	11%
Chest Pain	22%	21%	23%	18%	19%	15%	23%	26%*	16%	19%	22%	26%	31%	15%
Abdominal pain	84%**	81%	82%	79%*	83%$^{**-}$	77%	79%	85%*	83%	78%*	79%	88%*	83%	74%
GIS issues														
Diarrhea	17%**	18%**	17%	23%	25%*	20%	24%	34%**	17%	24%	27%	28%	13%	16%
Vomiting	17%*	14%**	12%	14%**	18%	10%	17%	23%	22%	21%	20%	21%	7%*	14%
Amyloidosis	75%	78%	80%	86%	76%	76%	74%	70%*	81%	76%	68%	60%	61%	79%
Eryhtema	67%$^{**-}$	69%**	73%	73%$^{**-}$	69%*	69%	69%	66%	76%	72%	68%*	48%**	58%	79%
Age Groups														
Infant	2%	1%	3%	3%	1%	2%	2%	2%	2%	24%	1%	0%	2%	2%
Toddler	3%	4%	2%	5%	3%	3%	3%	3%	5%	23%	3%	4%	2%	2%
Kid	21%	22%	17%	30%	23%	30%	29%	24%	27%	26%	27%	21%	13%	36%
Teen	14%	15%	16%	17%	17%	18%	13%	16%	18%	26%	17%	15%	13%	16%
Adult	60%	58%	62%	45%	56%	47%	53%	55%	48%	23%	51%	59%	60%	44%

$p<0.05$ (*), $p\leq0.05$ ($^{*-}$), $p<0.001$(**), $p\leq0.001$ ($^{**-}$)

zygosity was not specified for a variant. Considering the number of people who were homozygous, R202Q was the most common homozygous variant. E148Q, M694V, V726A, and M680I followed R202Q in decreasing order. However, the second-highest rate of homozygosity (15.2%) was observed with the E148Q variant ($p < 0.001$). On the other hand, the M694V variant was leading the heterozygous and compound heterozygous variant categories. Of these heterozygotes for M694V, 3989 of them were compound heterozygotes. E148Q and M680I were the two most common co-variants with 783 and 769 people, respectively. The other most common heterozygous variants were R202Q, V726A, M680I, and E148Q in descending order. On the other hand, the highest rate of heterozygosity was observed within A744S which stands out as the highest percentage of the statistically significant variants with 99.1% ($p < 0.001$). The most common variants present in a compound heterozygous state were M694V, R202Q, E148Q, V726A, and M680I in decreasing order. M694V was the most common co-variant with R202Q, which was found in 1944 people.

Gender association

There were in total 14,116 females, 11,578 males, and 1810 gender unspecified participants in the study. The gender distribution of the data population can be seen in Fig. 4.

Table 1 (continued)

	E230K	G304R	E148V	E148D	M694K	M680L	M680V	R761C	T177I	E251K	A289V	V449M	S339F	I591T
Gender association														
Female	38%	55%	57%	100%	-	11%*	100%	55%	20%	67%	50%	0%	25%*	71%
Male	63%	46%	43%	0%	-	89%*	0%	45%	80%	33%	50%	100%	75%*	29%
Zygosity														
Heterozygous	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Homozygous	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	0%	0%	0%	0%
Compound Het.	75%**	45%*	50%	100%	0%	11%	50%	100%	40%	50%	100%	0%	71%*	84%**
Colchicine response	100%**	58%$^{*-}$	67%	-	-	14%	-	100%	100%	0%	100%	100%	50%	100%*
Fever	50%	46%	78%*	0%	-	33%	50%	100%	25%	33%	50%	0%	60%	43%
Arthralgia	50%	50%	65%	100%	-	29%	100%	0%	50%	50%	100%	100%	67%	59%
Back pain	10%	18%	23%	0%	-	0%	0%	0%	25%	0%	0%	0%	0%	9%
Chest Pain	36%	27%	30%	-	-	33%	-	0%	0%	0%	0%	0%	0%	13%
Abdominal pain	79%	83%	93%	100%	-	100%	50%	100%	50%	75%	100%	100%	100%	77%
GIS issues														
Diarrhea	44%	7%*	33%	-	-	0%	-	0%	25%	0%	50%	100%	0%	19%
Vomiting	10%	3%*	21%	0%	-	0%	0%	0%	0%	0%	50%	0%	0%	8%
Amyloidosis	100%	89%	75%	-	-	100%	-	100%	50%	100%	100%	100%	100%	88%
Eryhtema	100%	82%	67%	-	-	100%	-	100%	50%	100%	100%	100%	100%	50%
Age Groups														
Infant	0%	0%	5%	0%	-	0%	0%	0%	0%	0%	0%	0%	0%	12%
Toddler	0%	0%	5%	0%	-	0%	0%	50%	0%	20%	0%	0%	0%	5%
Kid	19%	32%	35%	0%	-	11%	0%	50%	40%	20%	50%	0%	33%	22%
Teen	25%	28%	0%	0%	-	67%	0%	0%	20%	60%	50%	0%	17%	10%
Adult	56%	40%	55%	100%	-	22%	100%	0%	40%	0%	0%	100%	50%	51%

$p<0.05$ (*), $p\leq0.05$ ($^{*-}$), $p<0.001$(**), $p\leq0.001$ ($^{**-}$)

Table 1 (continued)

	A89T	P633L	S179I	V722M	H478Y	E225D	E225G	I259V	L110P	S749C	G687D	K695M	K695N	G678E	H123Q
Gender association															
Female	100%	100%	33%	82%	86%**	0%	0%	60%	56%	-	50%	53%	100%	67%	0%
Male	0%	0%	67%	18%	14%**	100%	100%	40%	44%	-	50%	47%	0%	33%	100%
Zygosity															
Heterozygous	100%	-	100%	100%	100%	100%	-	100%	100%	-	100%	100%	100%	-	100%
Homozygous	0%	-	0%	0%	0%	0%	-	0%	0%	-	0%	0%	0%	-	0%
Compound Het.	20%	0%	75%	36%	79%**	67%	0%	33%	100%**	0%	100%*	47%	0%	78%*	100%
Colchicine response	33%	0%	-	100%	14%	100%	-	-	86%**	-	50%	38%	-	60%	100%
Fever	50%	0%	67%	38%	40%	-	0%	50%	65%	100%	67%	12%*	-	33%	100%
Arthralgia	100%	0%	0%	29%	40%	-	0%	50%	48%	-	33%	0%	100%	25%	100%
Back pain	100%*	0%	0%	0%	-	-	0%	25%	8%	-	0%	0%	-	0%	0%
Chest Pain	100%*	0%	0%	25%	-	-	0%	0%	32%	-	0%	0%	100%	33%	0%
Abdominal pain	50%	100%	100%	100%	100%	100%	100%	60%	88%	-	100%	86%	0%	100%	100%
GIS issues															
Diarrhea	0%	0%	0%	25%	100%*	-	0%	0%	5%	-	0%	0%	-	67%	0%
Vomiting	67%	0%	0%	0%	100%*	-	0%	20%	0%*	-	0%	0%	-	33%	0%
Amyloidosis	100%	-	-	60%	-	-	100%	-	95%	-	100%	0%	-	80%	100%
Eryhtema	100%	0%	-	75%	100%	-	100%	-	90%	-	100%	100%	100%	25%	100%
Age Groups															
Infant	0%	0%	0%	0%	0%	0%	0%	20%	5%	-	0%	0%	0%	0%	0%
Toddler	0%	0%	33%	11%	0%	0%	0%	0%	8%	-	0%	0%	0%	0%	0%
Kid	20%	0%	0%	22%	29%	100%	0%	20%	33%	-	25%	40%	0%	0%	0%
Teen	40%	0%	33%	0%	21%	0%	0%	0%	21%	-	50%	40%	100%	40%	0%
Adult	40%	100%	33%	68%	50%	0%	100%	60%	33%	-	25%	20%	0%	60%	100%

$p<0.05$ (*), $p\leq0.05$ (*-), $p<0.001$(**), $p\leq0.001$ (**-)

