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Novel Pathogenic SNPs within *MEFV* Gene as Diagnostic Markers to Predict Familial Mediterranean Fever: Using in Silico Analysis

Baraa Alqarem[1,*] & Ghaleb Adwan[1,*]

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Abstract Familial Mediterranean fever (FMF), is a monogenic hereditary disorder, and recorded to be the most common auto-inflammatory disease associated to certain variants in MEFV gene, affecting people of Mediterranean descent. The high prevalence found in specific ethnicities including: Armenians, Arabs, Greek, non-Ashkenazi Jews and Turks. The major signs and symptoms including: fever attacks, inflammation in the abdomen (serositis), chest, skin and joints. The first attack usually occurs before the age of 20 years. The most severe complication is amyloid A, considered secondary damage (specially occur in kidney). By creating functional assays using specific biomarkers, it going to be possible to determine the clinical value of the numerous novel gene variants detected by gene sequencing in FMF. Recently, several data bases constitute a huge number of data conducted on FMF. Until now 398 variants were identified as being linked to MEFV gene. However, it has been evident that the process of interpreting the results of a diagnostic test can be quite difficult because some individuals with FMF may show only one or none of the known MEFV mutations, and vice versa, clinical symptoms are not always present when MEFV variants are carried. This review used multiple in silico study tools to follow up the update in computational analysis regarding MEFV gene SNPs. These bioinformatics tools found multiple novel mutations which can cause FMF symptoms and could be used as diagnostic markers between Mediterranean region individuals.

Keywords: Amilial Mediterranean Fever, MEFV Gene, In Silico Analysis, Pattern of Inheritance, SNPs.

Introduction

Familial Mediterranean Fever (FMF) is a monogenic hereditary disorder, and recorded to be the most common auto-inflammatory disease, affecting people of Mediterranean descent. The high prevalence found in specific ethnicities including: Armenians, Arabs, Greek, non-Ashkenazi Jews and Turks [1, 2]. The major signs and symptoms including: fever attacks, inflammation in the abdomen (serositis), chest, skin and joints. The first attack usually occurs before the age of 20 years. The most severe complication is amyloidosis A, considered secondary damage (specially occur in kidney) [3].

FMF is the most frequent systematic auto inflammatory disorder that affect mainly childhood (5-19 years), associated to certain variants in MEFV gene (from MEditerranean FeVer), which is located on "16 p 13.3" the short arm of chromosome 16 [4, 5]. MEFV gene have 10 exons with 21600 base pair which can provide instructions of 781 codons, form pyrin protein "also called marenostrin or TRIM20"[6, 7]. Pyrin protein has a central role in regulating the immune response and controlling inflammation [8].

Certain variants on MEFV gene correlated with pyrin dysfunction, that causes uncontrolled IL-1 Beta production and triggers the inflammatory reaction [9, 10]. Finding of chronic recurrent attacks of inflammatory reaction with serositis, fever, synovitis, peritonitis and pleuritis was thought to be have a correlation with cardiovascular risk in FMF individuals [10]. FMF disease characterized by recurrent attacks of fever with self-limiting serositis [11]. In some cases, secondary Amyloidosis as a result of renal deposition represent the most threatening complication in more than 8.6% of patients [12, 13].

FMF typically follows an autosomal recessive inheritance pattern; however, recent investigations propose that certain heterozygotes manifest a spectrum of clinical presentations ranging from classic FMF to mild FMF [14]. In cases of autosomal recessive FMF, it is generally observed that both parents of an affected individual harboring biallelic pathogenic variants in the MEFV gene are unaffected heterozygotes [15]. Nevertheless, in populations characterized by heightened carrier frequencies and/or a prevalence of consanguineous unions, the possibility arises that one or both parents may bear biallelic pathogenic variants, resulting in their manifestation of the phenotype [16].

An update literature made in understanding FMF's clinical features, pattern of inheritance, pathogenesis, diagnosis and therapeutic approaches, find out many old paradigms related to FMF disease have proven in accurate. Some updated paradigm that MEFV variants in FMF lead to loss of function in pyrin protein turned out to be gain of function mutations [17]. So, for more accurate diagnosis and treatment, we need to replace the old breakthrough in identifying the gen association with disease pathogenicity with new and more precise insights. Several computational analyses conducted regarding MEFV gene SNPs to found multiple novel mutations which can cause FMF symptoms and could be used as diagnostic markers between Mediterranean region individuals.

1 Department of Biology, Faculty of Science, AN-Najah National University, Nablus, Palestine.
*Corresponding author email: Baraa.alqarem@aaup.edu; adwang@najah.edu

Materials and Methods

Data mining

The data correlated with human MEFV gene was accessed by National Center for Biological Information (NCBI). Using protein accession number, the SNP information related to MEFV gene was retrieved by NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp/).

SIFT (Sort Intolerant from Tolerant)

Using this sequence homology-based tool, the phenotypic effect of amino acid substitution was predicted on protein. According to amino acid found at each position in the alignment, this tool calculated the probability that amino acid at a position is tolerated substitution is predicted to be deleterious if the normalized value is less than a cutoff (SIFT score <0.05) which predicted by certain algorithms.

