

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY

**MEFV MUTATION ANALYSIS IN FAMILIAL MEDITERRANEAN
FEVER, GOUT AND ADULT-ONSET STILL'S DISEASES**

M.Sc. THESIS

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Molecular Biology-Genetics and Biotechnology Programme

Thesis Advisor: Assoc. Prof. Dr. Eda TAHIR TURANLI

MAY 2015

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

**AİLESEL AKDENİZ ATEŞİ, GUT VE ERİŞKİN
STİLL HASTALARINDA MEFV MUTASYON ANALİZİ**

YÜKSEK LİSANS TEZİ

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To my family,

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ABBREVIATIONS

µg	: Microgram
µl	: Microliter
µM	: Micromolar
AOSD	: Adult-Onset Still's Disease
AS	: Ankylosing Spondylitis
ASC	: Apoptosis-associated Speck-like Protein
BD	: Behçet's Disease
bp	: Base Pair
BSA	: Bovine Serum Albumine
CARD	: Caspase Recruitment Domain
CC	: Coiled-coil Domain
dH₂O	: Distilated water
DNA	: Deoxyribonucleic Acid
dNTP	: Deoxyribonucleotide
EDTA	: Ethylenediaminetetraacetic Acid
EtBr	: Ethidium Bromide
EtOH	: Ethanol
FG	: FMF vs. Gout
FH	: FMF vs. HC
FMF	: Familial Mediterranean Fever
FS	: FMF vs. AOSD
g	: Gram
GA	: Gouty Arthritis
GH	: Gout vs. HC
GS	: Gout vs. AOSD
HC	: Healthy Control
HLA	: Human Leukocyte Antigen
IBD	: Inflammatory Bowel Disease
IL	: Interleukin
IPAF	: ICE-protease Activating Factor
kDa	: Kilodalton
M	: Molar
MEFV	: Mediterranean Fever (Gene)
min	: Minute
ml	: Mililiter
mM	: Milimolar
mRNA	: Messenger Ribonucleic Acid

NF-Kb	: Nuclear Factor Kappa B
ng	: Nanogram
NLR	: NOD-like Receptor
NLRP3	: NOD-like Receptor Containing Pyrin Domain
nm	: Nanometer
NMD	: Nonsense-mediated Decay
NS	: Not Significant
NSAID	: Non-steroidal Anti-inflammatory Drug
OD	: Optical Density
P/M	: Pyrin/Marenostrin (Protein)
PAMP	: Pathogen-associated Molecular Patterns
PBS	: Phosphate Buffered Saline
PCR	: Polymerase Chain Reaction
pmol	: Picomole
RA	: Rheumatoid Arthritis
RBCL	: Red Blood Cell Lysis Buffer
rpm	: Revolutions Per Minute
SDS-PAGE	: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
sec	: Second
SH	: AOSD vs. HC
SLC2A9	: Solute Carrier Family 2 Facilitated Glucose Transporter Member 9
SNP	: Single Nucleotide Polymorphism
TAE	: Tris Acetate EDTA
TE	: Tris EDTA
TLR	: Toll-like Receptor
UV	: Ultra Violet
Vs.	: Versus
WBCL	: White Blood Cell Lysis Buffer

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MEFV MUTATION ANALYSIS IN FAMILIAL MEDITERRANEAN FEVER, GOUT AND ADULT-ONSET STILL'S DISEASES

SUMMARY

The “autoinflammatory” term is first defined and developed in 1999 by a group of researcher who were studying hereditary periodic fever syndromes. The main cause of the syndromes is mutations/variatioins in genes involved in innate immunity and their alteration of the signaling or cytokine activation pathways. Familial Mediterranean fever (FMF, OMIM ID: 249100 and 134610) is one of the most common and best characterized inflammatory diseases that is inherited autosomal recessively and characterized by recurrent attacks of fever, abdominal pain, rashes, and arthritis while amyloidosis is the most severe complication of the autoinflammatory disorder. Gout is common inflammatory arthritis caused by hyperuricemia and the deposition of uric acid crystals in tissues and fluids within the body which results with metabolic, cardiovascular and renal morbidity especially in men over age of 40. Adult-Onset Still's Disease (AOSD) is a rare inflammatory disorder of unknown etiology and characterized by daily spiking fever, arthritis and rash.