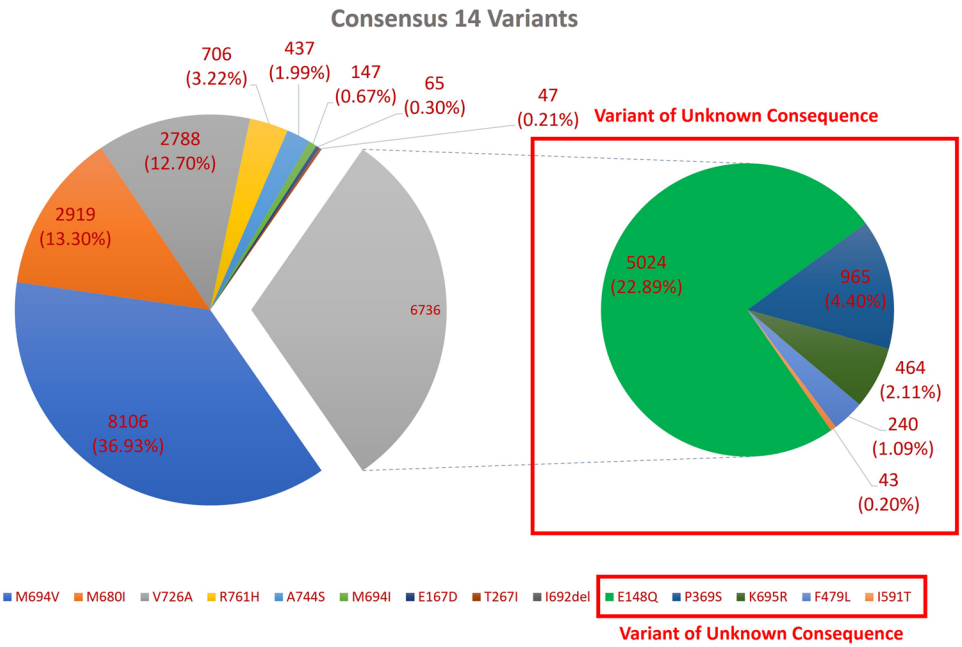

Fig. 3 The distribution of the "Consensus 14" variants in the study population. Nine pathogenic variants, M694V, M694I, M680I, V726A, R761H, A744S, E167D, T267I, and I692del and 5 variants of uncertain significance (VUS) K695R, E148Q, P369S, F479L, and I591T are shown together with the corresponding number of people for each variant. I692del was not represented in the population.

The top 10 variants in both genders were the same (Fig. 5). M694V, E148Q, and R202Q are the top 3 variants for both genders with M694V drastically taking the lead in both genders. M694V was the variant with the highest statistical association ($p < 0.001$) with gender; 52.3% of this variant was present in females. I591T and P369S were the two variants most commonly associated with females, 70.7% ($p = 0.047$) and 60.4% ($p = 0.002$), respectively. E148Q, M680I, and R202Q also had a statistically significant relationship with gender and all three of them were also present more in females than males.

Age groups

Nearly 50% (13,502) of the variants have been reported in adults (20+ years). Only about 1.5% (439) of the cases were seen in infants (0–2 years). In nearly 8% (2194) of the cases, the age group of the patient was not reported. Toddlers (2–4 years), kids (4–13 years), and teens (13–20 years) were represented with 3% (912), 23% (6427), and 15% (4030), respectively. The age distribution of detected variants is shown in Fig. 6.

Statistical analysis

Many of the statistical data given for different clinical features can be found in Tables 1 and 2. Table 1 provides the percentage of each variant for each clinical feature, age group, and gender together with the degree of statistical significance when relevant. Table 2 provides the results of the multivariate regression analysis.

Fever

Only 39% of the people reported a fever, but nearly 37%—of the participants did not show this most characteristic symptom of the disorder. In 24% of the participants, the fever status was not mentioned. 42.3% of people with fever had the M694V variant, but only 52.2% of the people with this variant showed fever as a symptom ($p < 0.001$); 52.7% of the people with the R202Q variant also had fever and this makes up for nearly 30% of the people that reported a fever ($p < 0.001$). Conversely, 54.3% of the people with the E148Q variant had no fever, and 25.5% of the participants without a fever had the E148Q variant ($p < 0.001$); 59.9% of the participants with the K695R variant also had no fever but that only accounted for 2.4% of all people without a fever ($p = 0.001$). Gender had no correlation with the fever status. Kids, teens, and adults were less likely than toddlers to have a fever (kids $p = 0.008$, teens and adults $p < 0.001$). M694I and F479L are likely to increase the probability of reporting fever 1.995 and 1.830 times, respectively ($p = 0.001$ and $p = 0.003$). M680I, M694V, V726A, A744S, and R761H also had a strong positive correlation with fever, making fever more likely if they are present (see Table 2 for multivariate regression analysis). Fever correlated significantly with colchicine response, arthralgia, erythema, amyloidosis, family history, and kidney issues ($p < 0.001$).

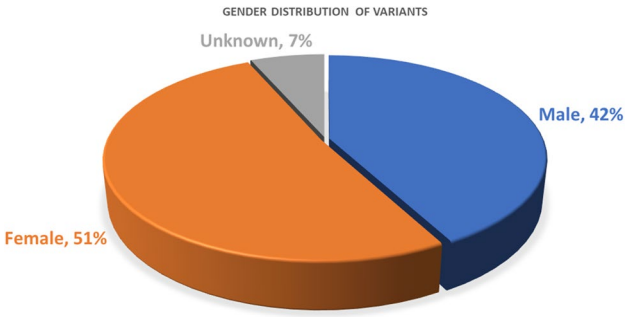

Fig. 4 The gender distribution of the study population. Fifty-one percent of the study population was female, and 42% of the study population was male. In 7% of the study population, the gender of the participant was not recorded or mentioned

Abdominal pain

Abdominal pain criterion was reported in 63% of the study population. Of this group, nearly 80% reported having abdominal pain. All age groups also showed a statistical relationship with abdominal pain ($p < 0.001$). Abdominal pain was strongly associated with V726A ($p < 0.001$), M680I ($p < 0.001$), and R761H ($p = 0.004$), and these three variants also made abdominal pain approximately 1.4-fold more likely ($p < 0.001$ for V726A and M680I, $p = 0.013$ for R761H). K695R also made abdominal pain 1.5-fold more

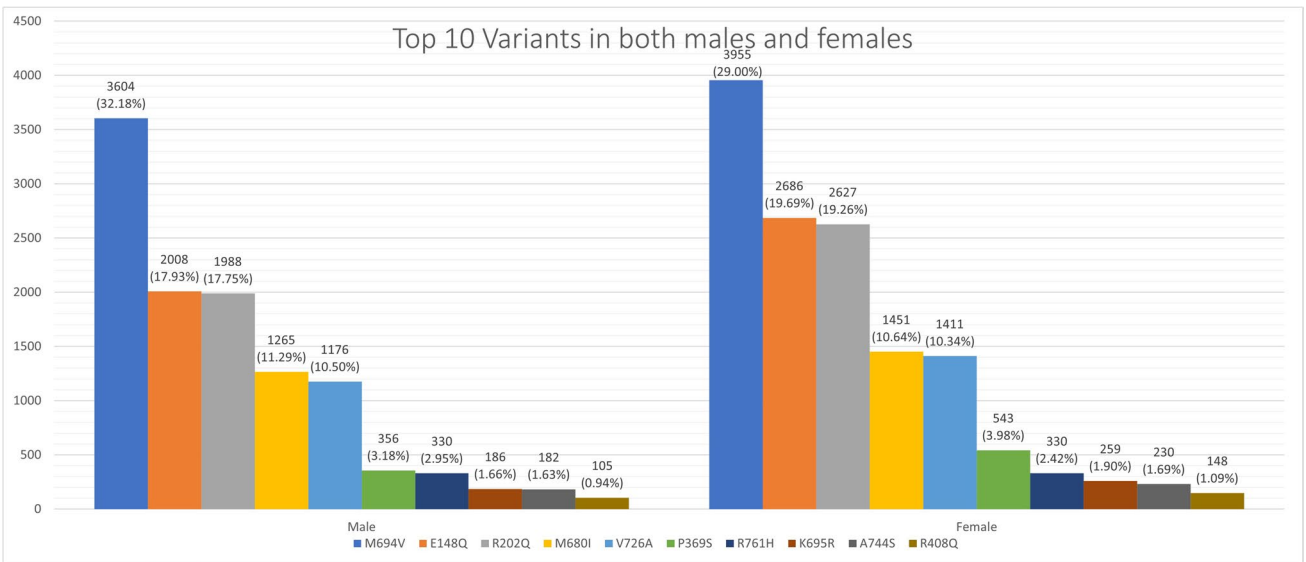

Fig. 5 The top 10 variants in both genders. The top 10 variants in both genders were exactly the same as each other. M694V, E148Q, and R202Q were the top 3 but M694V is by far the most common variant in both genders