GeneMANIA (A Real Time Multiple Association Network Integrating Algorithm for predicting gene function).

Integrating multiple genomics and proteomics data sources to make prediction about unknown protein function (http://www.genemania.org/).

INFEVERS: Using specific database for registry of hereditary auto inflammatory diseases, many statistical data related to MEFV gene was accessed to achieve the update in this review (https://infevers.umai-montpellier.fr/web/index.php).

Results and Discussion

Gene's involvement in disease development.

MEFV gene: The MEFV gene located on "16 p 13.3" the short arm of chromosome 16 [4, 5]. It has 10 exons with 21600 base pair, produces pyrin protein, consists of 781-amino acid immune-regulatory molecule that interacts with inflammasome and triggers specific inflammatory response to microorganisms. Serosal and synovial fibroblasts, granulocytes and dendritic cells are expressing the pyrin in normal state [18].

Pyrin is influenced by four functional domains: C-terminal B30.2 domain "B30.2/SPRY", N-terminal eponymous PYD domain, central B-box zinc finger coiled-coil domains "bBOX CC" and bZIP transcription factor. Most variants correlated with FMF are found in the B30.2 domains (Figure 1). deletion mutation in B30.2 domain activates the most typical mutations associated to FMF. Several studies identify the central helical scaffold domain that lies upstream to B30.2 which considered as a second regulatory domain [19].

When pyrin is activated, it binds cellular proteins to form an oligomer known as a "pyrin inflammasome" [20]. In the normal state, pyrin inflammasome activates the caspase-1 cascade, leads to promote releasing of pro inflammatory "IL-1β and IL18" from their precursors [21].

In physiologic conditions, the interaction between inhibitory 14-3-3 proteins and pyrin has been shown to be important in the regulation of the immune response [22,19]. When 14-3-3 proteins bind to pyrin, they can inhibit its activity and prevent the activation of the inflammasome and the subsequent production of inflammatory cytokines [20]. This mechanism serves as a negative feedback loop to prevent excessive or prolonged inflammation. In FMF, mutations in pyrin can disrupt its interaction with inhibitory 14-3-3 proteins, lead to the inflammasome activation of the and the production of inflammatory cytokines [19,23].

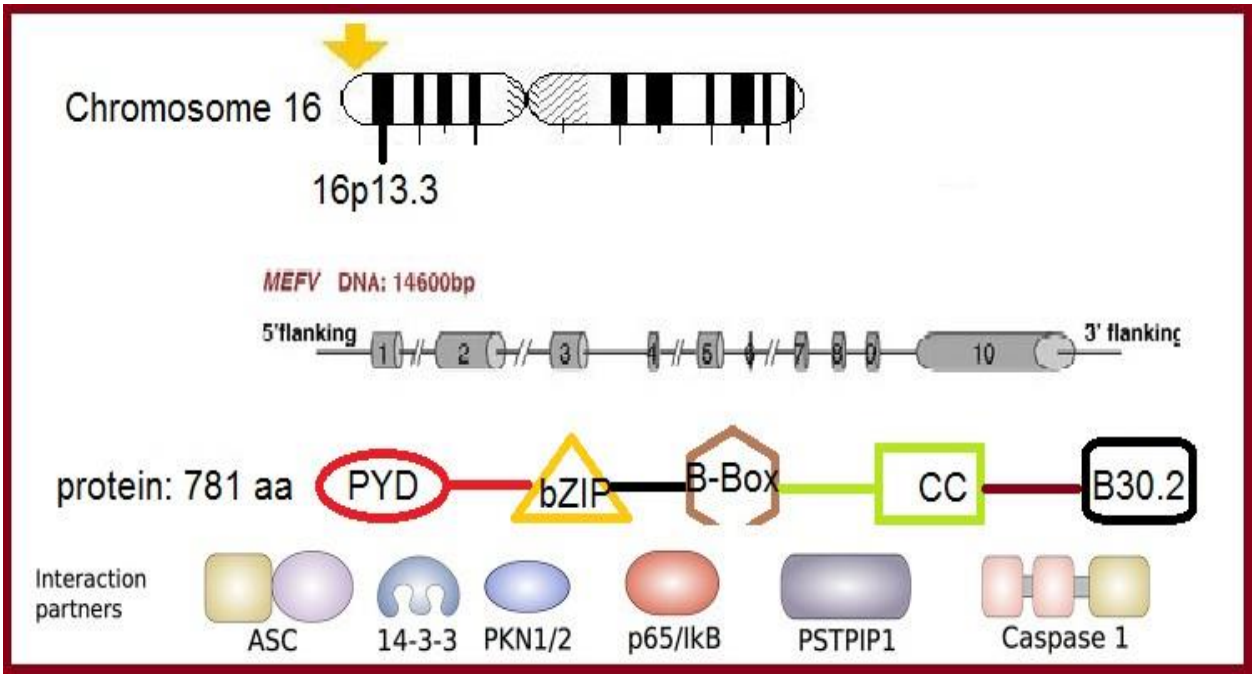

Figure (1): Schematic representation for *MEFV* gene with encoded protein "pyrin". In addition to the interaction domain of the pyrin that includes: Apoptosis associated speck like protein (ASC), 14-3-3 Protein, PKN1/2 "serine-threonine kinases PKN1 and PKN2", p65 "transcription factor p65", IκB "NF-κB inhibitor", PSTPIP1 "proline serine threonine phosphatase-interacting protein".