MEFV, MEditerranean FeVer gene is the first autoinflammatory gene that is introduced as a candidate gene for FMF. To date, 305 sequence variations are identified on MEFV gene. Still new mutations/variatioins and their association with FMF are understudied. Mutations/variatioins are located in exons 1, 2, 3, 5, 9 and 10 of MEFV. The majority of the mutations/variatioins are missense changes and clustering in exons 2 and 10. There are five common mutations/variatioins; V726A, M694V, M694I, M680I in exons 10 and E148Q in exon 2. MEFV transcripts encode a 781 amino acids protein called Pyrin or Marenosttrin (P/M). The main function of P/M protein is the regulation of caspase-1 activation, relatively interleukin-1 β (IL-1 β) production.

The association between FMF and MEFV gene mutations/variations is well established in the previous studies, still the existence of patients without mutations/variations and P/M role were not clarified. The aim of this study was to examine the specificity of MEFV gene to FMF or to general inflammatory pathway by comparing with other autoinflammatory diseases. MEFV exon 2 and 10 variations were explored in a group of FMF (N=75), Gout (N=30) and AOSD (N=28) patients and compared the frequencies between disease groups and healthy controls (N=54). MEFV gene product P/M is indicated to have a role in inflammatory pathway. The aim of the protein studies was the quantification of P/M levels in FMF (N=22) patients compared to healthy controls (N=9). DNA and protein samples were obtained from whole blood.

We first compared all mutations/variations between the patient and HC groups. MEFV gene mutations/variations were detected in 73 (97.3%) patients in FMF group, 21 (75%) in AOSD, 20 (66.6%) in Gout and 35 (64.8%) in HC group. MEFV mutations/variations were detected in 16.2% of alleles in FMF, 6.8% in Gout, 5.4% in AOSD patients and 7% in HC. The difference in the frequency of the MEFV mutations/variations between the patient groups and control group was statistically significant ($p < 0.0001$).

The common haplotype A165A-D102D-G138G was observed frequently in all groups, but the frequency was higher in FMF group (61.8%) compared to Gout (30%), AOSD (22.6%) and HC (33.6%) (non-significant).

One of the most common variations E148Q in exon 2 was observed at a frequency quite similarly between groups. The frequencies were 5.3%, 5.0%, 7.1%, 5.6% in FMF, AOSD, Gout patients and healthy controls, respectively (non-significant). In exon 2, only R202Q variation was found to be more frequent in FMF patients (42%) compared to other groups (19-25%) (FH: $p < 0.0001$, FG: $p = 0.0266$, FS: $p = 0.0032$). Out of 16.2% of all observed mutations/variations in FMF, R202Q was observed in 2.2%, whereas E148Q in 0.3%.

There was also significant difference in pathogenic mutations/variations between FMF and other groups. Out of 5.2% of all alleles, they were observed 3.5% in FMF, whereas 0.5-0.6% in other groups. Within these pathogenic mutations/variations, out of 19.7%, only E148Q (4.6%) and M694V (11.2%) were observed in all groups. The

most prominent pathogenic mutation/variation was M694V. It was observed 8.9% in FMF, 1.3% in Gout, 0.4% in AOSD and 0.6% in healthy controls as being the most frequent mutations/variations ($p < 0.0001$). As a further aspect, increased M694V frequency in Gout and AOSD patients compared to HC requires further analysis.

Second part of this study, we wanted to investigate the quantification of P/M levels in FMF (N=22) patients compared to healthy controls (N=9). Our preliminary results indicate approximately two fold decrease of P/M levels in FMF patients compared to HC group ($p = 0.3920$), which is the opposite of the previous results. To investigate the relationship between mutations/variations and P/M levels, MEFV mutation/variation analysis were also performed in protein study group. The allele frequencies of M694V in FMF and HC were 34.1% and 5.6%, respectively ($p = 0.0247$). E148Q, R202Q and R761H were also observed in both groups, however NS.

The average P/M quantity of the patients carrying M694V mutation/variation was 0.23, whereas non-carriers had 0.33. One of the FMF patients was in her/his attack period when the sample was collected and the P/M level was highest even though he/she was not M694V mutation/variation carrier. We have observed much higher P/M level and total mutation/variation carrying allele in attack patient compared to attack free patients. The protein study requires optimization with enlarged sample sizes and replicated for more accurate statistical results.