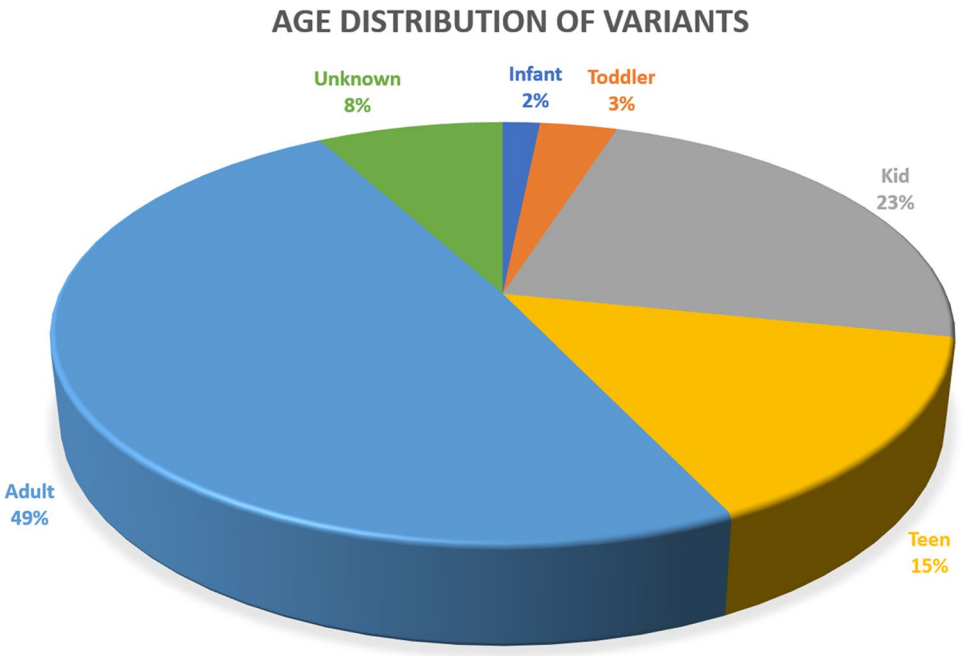

Fig. 6 The age distribution of the study population. Forty-nine percent of the variants in the study population were detected in adults. While the age group of the patient was not reported in approximately 8% of cases, the distribution of detected variants in infants, toddlers, kids, and teens were 2%, 3%, 23%, and 15%, respectively

likely (p =0.029). Abdominal pain was also associated with all the other clinical criteria in the study ($p < 0.001$). The female gender was also statistically less associated with abdominal pain by 0.845-fold ($p = 0.001$).

Arthralgia/Arthritis

Thirty-eight of the participants did not report arthralgia, while 32 percent of the participants were suffering from arthralgia. In nearly 30% of the participants, arthralgia status was unknown. Variants with a statistically significant relationship with articular involvement were E148Q, A744S, T267I, E167D, F479L, K695R, R408Q, and R202Q. E148Q and K695R had a negative relationship with arthralgia. 58.4% ($p = 0.003$) of the people with E148Q and 66.4% ($p = 0.001$) of the people with K695R had no joint pain and/or inflammation. Although E148Q accounts for 25.3% of all people without arthralgia, K695R only accounts for 2.2%. R202Q was a variant that was strongly associated with arthralgia, as 53.6% of the participants with this variant had joint involvement. Additionally, R202Q accounted for 31.6% of all people who reported arthralgia ($p < 0.001$). The other variants that were significantly associated with arthralgia only accounted for 6.5% of all the people that reported arthralgia. Carrying the T267I or the E167D alleles meant a 2.5-fold risk to experience arthralgia. R202Q is also associated with 2 times greater odds of developing arthralgia. M680I, M694V, A744S, E148Q, and P369S were also associated with a statistically significant increased chance of developing arthralgia. Males were less likely than females to present with arthralgia by 0.7 times ($p < 0.001$) Arthritis correlated strongly with erythema, amyloidosis, family history, and renal involvement ($p < 0.001$).

Back pain and chest pain

Back and chest pain were the two categories with the least amount of available patient data. In 59% of the cases back pain status was not reported, while the chest pain status of 60% of the participants was unknown. M694V was the only variant that was statistically associated with back pain as 85.6% of people with this variant reported back pain. Patients with this variant accounted for 39.2% of the people with this symptom. M694V, M680I, R761H, and R202Q had a negative correlation with chest pain. People with these variants made up 88.5% of people without chest pain with M694V in the lead with 39% ($p = 0.013$). R202Q was the most significant with a p value of 0.011 and accounted for 31.3% of people without chest pain. F479L variant increased the chance of backache by nearly 2.2 times ($p = 0.039$). Kids were 2.3 times more likely to have back pain than other age groups ($p = 0.023$). Males were 0.75 times less likely to have back pain ($p < 0.001$). R761H variant was the only variant that would significantly increase chest pain by 1.4 times ($p = 0.046$). Gender was not a significant factor in chest pain. Back pain and chest pain were correlated with each other. Back pain was correlated with renal involvement, family history of FMF, and diarrhea. Likewise, chest pain was correlated with the same issues as back pain, but additionally, it was also correlated with amyloidosis ($p < 0.001$).

Table 2 Multi-variate regression analysis. Consensus 14 variants and R202Q, age groups and gender were compared together to different clinical features. The statistical significance was indicated under the Sig title and the cut of value for significance was taken as $p < 0.05$. For significance, 0 = ($p < 0.001$). The fold values used in the text was taken from the Exp(*B*). For a *p* value <0.05, when the Exp(*B*) values are larger than 1, that clinical feature is more likely with that variant/gender/age group, and when the values are less than 1, the clinical feature is less likely with variant/gender/age group

	B	SE	Wald	*df*	Sig.	Exp(*B*)	95% CI for EXP(*B*)	
							Lower	Upper
Colchicine response								
Variants								
M680I	0.983	0.083	141.392	1	0	2.672	2.272	3.142
M694V	0.603	0.064	89.516	1	0	1.827	1.613	2.07
M694I	0.744	0.342	4.745	1	0.029	2.104	1.077	4.11
V726A	0.447	0.086	27.067	1	0	1.564	1.321	1.85
A744S	1.477	0.205	51.801	1	0	4.382	2.93	6.552
R761H	0.624	0.143	19.069	1	0	1.867	1.411	2.471
E167D	0.93	0.632	2.166	1	0.141	2.533	0.735	8.737
T267I	1.119	0.684	2.678	1	0.102	3.062	0.802	11.698
K695R	−0.436	0.247	3.116	1	0.078	0.646	0.398	1.049
E148Q	0.357	0.078	21.131	1	0	1.43	1.228	1.665
P369S	0.496	0.143	12.035	1	0.001	1.643	1.241	2.175
F479L	0.649	0.331	3.85	1	0.05	1.915	1.001	3.663
I591T	21.737	17,961.831	0	1	0.999	2,756,714,012.348	0	
R202Q	1.043	0.07	224.075	1	0	2.838	2.476	3.254
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	−1.248	0.322	15.071	1	0	0.287	0.153	0.539
Infant vs. kid	−0.476	0.26	3.358	1	0.067	0.621	0.373	1.034
Infant vs. teen	−0.531	0.263	4.073	1	0.044	0.588	0.351	0.985
Infant vs. adult	−0.34	0.258	1.73	1	0.188	0.712	0.429	1.181
Gender								
Female vs. male	−0.071	0.055	1.646	1	0.2	0.932	0.836	1.038
Fever								
Variants								
M680I	0.569	0.056	104.643	1	0	1.766	1.584	1.97
M694V	0.345	0.042	67.622	1	0	1.412	1.3	1.533
M694I	0.691	0.208	10.991	1	0.001	1.995	1.326	3.001
V726A	0.278	0.056	24.632	1	0	1.321	1.183	1.474
A744S	0.384	0.129	8.816	1	0.003	1.469	1.14	1.893
R761H	0.21	0.098	4.554	1	0.033	1.233	1.017	1.495
E167D	−0.429	0.34	1.587	1	0.208	0.651	0.334	1.269
T267I	0.529	0.331	2.546	1	0.111	1.697	0.886	3.249
K695R	−0.195	0.133	2.158	1	0.142	0.823	0.634	1.067
E148Q	0.056	0.051	1.225	1	0.268	1.058	0.958	1.168
P369S	0.174	0.089	3.814	1	0.051	1.19	0.999	1.418
F479L	0.604	0.204	8.81	1	0.003	1.83	1.228	2.728
I591T	−0.339	0.386	0.769	1	0.381	0.713	0.334	1.519
R202Q	0.262	0.045	34.19	1	0	1.299	1.19	1.418
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	0.187	0.166	1.265	1	0.261	1.205	0.871	1.668
Infant vs. kid	−0.358	0.135	7.071	1	0.008	0.699	0.537	0.91
Infant vs. teen	−0.947	0.137	47.472	1	0	0.388	0.296	0.508
Infant vs. adult	−0.915	0.133	47.165	1	0	0.4	0.308	0.52
Gender								
Female vs.	0.006	0.036	0.024	1	0.876	1.006	0.937	1.079
Arthralgia/Arthritis								
Variants								
M680I	0.192	0.057	11.295	1	0.001	1.212	1.083	1.355
M694V	0.089	0.044	4.188	1	0.041	1.093	1.004	1.191
M694I	0.282	0.211	1.792	1	0.181	1.326	0.877	2.003
V726A	0.042	0.058	0.531	1	0.466	1.043	0.931	1.169
A744S	0.482	0.133	13.226	1	0	1.619	1.249	2.1