***MEFV/MDK* overlapping**

In several previous studies, some cases with FMF phenotype presented without MEFV pathogenic variants (MEFV negative) led the researchers to search in other genes that have an overlapping in the same pathway related to auto inflammatory diseases (Table 1). Recently, families with mevalonate kinase

deficiency showed an overlapping symptom with MEFV pathogenic variants [24].

New rare variants reported in ClinVar data bases as a variant with uncertain significant, characterized in complete penetrance in some families with autosomal dominant pattern. Using in silico analysis, several computational searches show the interaction between different gene's products, which overlap in the same auto inflammatory effect which given by reactome database. (https://reactome.org/PathwayBrowser/#/R-HSA 168256&DTAB=MT).

Previously, Proline- Serine- Threonine Phosphatase-Interacting Protein 1 (PSTPIP1) variant (p. Arg228Cys) showed strong interaction with pyrin protein [25, 26, 27]. Which resulted in autoinflammatory response characterized by pyogenic arthritis and in some cases pyoderma gangrenosum [28, 29, 30, 31, 32 33].

Pattern of heredity with correlation between genotype and phenotype.

The MEFV gene has more than 398 nucleotide variants reported in the INFEVERS database. The majority of variants related to FMF are located on exon 10 that codes for the "B30.2" domain [20]. These have an autosomal-recessive inheritance pattern [34]. Whereas the variants in exons 2, 3, and 5 more frequently show an autosomal-dominant inheritance pattern (Figure 2) [35].

Table (1): List of the main conditions with overlapping gene's variants.

Infevers database (Internet Fevers; http://fmf.igh.cnrs.fr/ISSAID/infevers), a database dedicated to variants responsible for hereditary autoinflammatory diseases	
Monogenic-auto inflammatory diseases	Multifactorial auto inflammatory diseases
Blau's disease/ Early onset Sarcoidosis	Behcet's disease
NLRP3-associated auto inflammatory disease	Chronic nonbacterial osteomyelitis/osteitis (CNO)
Mevalonate kinase deficiency (MKD)	Periodic fever, aphtha's stomatitis, pharyngitis and adenitis (PFAPA)
NLRP12-related disease	Schnitzler syndrome
PSTPIP1-associated Pyoderma gangrenosum, acne, pyogenic arthritis syndrome (PAPA)	Recurrent idiopathic pericarditis
TNF receptor associated periodic syndrome (TRAPS)	Systemic onset juvenile idiopathic arthritis

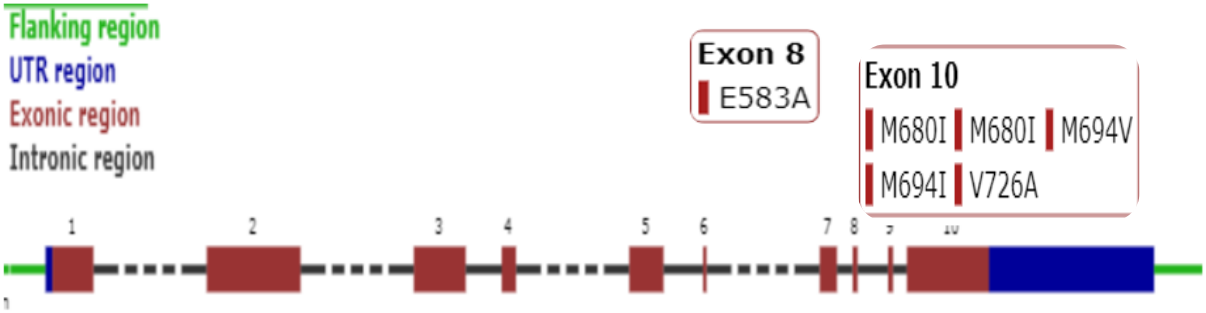

Figure (2.A): pathogenic variants. Red color beside each variant means substitution mutation.