AİLESEL AKDENİZ ATEŞİ, GUT VE ERİŞKİN STİLL HASTALARINDA MEFV MUTASYON ANALİZİ

ÖZET

Otoenflamatuvar terimi ilk olarak 1999 yılında kalıtsal periyodik ateş sendromları çalışan araştırmacı grup tarafından ortaya atılmış ve geliştirilmiştir. Sendromların asıl nedeninin doğal bağışıklık sisteminde görevli genlerde meydana gelen mutasyon/varyasyonlar ve değişen sinyal ve sitokin aktivasyon yolları olduğu öne sürülmektedir. Ailevi Akdeniz ateşi (AAA, OMIM ID: 249100 ve 134610) otozomal resesif olarak kalıtılan, tekrarlayan ateş atakları, karın ve eklem ağrıları ile kendini gösteren, hastalığın daha ileri seyrinde amiloidoz görülebilen yaygın ve en iyi karakterize edilmiş otoenflamatuvar bir hastalıktır. Gut, ürik asit kristallerinin dokularda ve sıvılarda birikmesi ile oluşan, genellikle 40 yaşı aşmış erkek bireylerde görülen ve metabolik, kardiyovasküler ve böbrek yetmezliği ile sonuçlanabilen, eklem bölgelerinde ödem, kızarıklık ve şiddetli ağrı ile kendini gösteren enflamatuvar bir hastalıktır. Erişkin Still hastalığı (ESH) ise nadir enflamatuvar hastalık olup etiyolojisi bilinmemektedir.

MEFV, MEditerranean FeVer geni, tanımlanan ilk otoenflamatuvar gen olup, AAA ile ilişkilendirilmiştir. Günümüzde MEFV geninde tanımlanmış olan 305 sekans varyansları mevcuttur. Yeni mutasyon/varyasyonlar ve AAA ile ilişkileri halen çalışılmaktadır. MEFV geni MEFV geni 10 ekzondan oluşmakta olup, mutasyon/varyasyonlar genin 1, 2, 3, 5, 9 ve 10. ekzon bölgelerinde bulunmaktadır. Mutasyon/varyasyonların çoğu yanlış anlamlı olup, 2. ve 10. Ekzon bölgeleri çevresinde yer almaktadır. En sık görülen mutasyon/varyasyonlar, 10. ekzonda yer alan V726A, M694V, M694I, M680I ve 2. Ekzonda yer alan E148Q, hastalıkla ilişkili allellerin yaklaşık %70'ini oluşturmaktadır. MEFV geninin ürünü olan Pyrin/Marenostrin (P/M) proteini, 781 aminoasitten oluşmakta ve başlıca granülosit, monosit, dendritik hücre ve eklem sıvısında bulunan fibroblast hücrelerinde anlatımı yapılmaktadır. Proteinin her alt birimi farklı proteinlerle etkileşime girerek hücre

ölümü, sitokin salınımı, transkripsiyonun düzenlenmesi gibi farklı fonksiyonlar göstermekte, ancak asıl görevi inflamasyonun regülasyonu olduğu açıklanmaktadır. MEFV genindeki mutasyon/varyasyonların hastalıkla ilişkili olduğu ve klinik tabloyu etkilediği gösterilmektedir. AAA hastası olmasına rağmen, MEFV mutasyon/varyasyonları taşımayan bireylerin olması, hastalığın genetiğinin daha kompleks olabileceğini düşündürmektedir. Ayrıca P/M rolü tam olarak açıklanmamıştır.

Bu çalışmada MEFV geninin AAA'ya ve ya genel inflamatuvar yolağına spesifikliğinin diğer otoenflamatuvar hastalıklarla karşılaştırılarak araştırılması amaçlanmıştır. MEFV geninin ikinci ve onuncu ekzon bölgelerindeki mutasyon/varyasyon frekansları AAA (N=75), Gut (N=30) ve ESH (N=28) hastalarında ve sağlıklı kontrol grubunda (N=54) karşılaştırılmıştır. Çalışmanın ikinci kısmında ise, P/M protein seviyeleri AAA (N=22) ve sağlıklı kontrollerde (N=9) araştırılmıştır. DNA ve protein örnekleri tüm kandan elde edilmiştir.

İlk olarak tüm mutasyon/varyasyon frekansları gruplar arası karşılaştırılmıştır. MEFV gen mutasyon/varyasyonları AAA grubunda 73 (97.3%), ESH grubunda 21 (75%), Gut grubunda 20 (66.6%) ve sağlıklı kontrollerde 35 (64.8%) kişide bulunmuştur. AAA grubu alellerinin %16.2'si, Gut grubunun %6.8'i, ESH grubunun %5.4'ü ve sağlıklı kontrollerin %7'si mutasyon/varyasyon taşıyan alelleri kapsamaktadır. Hasta grupları ile sağlıklı kontrol grubu arasındaki MEFV mutasyon/varyasyon frekans farkı istatistiksel olarak anlamlıdır ($p < 0.0001$).