Table 2 (continued)

	B	SE	Wald	*df*	Sig.	Exp(*B*)	95% CI for EXP(*B*)	
							Lower	Upper
R761H	0.084	0.101	0.694	1	0.405	1.088	0.892	1.327
E167D	0.753	0.349	4.665	1	0.031	2.124	1.072	4.206
T267I	0.915	0.334	7.48	1	0.006	2.496	1.296	4.807
K695R	−0.231	0.144	2.568	1	0.109	0.794	0.599	1.053
E148Q	0.126	0.052	5.797	1	0.016	1.134	1.024	1.257
P369S	0.232	0.091	6.536	1	0.011	1.261	1.056	1.505
F479L	0.249	0.204	1.489	1	0.222	1.283	0.86	1.916
I591T	0.384	0.393	0.957	1	0.328	1.468	0.68	3.169
R202Q	0.702	0.046	228.492	1	0	2.017	1.842	2.21
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	0.272	0.192	2.017	1	0.155	1.313	0.902	1.913
Infant vs. kid	0.942	0.154	37.221	1	0	2.566	1.896	3.472
Infant vs. teen	1.075	0.157	47.073	1	0	2.931	2.156	3.986
Infant vs. adult	1.273	0.153	69.268	1	0	3.571	2.646	4.819
Gender								
Female vs. male	−0.301	0.037	64.567	1	0	0.74	0.688	0.796
Back pain								
Variants								
M680I	−0.023	0.11	0.045	1	0.832	0.977	0.787	1.212
M694V	0.111	0.081	1.906	1	0.167	1.118	0.954	1.309
M694I	−0.419	0.477	0.771	1	0.38	0.658	0.258	1.676
V726A	0.101	0.113	0.804	1	0.37	1.107	0.887	1.38
A744S	0.309	0.23	1.795	1	0.18	1.362	0.867	2.138
R761H	0.222	0.203	1.187	1	0.276	1.248	0.838	1.859
E167D	−0.898	0.603	2.218	1	0.136	0.407	0.125	1.328
T267I	−0.126	0.54	0.055	1	0.815	0.881	0.306	2.538
K695R	0.043	0.298	0.021	1	0.884	1.044	0.583	1.872
E148Q	0.152	0.102	2.21	1	0.137	1.164	0.953	1.421
P369S	−0.071	0.188	0.143	1	0.706	0.932	0.645	1.346
F479L	0.799	0.388	4.241	1	0.039	2.224	1.039	4.759
I591T	−0.409	0.752	0.296	1	0.586	0.664	0.152	2.899
R202Q	0.096	0.084	1.285	1	0.257	1.1	0.933	1.298
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	0.186	0.474	0.153	1	0.695	1.204	0.475	3.05
Infant vs. kid	0.842	0.371	5.146	1	0.023	2.321	1.121	4.806
Infant vs. teen	1.264	0.373	11.472	1	0.001	3.541	1.704	7.36
Infant vs. adult	1.779	0.363	23.975	1	0	5.924	2.906	12.076
Gender								
Female vs. male	−0.276	0.075	13.505	1	0	0.759	0.655	0.879
Chest pain								
Variants								
M680I	0.067	0.091	0.543	1	0.461	1.069	0.895	1.277
M694V	0	0.071	0	1	0.998	1	0.87	1.149
M694I	0.298	0.341	0.765	1	0.382	1.348	0.691	2.63
V726A	−0.08	0.099	0.647	1	0.421	0.923	0.76	1.122
A744S	0.143	0.209	0.464	1	0.496	1.153	0.765	1.738
R761H	0.337	0.169	3.989	1	0.046	1.401	1.006	1.95
E167D	0.666	0.575	1.341	1	0.247	1.946	0.631	6.007
T267I	−0.167	0.636	0.069	1	0.793	0.846	0.243	2.941
K695R	−0.045	0.271	0.028	1	0.867	0.956	0.562	1.625
E148Q	−0.078	0.089	0.76	1	0.383	0.925	0.776	1.102
P369S	0.085	0.157	0.29	1	0.591	1.088	0.8	1.481

Table 2 (continued)

	B	SE	Wald	*df*	Sig.	Exp(*B*)	95% CI for EXP(*B*)	
							Lower	Upper
F479L	−0.135	0.391	0.119	1	0.73	0.874	0.406	1.879
I591T	−0.263	0.78	0.114	1	0.736	0.768	0.167	3.546
R202Q	0.055	0.077	0.51	1	0.475	1.057	0.908	1.229
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	−0.365	0.419	0.757	1	0.384	0.694	0.305	1.58
Infant vs. kid	0.956	0.294	10.587	1	0.001	2.601	1.462	4.627
Infant vs. teen	1.546	0.295	27.392	1	0	4.695	2.631	8.377
Infant vs. adult	1.785	0.289	38.251	1	0	5.957	3.384	10.486
Gender								
Female vs. male	−0.088	0.062	2.017	1	0.156	0.916	0.811	1.034
Diarrhea								
Variants								
M680I	−0.453	0.104	19.118	1	0	0.636	0.519	0.779
M694V	−0.455	0.072	39.544	1	0	0.634	0.55	0.731
M694I	−0.299	0.379	0.622	1	0.43	0.741	0.352	1.559
V726A	0.201	0.097	4.277	1	0.039	1.222	1.011	1.479
A744S	−0.013	0.203	0.004	1	0.949	0.987	0.663	1.469
R761H	0.592	0.157	14.289	1	0	1.808	1.33	2.458
E167D	−1.3	0.706	3.389	1	0.066	0.273	0.068	1.088
T267I	−0.65	0.632	1.058	1	0.304	0.522	0.151	1.802
K695R	−0.309	0.27	1.307	1	0.253	0.734	0.432	1.247
E148Q	0.062	0.087	0.506	1	0.477	1.064	0.897	1.261
P369S	0.114	0.146	0.607	1	0.436	1.121	0.842	1.492
F479L	0.526	0.36	2.132	1	0.144	1.692	0.835	3.428
I591T	−0.409	0.644	0.402	1	0.526	0.665	0.188	2.35
R202Q	0.049	0.078	0.397	1	0.528	1.05	0.902	1.223
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	−0.432	0.221	3.827	1	0.05	0.649	0.421	1.001
Infant vs. kid	−0.007	0.164	0.002	1	0.964	0.993	0.72	1.368
Infant vs. teen	−0.087	0.173	0.255	1	0.614	0.916	0.653	1.287
Infant vs. adult	0.019	0.161	0.014	1	0.906	1.019	0.743	1.397
Gender								
Female vs. male	0.052	0.061	0.738	1	0.39	1.054	0.935	1.187
Vomiting								
Variants								
M680I	−0.241	0.098	6.042	1	0.014	0.786	0.649	0.952
M694V	−0.419	0.075	31.192	1	0	0.657	0.568	0.762
M694I	−0.595	0.411	2.094	1	0.148	0.552	0.246	1.235
V726A	−0.125	0.1	1.554	1	0.213	0.883	0.725	1.074
A744S	−0.272	0.217	1.571	1	0.21	0.762	0.498	1.166
R761H	0.196	0.162	1.464	1	0.226	1.217	0.886	1.672
E167D	−1.517	0.697	4.732	1	0.03	0.219	0.056	0.861
T267I	−0.58	0.487	1.415	1	0.234	0.56	0.215	1.456
K695R	−0.023	0.229	0.01	1	0.919	0.977	0.623	1.532
E148Q	−0.02	0.088	0.054	1	0.817	0.98	0.825	1.164
P369S	−0.098	0.148	0.433	1	0.511	0.907	0.678	1.213
F479L	0.202	0.381	0.28	1	0.597	1.224	0.58	2.582
I591T	−0.848	0.746	1.294	1	0.255	0.428	0.099	1.846
R202Q	−0.499	0.079	39.572	1	0	0.607	0.52	0.71
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	−0.486	0.22	4.897	1	0.027	0.615	0.4	0.946