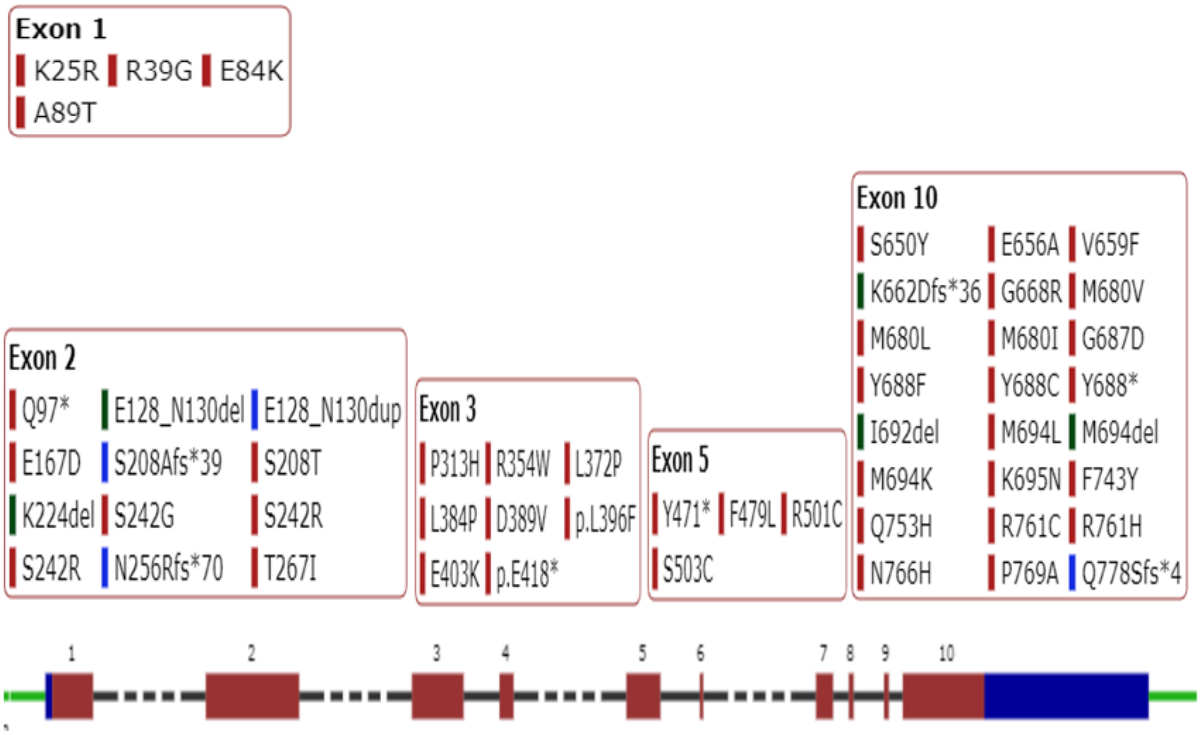

Figure (2.B): likely pathogenic variants. Red bar (substitution), green bar (deletion), blue bar (duplication).

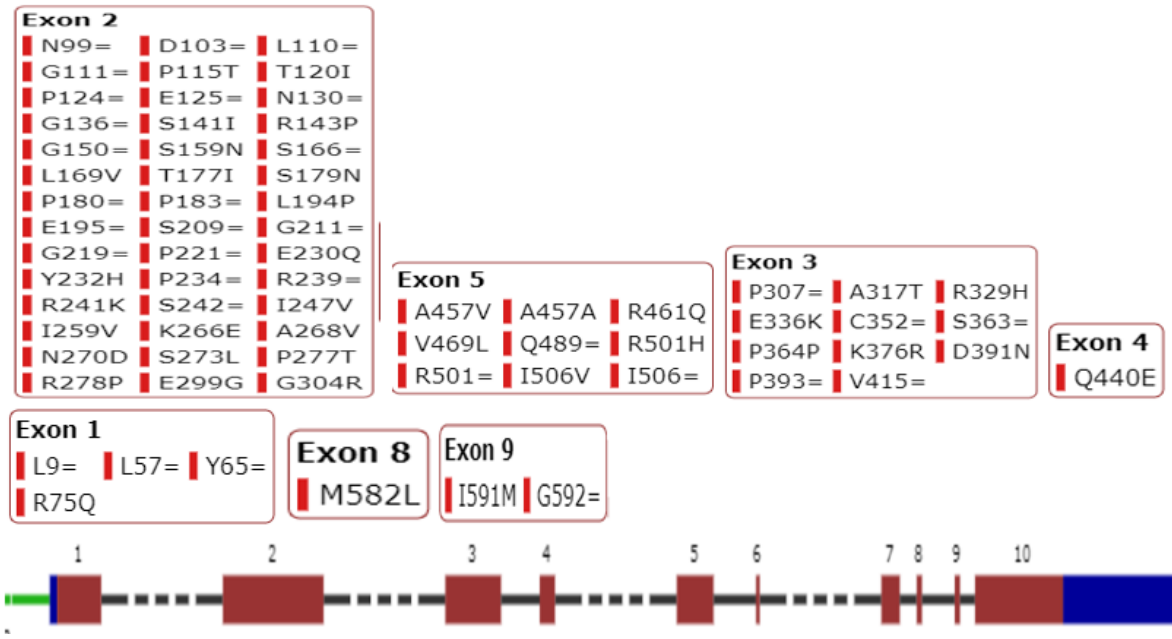

Figure (2.C): likely benign variants, all are substitutive mutations.