A165A-D102D-G138G ortak haplotipi tüm gruplarda gözlemlenmiş olup, AAA grubunda diğer gruplara göre, Gut (30%), ESH (22.6%) ve sağlıklı kontrol (33.6%), daha yüksek frekansta gözlenmiştir (%61.8) (istatistiksel olarak anlamlı bir fark bulunmamaktadır).

Yaygın varyasyonlardan ikinci ekzon üzerinde bulunan E148Q gruplar arasında yaklaşık olarak aynı frekansta gözlenmiştir. Alel frekansları sırasıyla %5.3, %5.0, %7.1, %5.6 olarak AAA, Gut ESH hastaları ve sağlıklı kontrollerde görülmüştür (istatistiksel olarak anlamlı bir fark bulunmamaktadır). İkinci ekzonda sadece R202Q varyasyonu AAA hastalarında daha sık (%42), diğer gruplarda ise %19-25 oranında görülmüştür (FH: $p < 0.0001$, FG: $p = 0.0266$, FS: $p = 0.0032$). AAA grubu alellerinin %16.2'sinde mutasyon/varyasyon görülmüş olup, bunların %2.2'si R202Q, %0.3'ü ise E148Q'dur.

Patojenik mutasyon/varyasyonların AAA grubunda diğer gruplarla arasında istatistiksel olarak anlamlı bir fark olduğu bulunmuştur. Tüm alellerinin %5.2'sinde mutasyon/varyasyon görülmüş olup, bunların %3.5'i AAA grubuna, diğerleri ise %0.5-0.6 oranında diğer gruplarda görülmüştür. Patojenik mutasyon/varyasyonlar tüm gruplardaki alellerin %19.7'sini oluştururken, sadece E148Q (%4.6) ve M694V (%11.2) tüm gruplarda ortak olarak görülmüştür. En yaygın mutasyon/varyasyon M694V olarak bulunmuş olup, AAA grubunun %8.9'unu, Gut grubunun %1.3'ünü, ESH grubunun %0.4'ünü ve sağlıklı kontrollerin %0.6'sini oluşturmaktadır ($p < 0.0001$). Gut ve ESH hastalarında artan M694V alel frekanslarının daha detaylı olarak araştırılması gerekmektedir.

Çalışmanın ikinci kısmında, AAA hastalarında (N=22) P/M protein seviyeleri sağlıklı kontrol ile (N=9) karşılaştırılarak incelenmiştir. AAA hastaları ve sağlıklı kontrollerde P/M protein seviyelerine bakıldığında, iki grup arasında istatistiksel olarak anlamlı bir fark bulunmamasına karşın, AAA hastalarında protein seviyelerinin sağlıklılara göre yaklaşık iki kat az olduğu gözlenmiştir ($p = 0.3920$). Öncül çalışmamızın sonuçları literatürde yer alan önceki çalışmalara göre zıt bir durum ortaya çıkarmaktadır. Mutasyon/varyasyonlar ile P/M seviyesi arasında olası ilişkiyi araştırmak üzere MEFV mutasyon/varyasyon analizi protein çalışması grubundaki bireylere de yapılmıştır. M694V alel frekansı AAA grubunda %34.1 iken sağlıklılarda %5.6 olarak gözlenmiştir ($p = 0.0247$). Her iki grupta da gözlenen diğer mutasyon/varyasyonlar E148Q, R202Q ve R761H olmasına karşın, istatistiksel olarak anlamlı bir fark görülmemiştir.

M694V taşıyan AAA hastalarında ortalama P/M seviyesi 0.23 iken, taşımayan hastalarda ortalama 0.33 olarak gözlenmiştir. Hasta grubunda bir kişiden ataklı döneminde örnek alınmıştır. Bu birey M694V mutasyon/varyasyonu taşımasına karşın, grupta en yüksek P/M seviyesine sahip bireydir. Ataklı hastada ataklı olmayan diğer hastalara göre daha yüksek P/M seviyesi ve toplam mutasyon/varyasyon taşıyan alel sayısı olduğu gözlenmiştir. Protein çalışmasında istatistiksel olarak anlamlı sonuçlar elde etmek için deneyler daha fazla örnek sayısı ile tekrarlanarak optimize edilmelidir.