Table 2 (continued)

	B	SE	Wald	*df*	Sig.	Exp(*B*)	95% CI for EXP(*B*)	
							Lower	Upper
Infant vs. kid	−0.1	0.165	0.366	1	0.545	0.905	0.655	1.251
Infant vs. teen	−0.236	0.174	1.852	1	0.174	0.79	0.562	1.11
Infant vs. adult	−0.632	0.164	14.809	1	0	0.531	0.385	0.733
Gender								
Female vs. male	−0.11	0.063	3.037	1	0.081	0.896	0.792	1.014
Erythema								
Variants								
M680I	−0.271	0.079	11.622	1	0.001	0.763	0.653	0.891
M694V	−0.314	0.064	23.811	1	0	0.73	0.644	0.829
M694I	0.059	0.33	0.032	1	0.857	1.061	0.556	2.025
V726A	−0.24	0.084	8.077	1	0.004	0.787	0.667	0.928
A744S	−0.202	0.18	1.253	1	0.263	0.817	0.574	1.164
R761H	−0.277	0.147	3.57	1	0.059	0.758	0.568	1.01
E167D	0.575	0.548	1.104	1	0.293	1.778	0.608	5.202
T267I	0.486	0.588	0.684	1	0.408	1.626	0.514	5.146
K695R	0.023	0.246	0.009	1	0.926	1.023	0.632	1.656
E148Q	−0.109	0.078	1.932	1	0.165	0.897	0.77	1.046
P369S	−0.277	0.138	4.002	1	0.045	0.758	0.578	0.994
F479L	−1.251	0.277	20.418	1	0	0.286	0.166	0.492
I591T	−0.451	0.602	0.561	1	0.454	0.637	0.196	2.072
R202Q	0.249	0.072	11.967	1	0.001	1.283	1.114	1.477
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	2.572	0.221	135.128	1	0	13.09	8.484	20.196
Infant vs. kid	2.334	0.169	191.405	1	0	10.322	7.416	14.368
Infant vs. teen	2.159	0.175	152.93	1	0	8.658	6.15	12.19
Infant vs. adult	2.058	0.165	155.661	1	0	7.828	5.666	10.815
Gender								
Female vs. male	0.082	0.055	2.184	1	0.139	1.085	0.974	1.209
Amyloidosis								
Variants								
M680I	−0.113	0.095	1.431	1	0.232	0.893	0.741	1.075
M694V	−0.108	0.078	1.892	1	0.169	0.898	0.77	1.047
M694I	0.225	0.387	0.337	1	0.561	1.252	0.586	2.672
V726A	−0.148	0.101	2.16	1	0.142	0.862	0.707	1.051
A744S	−0.114	0.207	0.306	1	0.58	0.892	0.595	1.338
R761H	−0.347	0.17	4.154	1	0.042	0.707	0.506	0.987
E167D	0.12	0.61	0.039	1	0.844	1.128	0.341	3.731
T267I	0.296	0.572	0.268	1	0.605	1.344	0.438	4.121
K695R	0.133	0.302	0.194	1	0.66	1.142	0.632	2.066
E148Q	−0.117	0.091	1.624	1	0.203	0.89	0.744	1.065
P369S	−0.5	0.153	10.656	1	0.001	0.607	0.449	0.819
F479L	−0.832	0.38	4.781	1	0.029	0.435	0.207	0.917
I591T	0.363	0.768	0.223	1	0.637	1.437	0.319	6.471
R202Q	0.793	0.089	79.592	1	0	2.21	1.857	2.631
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	0.746	0.229	10.581	1	0.001	2.109	1.345	3.306
Infant vs. kid	0.913	0.18	25.799	1	0	2.492	1.752	3.544
Infant vs. teen	0.796	0.188	17.998	1	0	2.217	1.535	3.203
Infant vs. adult	0.943	0.176	28.729	1	0	2.567	1.818	3.623
Gender								
Female vs. male	−0.089	0.065	1.871	1	0.171	0.915	0.805	1.039

Table 2 (continued)

	B	SE	Wald	*df*	Sig.	Exp(*B*)	95% CI for EXP(*B*)	
							Lower	Upper
FMF history								
Variants								
M680I	0.036	0.098	0.137	1	0.711	1.037	0.856	1.257
M694V	0.036	0.078	0.215	1	0.643	1.037	0.889	1.209
M694I	0.396	0.388	1.043	1	0.307	1.486	0.695	3.178
V726A	−0.012	0.101	0.014	1	0.905	0.988	0.811	1.204
A744S	−0.074	0.206	0.127	1	0.722	0.929	0.62	1.392
R761H	−0.001	0.188	0	1	0.995	0.999	0.691	1.444
E167D	2.538	0.838	9.168	1	0.002	12.658	2.448	65.454
T267I	0.222	0.522	0.182	1	0.67	1.249	0.449	3.473
K695R	0.558	0.304	3.376	1	0.066	1.747	0.963	3.166
E148Q	−0.017	0.091	0.037	1	0.848	0.983	0.822	1.175
P369S	0.113	0.166	0.465	1	0.495	1.12	0.809	1.549
F479L	−1.282	0.315	16.576	1	0	0.277	0.15	0.514
I591T	−0.308	0.549	0.315	1	0.575	0.735	0.25	2.156
R202Q	0.777	0.088	77.699	1	0	2.175	1.83	2.585
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	2.919	0.273	114.422	1	0	18.524	10.85	31.624
Infant vs. kid	2.884	0.209	191.141	1	0	17.895	11.889	26.935
Infant vs. teen	2.781	0.215	166.872	1	0	16.134	10.58	24.603
Infant vs. adult	2.638	0.205	166.165	1	0	13.987	9.365	20.89
Gender								
Female vs. male	0.116	0.066	3.047	1	0.081	1.123	0.986	1.278
Renal involvement								
Variants								
M680I	−0.303	0.093	10.574	1	0.001	0.739	0.616	0.887
M694V	−0.381	0.073	27.312	1	0	0.683	0.592	0.788
M694I	−0.094	0.366	0.066	1	0.797	0.91	0.444	1.867
V726A	−0.318	0.102	9.77	1	0.002	0.727	0.596	0.888
A744S	−0.237	0.216	1.201	1	0.273	0.789	0.517	1.205
R761H	−0.637	0.17	14.019	1	0	0.529	0.379	0.738
E167D	0.438	0.565	0.602	1	0.438	1.55	0.512	4.689
T267I	−0.154	0.545	0.08	1	0.777	0.857	0.294	2.495
K695R	−0.366	0.272	1.804	1	0.179	0.694	0.407	1.183
E148Q	−0.21	0.091	5.382	1	0.02	0.811	0.679	0.968
P369S	−0.578	0.157	13.486	1	0	0.561	0.412	0.764
F479L	−1.754	0.318	30.464	1	0	0.173	0.093	0.323
I591T	−0.459	0.823	0.312	1	0.577	0.632	0.126	3.169
R202Q	0.198	0.08	6.179	1	0.013	1.219	1.043	1.425
692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	2.582	0.24	115.301	1	0	13.223	8.254	21.183
nfant vs. kid	2.318	0.185	156.464	1	0	10.159	7.064	14.608
Infant vs. teen	2.234	0.193	133.843	1	0	9.335	6.394	13.629
Infant vs. adult	2.346	0.182	165.76	1	0	10.442	7.306	14.924
Gender								
Female vs. male	0.098	0.064	2.303	1	0.129	1.103	0.972	1.251
Abdominal pain								
Variants								
M680I	0.352	0.078	20.155	1	0	1.422	1.219	1.658
M694V	0.177	0.057	9.723	1	0.002	1.194	1.068	1.335
M694I	0.477	0.305	2.446	1	0.118	1.611	0.886	2.928
V726A	0.368	0.082	20.034	1	0	1.444	1.229	1.696