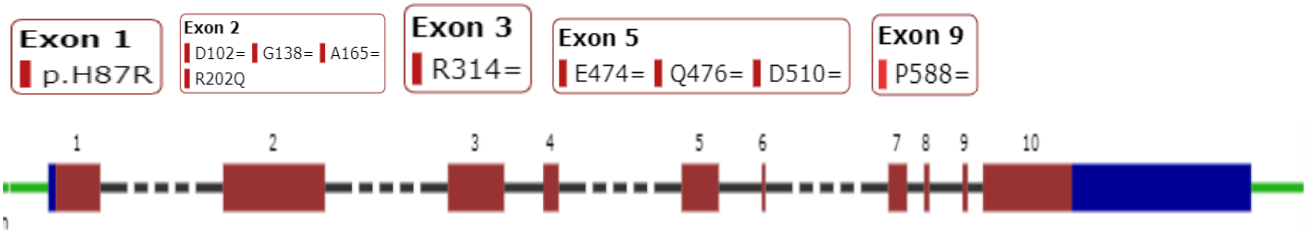

Figure (2.D): Benign variants, all are substitutive.

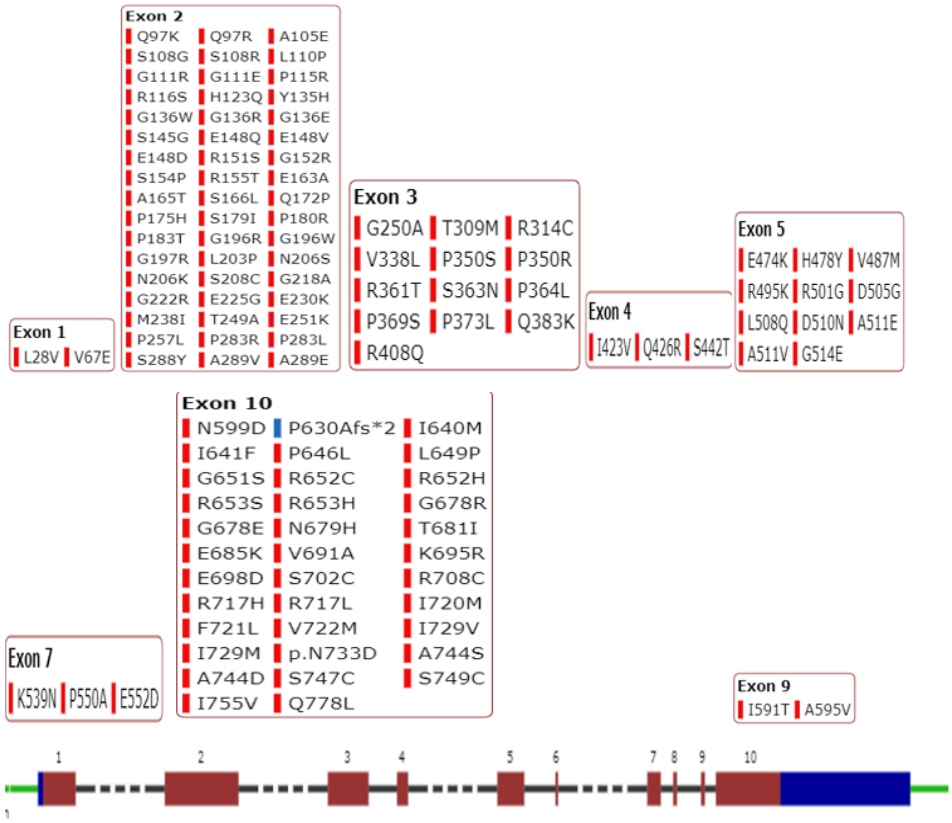

Figure (2.E): variant of uncertain significant.

Figure (2): *MEFV* mutational spectrum, characterized as pathogenic mutations (Graph A) Common pathogenic variants are found on exons 2 and 10 and represent 6 sequences among 399, likely pathogenic (Graph B) represent 57 sequences among 399, Likely benign that represent 125 sequences among 398 (Graph C), Benign variants and represent 10 sequence (Graph D) and VUS (Variant of uncertain significant) that represent 121 sequences among 399 (Graph E). Source of data created by the free source INFEVER online database.

Pathogenic Vs Benign Variants

The most common variants on Exon 10:

The majority of the identified pathogenic and likely pathogenic of MEFV variants are located on exon 10 which encodes B30.2/SPRY domain [34]. Which including: E148Q, M694V, M680I and V726A. The specific pathogenic variants that a person inherits can affect the severity and frequency of FMF episodes [36]. Most relevant severity of FMF manifestations are related to B30.2 variants.

This domain plays a key role in protein -protein interactions with other apoptosis proteins which correlated in inflammatory reaction regulation [37, 38, 39 36]. Several in silico studies are performed to identify the different interactions in the inflammatory pathway. B30.2/SPRY-Casp1/P20 complex showed a dynamic equilibrium with B30.2/SPRY-P20 complex of the studied variants, which could be a new computational model in pathogenicity of FMF in several data base programs [34].