1. INTRODUCTION

1.1 Autoinflammatory Diseases

The “autoinflammatory” term is first defined and developed in 1999 by a group of researcher who were studying hereditary periodic fever syndromes (McDermott et. al., 1999; Galon et. al., 2000). The main cause of the syndromes is mutations/variations in genes involved in innate immunity and their alteration of the signaling or cytokine activation pathways (McGonagle et. al., 2006, Stojanov et. al., 2005). The maturation of the pro-inflammatory cytokine IL-1 β is one of the main signaling pathways that give rise to the development of autoinflammatory diseases (Petrilli and Martinon, 2007).

1.1.1 Familial mediterranean fever (FMF)

Familial Mediterranean fever (FMF, OMIM ID: 249100 and 134610) is one of the most common and best characterized inflammatory diseases that is inherited autosomal recessively and characterized by recurrent attacks of fever, abdominal pain, rashes, and arthritis while amyloidosis is the most severe complication of the autoinflammatory disorder (Ben-Chetrit and Touitou, 2011). The disease is prevalent among eastern Mediterranean populations, mainly non-Ashkenazi Jews, Armenians, Turks, and Arabs (Ben-Chetrit and Touitou, 2009). The frequency is estimated as 1/1000 and the carrier rate as 1/5 in Turkey (Tunca et al., 2005). Diagnosis is based on clinical criteria, family history and laboratory results. In addition, *MEFV* gene mutations/variations, the responsible gene for the disease, are analyzed to support the diagnosis (Ben-Chetrit and Touitou, 2011; Fonnesu et. al., 2009). Since 1972, the effective treatment is colchicine, which controls the acute attacks and prevents the development of amyloidosis (Goldfinger, 1972).

1.1.2 Gout disease

Gout is common inflammatory arthritis caused by hyperuricemia and the deposition of uric acid crystals in tissues and fluids within the body which results with metabolic, cardiovascular and renal morbidity especially in men over age of 40

(Richette and Bardin, 2010; Kim et al. 2003). The leading symptoms are recurrent attacks of swelling, erythema, warmth, and intense pain whereas advanced gout may further cause persistent pain, nerve compression, ulceration of the skin, joint destruction and deformities, and more rarely, renal manifestations including urolithiasis (Khanna et. al., 2012). The disease is prevalent among Western countries and the frequency is estimated as 0.31% in Turkey, while the ratio between male and female is ranging from 3:1 to 9:1 (Birlik et. al., 2014; Kramer et. al., 2002). The *GLU9* gene (*SLC2A9*) is indicated to be highly significantly linked with hyperuricemia and gout by using a whole-genome approach (Vitart et. al., 2008). Diagnosis is not only based on histological crystal diagnosis but also imaging is used to monitor treatment response (Chowalloor et. al., 2014). The current treatments of gout goals to resolve acute gouty flares, prevent future attacks, the formation of tophi, and reduce gout-related morbidity, which includes medications named as allopurinol, febuxostat, and probenecid (Wang et. al., 2013).

1.1.3 Adult-Onset Still's disease (AOSD)

Adult-Onset Still's Disease (AOSD) is a rare inflammatory disorder of unknown etiology and characterized by daily spiking fever, arthritis and rash (Bywaters, 1971). The incidence in France has been estimated at 0.16, in Japan 0.22 and in Norway 0.4 per 100,000 persons. The reported prevalence in the Japanese and the European populations rates range from 1 to 34 cases per 1 million persons (Valentin et. al., 2014). Yet, there is no indication about the frequency in Turkey. There are no clinical or laboratory test specific for AOSD. However, serum ferritin is used as a diagnostic and disease activity marker. In order to control fever, arthritis, and systemic disease non-steroidal anti-inflammatory drug (NSAIDs), steroids, and antirheumatic agents are used a treatment (Efthimiou et. al., 2006). The exact mechanism is still unknown; however HLA antigens are shown to be associated with the emergence and progression of the disease (Pouchot et. al., 1991).