Table 2 (continued)

	B	SE	Wald	*df*	Sig.	Exp(*B*)	95% CI for EXP(*B*)	
							Lower	Upper
A744S	0.041	0.163	0.064	1	0.800	1.042	0.756	1.436
R761H	0.361	0.145	6.182	1	0.013	1.435	1.079	1.907
E167D	−0.366	0.469	0.607	1	0.436	0.694	0.276	1.741
T267I	−0.433	0.374	1.338	1	0.247	0.649	0.312	1.350
K695R	0.443	0.203	4.770	1	0.029	1.558	1.047	2.320
E148Q	0	0.068	0	1	0.998	1.000	0.875	1.143
P369S	−0.012	0.117	0.010	1	0.919	0.988	0.786	1.242
F479L	0.647	0.325	3.973	1	0.046	1.911	1.011	3.611
I591T	0.118	0.448	0.069	1	0.793	1.125	0.468	2.706
R202Q	−0.032	0.060	0.280	1	0.597	0.969	0.860	1.090
I692del*	–	–	–	–	–	–	–	–*
Age groups								
Infant vs. toddler	0.791	0.167	22.532	1	0	2.206	1.591	3.059
Infant vs. kid	1.575	0.136	134.220	1	0	4.832	3.701	6.307
Infant vs. teen	1.435	0.142	102.611	1	0	4.199	3.181	5.542
Infant vs. adult	1.099	0.132	69.658	1	0	3.002	2.319	3.886
Gender								
Female vs. male	−0.169	0.049	11.976	1	0.001	0.845	0.767	0.929

*Since the I692del variant could not be detected in our patient group, no statistical evaluation could be made for this variant

Colchicine response in different variants

Only around 15% of the people reported a favorable colchicine response, but in nearly 62% of the cases, the colchicine response was not mentioned at all. Twenty-three percent of the people reported partial or complete lack of colchicine response; 26.6% of the people with an unfavorable colchicine response were reported to have the E148Q ($p < 0.001$). R202Q followed E148Q as a close second, making up 20.3% of the unfavorable colchicine response group (p <0 .001). All the participants with the I591T variant had a favorable response to colchicine ($p = 0.002$); 75% and 85.7% of the people with R408Q and L110P variants also showed favorable colchicine response, respectively ($p < 0.001$ for both). Colchicine response had no significant relationship with gender regardless of the variant. Being a toddler or a teen decreased the likelihood of colchicine response ($p < 0.001$ and $p = 0.044$, respectively). Having the A744S variant increased the chance of a favorable colchicine response by 4.382 times ($p < 0.001$). R202Q and M680I variants made it more likely to have a favorable colchicine response by 2.838- and 2.672-fold, respectively ($p < 0.001$ for both). The presence of colchicine response was strongly correlated with the presence of a fever, arthralgia, and amyloidosis ($p < 0.001$ for all except amyloidosis, $p = 0.025$).

Certain gastrointestinal issues

Only 7% of the participants reported gastrointestinal (GI) issues such as attacks of diarrhea and/or constipation, sometimes suggestive of irritable bowel syndrome; 27.2% reported that they did not experience these symptoms. However, in 65% of the cases, the presence or absence of these complaints was not mentioned. V726A, E148Q, and M694V were the three variants that were associated with less GI symptoms in a statistically significant manner. M694V had a stronger association with a p value less than 0.001. 82% of people with the M694V reported no GI issue. R761H was the variant with the highest association with GI symptoms, making it 1.8 times more likely ($p < 0.001$). On the other hand, M680I and M694V made it less likely (0.6 times) to have GI complaints ($p < 0.001$). These symptoms had no significant correlation with gender or different age groups. On the other hand, these complaints were correlated with erythema, a family history of FMF, and kidney problems ($p < 0.001$).

Nausea/Vomiting

Of the participants, 6.9% reported nausea/vomiting. On the contrary, 32.4% of the participants did not experience nausea/vomiting. However, in 60.7% of the participants, no data about nausea/vomiting was available. E148Q, M694V, and

R202Q were all statistically significant with regard to vomiting. The majority of the participants with these variants were not affected by nausea/vomiting ($p < 0.001$). One hundred percent of participants with the L110P variant reported no nausea/vomiting, though the participant size for this variant group was only 43 people (FE 2-sided $p = 0.009$). There were no variants positively correlated with nausea/vomiting. M680I was least associated with nausea/vomiting in terms of statistical significance ($p = 0.014$). Gender had no significant association with nausea/vomiting. Nausea/vomiting was correlated with erythema, a family history of FMF, and kidney problems ($p < 0.001$).

Erythema

Twenty-five percent of the study population reported erythema, while 11% had no such history. There was no information regarding the presence of erythema in 64% of the participants. R202Q ($p = 0.001$) was associated with an increased likelihood of erythema M680I, M694V, and V726A P369S decreased the chance of erythema by approximately 0.7 times ($p = 0.001$, $p < 0.001$, $p = 0.004$, and $p = 0.045$, respectively). All age groups were more than 5 times likely to have erythema compared to infants ($p < 0.001$). Gender was not an important factor in erythema. Erythema was correlated with nearly all the clinical criteria except for colchicine response ($p < 0.001$).

Amyloidosis

Twenty percent of the participants had amyloidosis and 6% reported no amyloidosis. Unfortunately, in 73% of the participants, the amyloidosis status was unknown; 85.8% of the participant with R202Q reported amyloidosis. These participants made up nearly 31.4% of all people with amyloidosis ($p < 0.001$). R202Q, E148Q, P369S, M680I, R761H, and F479L all had a statistically significant relationship with amyloidosis and more than 60% of people with these variants showed amyloidosis. Together, they made up 76.1% of all people with amyloidosis. R202Q increased the chance of amyloidosis by 2.2 times ($p < 0.001$) whereas R761H and P369S decreased the chance of amyloidosis by 0.7 ($p = 0.042$) and 0.6 ($p = 0.001$) times, respectively. Gender had no significant role in developing amyloidosis. Amyloidosis was correlated with colchicine response, fever, arthralgia, chest pain, vomiting, positive family history, and kidney issues ($p < 0.001$).

Family history of renal involvement

Of the people, 23.6% reported a positive family history of kidney problems where 7.1% reported no kidney problems in their family members. In 69.3% of the cases, kidney disease history was not mentioned. Participants with M694V had the highest percentage of family history of kidney issues with 75.4%. These individuals made up 38.4% of the positive family group ($p = 0.021$). 56.7% of the people with the variant F479L reported no family history of renal involvement ($p < 0.001$). R202Q was the only variant that increased the chance of kidney issues by 1.2 times ($p = 0.013$). Family history of renal involvement was correlated with all the clinical criteria ($p < 0.001$).