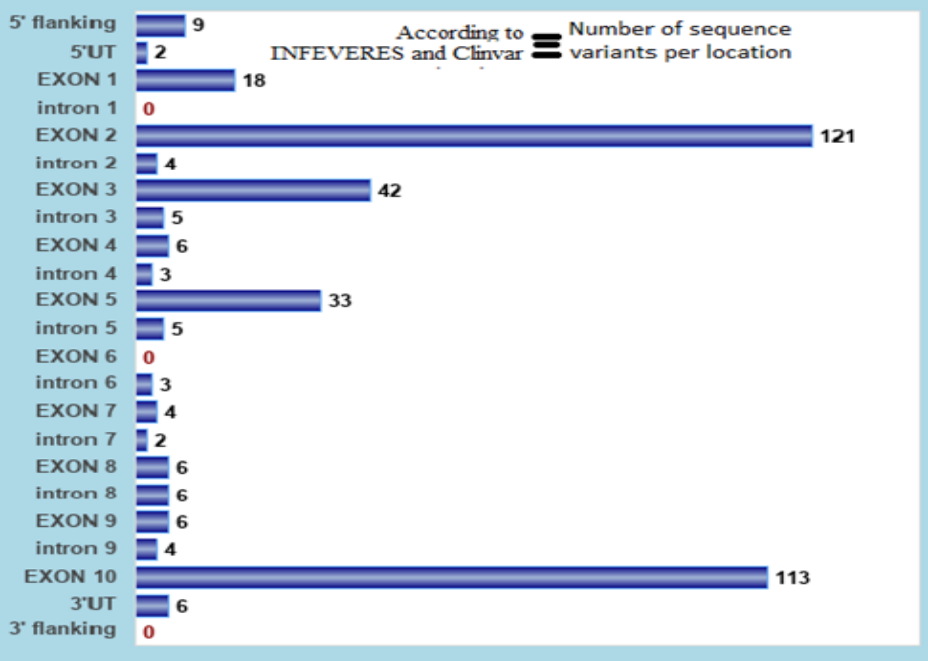

Figure (3): Representation of sequence variants distribution within the *MEFV* gene. Data obtained by computational analysis (clinvar and infeveres data bases).

The most common variants on Exon 2

The variations, that are typically located on exon 2, are considered as benign or likely benign mutations, because they don't have a significant effect to alter the pyrin protein (Figure 3). These variants including: K695R (p. Lys695Arg) F479L (p. Phe479Leu), M694I (p. Met694Ile) and P369S (p. Pro369Ser) [5]. Some Genetic testing in MEFV gene, may detect a variants of uncertain significance "VUS". which means that the change in genetic material are related to FMF or has any clinical significance at all [40]. In this case, it's important to choose another way to determine whether these mutations are likely to be pathogenic or benign [5]. R202Q, E148Q and P369S are commonly found in People with Mediterranean ancestry and known as the most prevalent VUSs found in the MEFV gene [41].

Monoallelic Vs Heteroallelic Inheritance in Correlation to Pathogenicity

Primarily, FMF is inherited in an autosomal recessive pattern. But in some cases, monoallelic FMF have been reported. That occurs when a patient only has one copy of mutated allele rather than two, and still exhibits some of the main signs of disease [1]. However, monoallelic pattern of FMF typically has milder or fewer signs and symptoms, and the chance for complications like amyloidosis (AA) is a much lower.

In contrast, Heterozygous or heteroallelic FMF, which occurs when a person has two different mutations in the MEFV gene. This pattern of inheritance has a range of symptoms and severity, depending on the type of specific mutations involved [35].

On the other hand, if patient carries two copies of the same variant in the MEFV gene, this called a homozygous FMF. This type considered more serious heterozygous or compound heterozygous [42]. Person who has homozygous FMF, typically experience inflammation in the membranous system such as meningitis, skin rash, frequent attacks of fever, abdominal pain, chest pain, and joint pain. With high risk for developing complications such as amyloidosis [43].

The common homozygous variants in the MEFV gene including: "M694V, V726A and M680I", that located on exon 10 of the MEFV gene. The most common variant associated with FMF is M694V, about 50% of cases in Mediterranean patients [44]. which results in a substitution of amino acid from methionine to valine at position 694 of the pyrin protein. Another homozygous mutation is V726A, that results in a change of valine to alanine at 726 position on the pyrin protein. that is commonly detected in Armenian, Turkish, and Jewish ancestry [45].

In addition, a substitution of methionine to isoleucine is resulted in M680I variant. Frequently observed in people of Turkish, Arab, or Jewish. Heterozygotes, are carriers who receive one copy of the mutated gene from their parents. Even though heterozygotes do not get FMF, they can raise the risk of

FMF in future generations by passing on the mutation to their progeny [46]. In heterozygous carriers of FMF mutations, the prevalence can vary from less than1%to morethan30%, contingent upon the particular variant and the population under investigation [47].

The MEFV gene has been linked to a number of heterozygous variants, including the MEFV E148Q variant, which is thought to be the most prevalent FMF-associated variant in many populations [48]. Another type of FMF that occurs when a person has two different mutations in the FMF gene, is called Compound heterozygous FMF. Compound heterozygous FMF can cause more severe symptoms than heterozygous or monoallelic FMF. Medical records show that the most frequent variants identified in 32% of examined alleles are including: M694V, E148Q in 26%, V726A in 17% of examined allelesand M680I 14% of them, these identified mutations of compound heterozygous are characterized as rare mutations [49].