Other relatives with FMF

Of the participants, 31.1% reported family history, and 6.1% of the participants reported no family history. However, in 62.8% of the participants, family history was not mentioned at all. 88% of people with R202Q reported having relatives with FMF and this variant made up 33.3% of all people where family history was reported ($p < 0.001$). F479L was one variant where the family history was absent with 67.2% of the people with this variant not reporting a family history ($p < 0.001$). E167D variant had a 12.65 ($p = 0.002$) higher chance of presenting with a family history of FMF. R202Q variant also had a 2.17 ($p < 0.001$) chance of presenting with a family history. Positive family history was correlated with all the clinical criteria ($p < 0.001$)

Discussion

FMF, one of the most commonly described monogenic periodic fever syndromes, is an autoinflammatory disease commonly seen in the Turkish, Arab, Jewish, and Armenian populations (Soriano and Manna 2012; Ozdogan and Ugurlu 2019). FMF remains a disease with a complex genotype-phenotype relationship and a complex inheritance pattern. Heterozygous variant carriers in the *MEFV* gene could still display a clinical FMF phenotype, and as high as 25–33% of the people clinically diagnosed with FMF carry only one variant in the *MEFV* gene. Therefore, the autosomal dominant transmission of FMF has also been recognized as a valid inheritance model (Shohat 1993). The distribution of variants in the *MEFV* gene, from one population to the next, or even within the same population, may vary profoundly (Ozen and Batu 2015). Most of the *MEFV* gene variants have been investigated in detail throughout the literature, but their relationship with the clinical aspects of FMF needs to be studied in larger cohorts. Cekin et al. presented a study in 2017, examining the relationship of *MEFV* gene variants with FMF clinical symptoms, but it was limited to only 514 patients and 4 major clinical symptoms (Cekin et al. 2017). Yasar Bilge et al. also asserted that their study in 2019 represented one of the largest samples in Turkey. The data of 1719 FMF patients has been obtained from 15 different

rheumatology clinics in different regions in Turkey. The authors reported the prevalence and clinical significance of common *MEFV* variants in the study, and they concluded that M694V was the most common pathogenic variant in Turkish FMF patients (Yaşar Bilge et al. 2019). Our study with a cohort of more than 27,000 patients investigates the variant profiles in the Turkish population in detail and sheds a light on the complex relationship between the *MEFV* gene variants and the clinical FMF symptoms. Although the number of patients is not evenly distributed across the regions, we hope that our study reflects a general perspective of the national FMF burden. In Turkey, it is thought that the patients mostly originate from the Central and Eastern Anatolian regions and the Black Sea Region (Tunca et al. 2005; Ozdogan and Ugurlu 2019). This data overlaps with ours as the majority of the centers that contributed to our study are from Central Anatolian and the Black Sea Regions.

Nearly 86% of our study population had at least one of the five most frequent variants. These variants are M694V, M680I, and V726A in exon 10; E148Q and R202Q in exon 2. This is in accordance with the literature that exons 10 and 2 variants make up 85% of the variants in the Mediterranean region (Soriano and Manna 2012; Alghamdi 2017). Concordant with an earlier Turkish study of 2838 patients, our findings also demonstrate M694V as the leading pathogenic variant in the Turkish population (Tunca et al. 2005). Additionally, E148Q was reported to be a common variant in the Turkish population (Yildirim et al. 2019). In contrast to M694V, which is associated with a more severe disease phenotype, E148Q and V726A have mostly been reported as variants with a milder phenotypic outlook (Gangemi et al. 2018). There are conflicting reports regarding the pathogenicity of E148Q. It is observed not only in FMF patients but also in the healthy population. Therefore, it is considered a variant showing reduced penetrance. Overall, this variant is currently classified as Variant of Uncertain Significance (VUS) (Eyal et al. 2020). Nearly one-third of variants in the *MEFV* gene are still classified as a variant of unknown significance (Accetturo et al. 2020). Just like M694V, M680I has also been associated with an increase in the severity of the clinical symptoms (Gangemi et al. 2018). Even though R202Q is present in most of the selected targeted-specific sequencing panels and included in our analyses, it is mostly considered as a benign variant. However, there are also publications that emphasize its increased frequency in FMF patients compared to healthy controls and suggest a contributing role in FMF phenotype (Yigit et al. 2012). Milenković et al. (2016) demonstrated higher values of oxidative stress markers and higher incidence of symptoms suggestive of autoinflammation in R202Q homozygous carriers compared to controls.

As mentioned earlier, geography has a major influence on FMF prevalence and severity. Several variants have a characteristic distribution in certain countries. M680I has been reported as a common variant in Turks as well as Armenians (Papadopoulos et al. 2008). On the other hand, V726A is more common in Ashkenazi Jews (Tunca et al. 2005; Ozdogan and Ugurlu 2019). In a study, Gumus examined the spectrum of *MEFV* gene variants in Sanliurfa province located in the Southeastern Anatolia region. He noticed that the results of his work differed from the region's previous results. He interpreted this finding could be a consequence of the Syrian Civil War, causing many immigrants from Syria to settle near Sanliurfa. He discussed that these results ensured a good example of the variation of gene frequency and genotype distributions in different communities (Gumus 2018).

Another concept contributing to the disease severity might be the modifier genes. Serum amyloid A (SAA) and MHC class I polypeptide-related sequence A (MICA) have been proposed to affect the FMF phenotype (Touitou et al. 2001; Bakkaloglu et al. 2004; Atoyan et al. 2016). SAA1 α/α genotype correlated with amyloidosis and more severe phenotype in several studies (Bakkaloglu et al. 2004; Atoyan et al. 2016). The presence of FMF was associated with MHC I-linked disorders including psoriasis, Behcet's disease, ankylosing spondylitis, Crohn's disease, and ulcerative colitis (Watad et al. 2019).

Environment or epigenetic factors could also play a role in the severity of the disease as well since Turkish patients in Germany and Armenian patients in the USA have less severe courses of FMF than their ethnic counterparts in their consecutive countries (Tunca et al. 2005; Cekin et al. 2017; Ozdogan and Ugurlu 2019). The influence of these factors in the phenotype of patients with reduced-penetrance variants such as E148Q was also discussed (Ben-Chetrit et al. 2000; Akpolat et al. 2012; Ozen and Bilginer 2014; Topaloglu et al. 2018). The contributions of epigenetics and environmental factors should be investigated in further studies.

Within the scope of our study, several methods are utilized in the diagnosis of FMF. Although NGS offers much more information including rare variants, it is still not possible to use NGS for testing every suspected patient due to its higher cost. Most centers prefer conventional methods such as Sanger sequencing, pyrosequencing, FMF strip assay, and RFLP at least as a first-line diagnostic approach. Using these methods, most centers sequence selected variants, which mostly contain Consensus-14 variants. Therefore, we performed some of our analyses based on these variants. Even though included in the list, I692del was not reported in a single patient in our cohort. This variant has been reported more commonly in the Arab population, though it occurs rarely in other populations (Mikula et al. 2008; Belmahi et al. 2012).

According to the zygosity analysis, it was found that people with homozygous variants constitute 3% of the study

population, which is not a large part of our cohort. In a study conducted by Yildirim et al. (2019), the variant with the highest number of homozygous patients was M694V; however, in our study, we report that the R202Q variant stands out with the highest number of homozygous patients. Other studies have also reported a low number of homozygous individuals as well (Cekin et al. 2017). The low proportion of homozygous variants could be explained by various phenomena. These phenomena include segregation analyses of unaffected family members, presence of other diseases in the differential diagnosis, rare variants unnoticeable without NGS, and autosomal dominant inheritance. Also, environmental or epigenetic factors and modifying genes could contribute to a lesser extent.

Some variants were more associated, in a statistically significant manner, with the female gender. One possible explanation could be the reduced-penetrance variants resulting in symptoms more frequently in females. However, no such effect of gender was established in the literature. Abdominal pain and fever seem to be the most prevalent symptoms in nearly all ethnic groups (Alghamdi 2017; Cekin et al. 2017). Fever can sometimes be the only detectable symptom of FMF in childhood. On the other hand, abdominal pain can be commonly seen in 90% of individuals with FMF (Soriano and Manna 2012). In our study, nearly half of the patients with M694V had fever, and this variant was present in more than half of the patients with fever. The presence of some variants such as M694I and F479L increased the chance of fever by nearly two-fold. The negative correlation of E148Q with fever is concordant with the milder phenotype associated with it in the literature (Shohat and Halpern 2011). The presence of fever made it more likely to report other symptoms such as arthralgia, erythema, and renal function abnormalities; as well as amyloidosis, and colchicine response. Fever was also associated with positive family history.

Abdominal pain can be seen in 90% of the cases and is a common clinical finding of FMF (Soriano and Manna 2012; Ozdogan and Ugurlu 2019). Cekin et al. (2017) have reported that M694V and M680I were the two variants with the more severe symptoms. P369S has also been reported to have a higher incidence of abdominal pain. However, one of the shortfalls of their study is the low number of participants. Kilic et al. (2015) have reported abdominal fever to be more common in people with M694V and E148Q variants. Our study has found M680I to be correlated with abdominal pain but the M694V, E148Q, and P369S were not among the variants associated with abdominal pain despite the higher number of people with these variants. The presence or absence of abdominal pain was only mentioned in 12,788 (46%) of the participants. Of those, only 10,204 had reported abdominal pain, making it about 80%. This difference could be due to the lack of reporting for this clinical finding in more than half the participants.

Articular issues such as arthralgia or arthritis can be seen in nearly 75% of the FMF cases and are most likely restricted to a single joint, particularly at one of the large joints of the lower extremity. It usually develops in childhood and is asymmetrical (Soriano and Manna 2012; Alghamdi 2017). Our results were different from the literature, where the K695R variant was reported to be more associated with arthritis (Cekin et al. 2017). Our findings demonstrate that 66.4% of the patients with this variant did not report any articular involvement. On the other hand, E148Q was less associated with arthritis, reflecting its milder clinical picture, similar to its relation with fever (Shohat and Halpern 2011). Males were slightly less likely to have arthralgia and/or arthritis. Our study includes some patients without a definitive diagnosis of FMF, and these patients could have other disorders with arthritis which are often more prevalent in females (Ortona et al. 2016; Kim and Kim 2020). Therefore, our results require further studies to confirm the aforementioned gender distribution of arthritis in FMF patients. Many common variants including M694V increased the likelihood of arthritis, also other less common variants such as T267I and E167D increased the chance of arthritis nearly 2.5-fold.

Our finding demonstrated M694V to be strongly correlated with back pain but not with chest pain. There is not enough data in the literature to establish M694V as a specific risk factor for back pain or chest pain.

A large majority of people with FMF are expected to suffer from generalized abdominal pain, though this is not the sole gastrointestinal complaint many patients have. Multiple gastrointestinal system symptoms have been reported in FMF with diarrhea being more common in children (Mor et al. 2003; Soriano and Manna 2012; Ortona et al. 2016; Ozdogan and Ugurlu 2019). R761H was found to increase the likelihood of diarrhea by 1.8 times, but a common and severe variant namely M694V showed an inverse correlation with diarrhea, as the majority of patients with this variant did not report diarrhea. No significant link to age groups or gender was found with diarrhea.

Dermatological findings constitute another significant aspect of FMF symptomatology. There is not enough data in the literature regarding the genotype-phenotype correlation in erythematous lesions in FMF. We report a positive correlation between R202Q and erythematous skin lesions. Our data also suggests that M680I, M694V, V726A, and P369S were associated with a lesser risk of developing erythema. Pathogenic variants in the *MEFV* gene may also cause pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND). The reported variants responsible for PAAND are S242R and E244K (Moghaddas et al. 2017). In our study, we did not observe these rare variants in participants presenting with the erythema.

FMF is one of the most frequent causes of secondary amyloidosis in the world, causing significant morbidity and even mortality. The disorder is the leading cause of secondary amyloidosis in Turkey (Akpolat et al. 2012). Amyloidosis is reported at a rate of 12% in Turkish FMF patients, though it is more prevalent in the Jewish and Armenian populations (Soriano and Manna 2012). Our study has reported a much higher rate of amyloidosis at 20%; however, the lack of detailed information of some patients may be the reason behind this result. M694V variant was more commonly associated with amyloidosis in the literature, especially when it is in a homozygous state. However, amyloidosis has also been described in cases carrying variants other than M694V (Soriano and Manna 2012; Akpolat et al. 2012). In our cohort, 60% of people with the R202Q variant reported amyloidosis, and these people made up nearly a third of all people with amyloidosis. Our data indicate that the R202Q variant would increase the chance of amyloidosis by 2.2 times. R202Q is mostly considered a benign variant, with a high population frequency, present even in the homozygous state in healthy individuals. So, it is not expected to be the single cause of amyloidosis in this large patient group. Our results reflect the opposite, though there may be several reasons behind that. The amyloidosis status of 69% of the participants is unknown. Another aspect to consider is the co-occurrence of other variants with R202Q. 3127 people also carried a second variant from the Consensus-14 group. M694V was the most common of these variants, which was observed with R202Q in 1944 patients. Besides, some patients were only tested for more frequent variants, which may hinder a rare pathogenic variant in R202Q-positive individuals. Our statistical analyses also included homozygous, heterozygous, and compound heterozygous variants altogether. We can either propose a very low penetrance of R202Q affected by environmental factors in the Mediterranean region, or an additive effect of R202Q in the presence of other pathogenic variants, with regard to amyloidosis. Additional variants such as the E148Q, P369S, M680I, R761H, and F479L also had a significant relationship with amyloidosis. However, P369S and R761H were more likely to decrease the chance of amyloidosis. M694V and V726A have not come out of our study as variants with a significant association with amyloidosis. The development of amyloidosis still remains a complex issue in FMF (Tunca et al. 2005).

The treatment regimen of FMF often depends on the response of the disease to colchicine. Dose adjustments and other agents may be required if the patient still reports attacks or is unable to tolerate colchicine. Anti-IL-1β monoclonal antibody canakinumab is the only drug approved by the FDA for the treatment of colchicine-resistant FMF. However, other treatment modalities inhibiting Interleukin-1 such as rilonacept and anakinra also gained some popularity (Shohat 1993; Alghamdi 2017). The fact that 23% of the people reported an unfavorable colchicine response points out the need for more aggressive treatment modalities. There was still a large number of people where the colchicine response was not recorded. M694V homozygous individuals tend to be more resistant to colchicine treatment and require higher doses. This genotype is also the most common one in people with the clinical phenotype 2 of FMF (Soylemezoglu et al. 2010; Akpolat et al. 2012; Ozdogan and Ugurlu 2019). Unexpectedly, instead of M694V, E148Q and P369S were more likely to have an unfavorable response to colchicine. This finding that does not comply with the literature may have various explanations. One of these explanations is that patients heterozygous for E148Q or compound heterozygous with E148Q and a pathogenic variant other than M694V may not receive colchicine (Shohat 1993). Another explanation is that the data about the colchicine response is insufficient to see the complete picture. However, R202Q would make it 2.8 times more likely to have a favorable colchicine response ($p < 0.001$). Variants such as L110P and R408Q had a better chance of responding well to colchicine. L110P was first reported in the Chuetas region in Spain in 2000 (Domingo et al. 2000). The variant has later been reported in the Hatay province in Turkey (only 0.1%) and has also been reported in Japan (Tomiyama et al. 2008; Gunesacar et al. 2014). Not much has been reported on the clinical implications of the L110P variant. A744S was the variant with the strongest association with a favorable response to colchicine. A744S has been reported as a rare variant in the Turkish population and was present in only 2.2% of our study population (Soylemezoglu et al. 2015).

Oztuzcu et al. (2014) investigated the distribution of the FMF mutation spectrum in the southeastern region of Turkey. They reported that molecular diagnosis of *MEFV* is a beneficial tool in clinical practice, and studies should be conducted on the genotype-phenotype correlation of FMF in larger groups in the Turkish population, including all *MEFV* gene variants (Oztuzcu et al. 2014). Yildirim et al. (2019) reported the prevalence of various *MEFV* variants, the frequency of clinical findings of the patients, and the high ratio of carriers in a large cohort. They have indicated that in such a large population, possible complications (especially amyloidosis), and their relationship with variants should also be analyzed with further studies (Yildirim et al. 2019). Our study modestly attempts to broaden the data regarding the genotype-phenotype correlation in FMF. One of the obstacles that come out in our study is that there is a lack of uniformity when it comes to reporting symptoms, zygosity, testing methods, and in general all the clinical features. In order to achieve a better database for the Turkish population, reporting of the variants, zygosity, and clinical features need to be standardized. The

retrospective nature of our study is another reason behind the heterogenicity and inadequacy of some of our data.

Our study may include patients that were referred with a pre-diagnosis of FMF, but, in fact, have other diseases with very similar features. Additionally, some FMF patients carry no detectable disease-causing *MEFV* variants. Patients with inconclusive conventional *MEFV* gene analysis results may undergo a broader genetic test using NGS investigating all exons of the *MEFV* gene, as well as genes like *MVK* and *TNFRSF1A* (Karacan et al.; Ozdogan and Ugurlu 2019).

Overall, our study attempted to provide the clinical and genetic data of FMF patients from all over the country in order to better reflect Turkey's national profile. When compared to studies with smaller populations, our study outperforms all by having a better population profile due to its size. Unlike many studies in the literature, our study investigates a broader symptomatic spectrum and the relationship between the genotype and phenotype data. In this sense, we aimed to guide all clinicians and academicians who work in this field to better establish a comprehensive data set for the patient. One of the biggest messages of our study is that lack of uniformity in some clinical and demographic data of participants may become an obstacle in approaching FMF patients and understanding this complex disease.

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Availability of data and material All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Conflict of interests The authors declare no conflict of interest.

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