Epidemiology

FMF is common in Mediterranean-coastal countries, primarily affecting Jews, Turks, Arabs, and Armenians. Turkey is most likely the nation with the highest prevalence, which varies by location and is estimated to be 1:1000 nationwide [50,51].

According to (figure 4.) a multicenter, country wide investigation conducted in Turkey demonstrates that the majority of patients with FMF are from non-Mediterranean locations, with the inner Black Sea regions and central and eastern Anatolia accounting for approximately70% of cases [52]. In other populations, such as those in North America or western Europe, the prevalence is much lower, ranging from1 in1,000 to1 in 10,000 individuals [53].

FMF usually diagnosed in childhood, the symptoms first appear between the ages of five and fifteen. On the other hand, FMF can be identified at any age, and in certain situations [54]. Therefore, the countries with high episodes, are typically with large populations of people of Mediterranean descent. Some of the countries with the highest reported prevalence of FMF include: Turkey, Armenia, Lebanon, Israel (particularly among Sephardic Jews) Syria, Greece, Jordan, Egypt, Tunisia and Morocco, respectively. FMF is a rare disorder, even in populations with high prevalence rates, the actual number of FMF patients in each country may be relatively small, and estimates may vary depending on the source of data [5, 55].

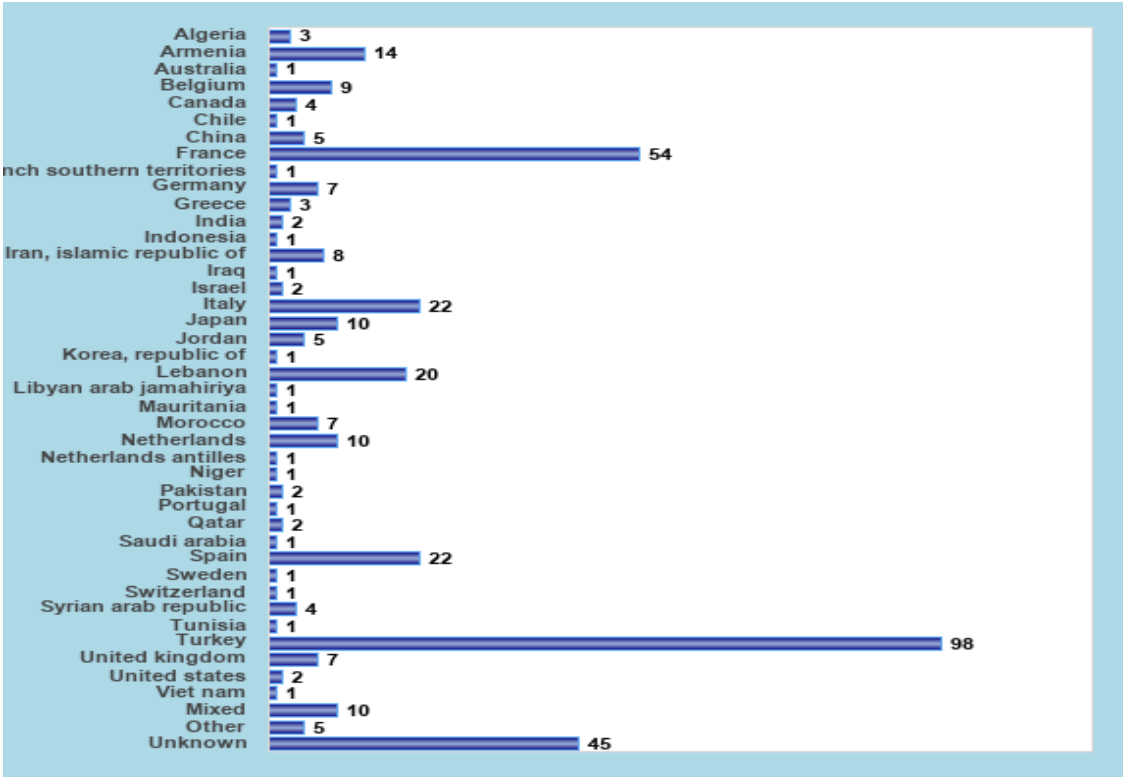

Figure (4): Distribution of 399 mutation associated familial Mediterranean fever (FMF) for Arab countries and non-Arab countries. This figure created by computational analysis of auto inflammatory diseases data bases (Eurofeveres and Infeveres data base).

In Palestine, like in many other countries, FMF is often under diagnosed or misdiagnosed due to its rarity and the lack of awareness among healthcare providers. However, there are efforts underway to increase awareness of FMF among healthcare providers and the general public in Palestine.

One study conducted in West Bank in 2023, found that among 124 patients with recurrent fever, R202Q, E148Q, M694V, A744S and V726A are the most common mutations.

A study conducted on MEFV gene mutations in 2014, among students of An-Najah National University, found that V726A, M680I and M694V is the most prevalent mutation [57].

S. Ayesh et al. study conducted on Palestinian patients, detects different MEFV mutations, including: M694V, E148Q, V726A, M694I, A744S, P369S, R408Q, and F479L, which detected in 4.8% of mutant alleles [58, 59]. Other study conductedin2019amongPalestinianchildrenwithperiodicsyndrom eM694V and E148Q detected with frequencies of 44.4% and 27.8%, respectively [60]. According to a study published between 2009-2014, FMF in Israel is estimated to have a prevalence of1 in1000 individuals, with the most common variants including: M694V (the most frequent), E148Q and V726A [61].

Diagnosis and Treatment

Genetic testing is available to detect certain mutations in the MEFV gene, which is linked to FMF. Based on clinical characteristics and family history, genetic testing is usually advised for those who have a strong suspicion of FMF [62]. In situations where the clinical symptoms are unclear or the

diagnosis is ambiguous, it can be helpful to confirm an FMF diagnosis. Furthermore, the exact mutations linked to FMF can be identified by genetic testing, yielding details regarding the severity of the illness and its possible response to treatment. According to sign and symptoms, fever is the typical inflammatory attack in more than 96 % of cases [63].

Sequencing of the MEFV gene is commonly used in genetic testing for FMF in order to find any mutations or variants that may be connected to the illness [64, 65]. Results can be obtained in a few weeks and can be obtained on a sample of saliva or blood [64, 66, 67].

Three distinct sets of criteria are used to identify familial Mediterranean fever (FMF): The Livneh criteria, the Turkish pediatric criteria, and the Tel Hashomer criteria (Table 2). The Tel Hashomer and Turkish pediatric clinical criteria are the most recent and simplest set of clinical criteria used to diagnose FMF (Table 3) [64, 68]. In cases where clinical symptoms are not conclusive, the diagnosis is confirmed by molecular testing identifying biallelic MEFV pathogenic mutations [67].

Table (2): Sets of the main diagnostic criteria of FMF [67].

Tel Hashomer revisited criteria set for the diagnosis of familial Mediterranean fever (FMF)	
Major criteria	Minor criteria
Recurrent febrile episodes *with* serositis	Recurrent febrile episodes *without* signs of serositis
AA Amyloidosis detection	Erysipelas-like erythema
Favorable response to colchicine	FMF in a first-degree relative

Turkish FMF Pediatric Criteria
Fever (axillary temperature >38°C, 6–72 hours of duration, ≥3 attacks)
Abdominal pain (6–72 hours of duration, ≥3 attacks)
Chest pain (6–72 hours of duration, ≥3 attacks)
Oligoarthritis (6–72 hours of duration, ≥3 attacks)
Family history of FMF

Table (3): Sets of main diagnostic criteria [64].

The main purpose of the available preventative care is to stop FMF attacks in the future. The corner stone of preventative care is colchicines, which effectively staves off attacks that play a key role as an anti-inflammatory drug [65,66,68]. The age and weight of the patient influence the required colchicines dosage [69- 73].

Recently, several studies conducted many analytical protocols to show the differences between colchicine-responsive and colchicine resistant FMF individuals. The resistance constitutes a problem in 5-10% of FMF patients. After colchicine resistant defined, anti-interleukin 1 therapy are currently used in management the colchicine tolerant individuals [74].

Conclusion

(FMF) is the major common inflammatory disorder worldwide, it is an autosomal recessive disease which primarily affects young individuals less than 20 years, specially whom from Mediterranean origin, but has been reported in other populations as well [75, 76].

Mutations in this gene mainly are a missense mutation that led to dysregulation of inflammation, resulting in the symptoms seen in FMF. Using certain bioinformatics tools, new different mutations have been found. That affected the stability of MEFV gene. Computational analysis conducted based on different parameters provided clues on the molecular level about the predicted effect of these variants. The majority of the identified MEFV variants are rare and have uncertain clinical significance. However, several pathogenic include: E148Q (p. Glu148Gln), M694V (p. Met694Val), M680I (p. Met680Ile) and V726A (p. Val726Ala). The specific pathogenic variant(s) that a person inherits can affect the severity and frequency of FMF episodes [77].

The primary treatment for FMF is colchicine, a medication that has been used for centuries to treat gout. Colchicine is highly effective in preventing attacks and reducing the severity of symptoms [74,78]. Recent research found that the concepts related to FMF is no longer appropriate in disease diagnosis, because the concepts that depends on clinical features alone are proved to be wrong. The recent paradigm of FMF, which the MEFV gene mutation led to a loss of function in the associated pyrin protein turned to be gain of function variants [76]. This replacement in the paradigms give a new and more accurate insights in diagnosis and treatment [76, 79, 80].

Disclosure Statement

- **Ethics approval and consent to participate:** Not applicable.
- **Consent for publication:** Not applicable.
- **Availability of data and materials:** The illustrations and body of the manuscript may contain the raw data required to replicate these finding.
- **Author's contribution:** Study's conceptualization and design: GA and BA; collection the data and computational statistics using several data bases: BA; analysis of collected data: GA; GA and BA prepare the draft manuscript; Both authors evaluated the conclusions of this review before approving the manuscript's final draft.
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