1.2 MEFV Gene

MEFV, MEditerranean FeVer gene (OMIM ID: 608107, Gene ID: 4210) is the first autoinflammatory gene that is introduced as a candidate gene for FMF and identified by positional cloning in 1971 (International FMF Consortium, 1997; The French

FMF Consortium, 1997). MEFV gene is located on chromosome 16p13.3 and comprises of 10 exons (Figure 1.1) (Touitou, 2001). MEFV is expressed mainly in neutrophils, eosinophils, cytokine activated monocytes, dendritic cells and synovial fibroblasts. It encodes a 781 amino acid protein known as Pyrin/Marenostrin (P/M), which plays role in the inflammatory response by regulating the processing of mature IL-1 β (Booty et. al., 2009).

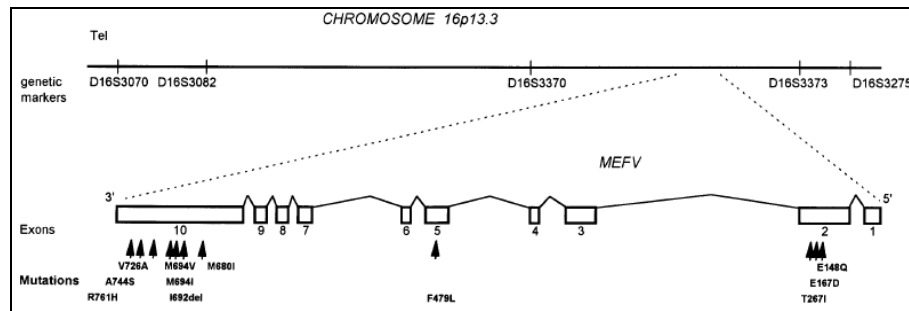


Figure 1.1 : Schematic Diagram of MEFV Gene (Bernet et. al., 1998).

1.2.1 MEFV mutations/variations

To date, 305 sequence variations are identified on MEFV gene. Still new mutations/variations and their association with FMF are understudied. Mutations/variations are located in exons 1, 2, 3, 5, 9 and 10 of MEFV. The majority of the mutations/variations are missense changes and clustering in exons 2 and 10 (Figure 1.2) (Touitou, 2008).

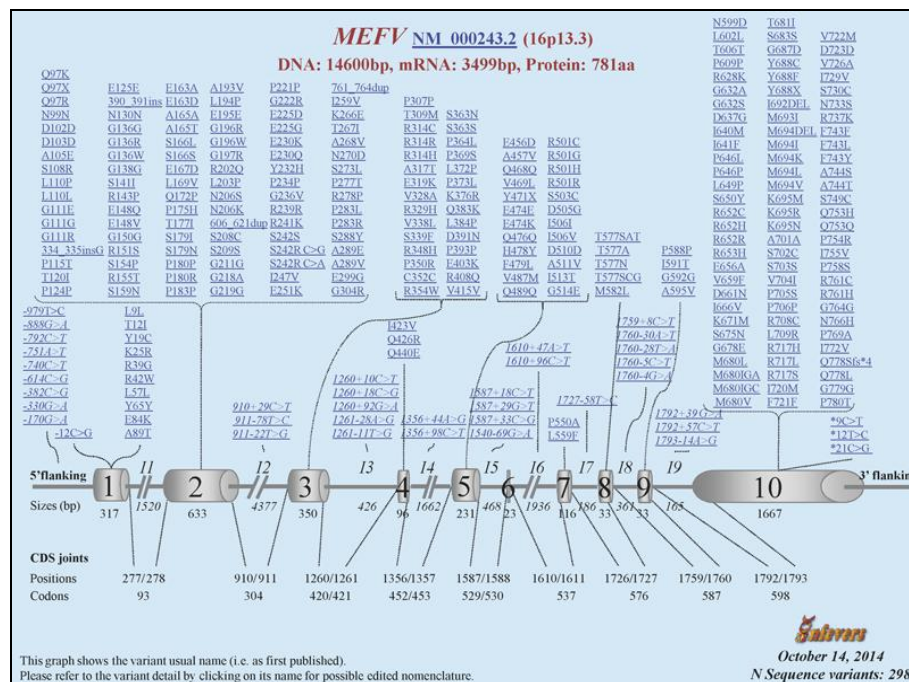


Figure 1.2 : MEFV Mutations/Variations (Infevers).

There are five common mutations/variations which accounts for 70% of the alleles associated with disease. These are; E148Q in exon 2 and V726A, M694V, M694I, M680I in exon 10, which corresponds to B30.2 domain of P/M protein (Touitou, 2001, Weinert et. al., 2009). These five mutations/variations are given in Table 1.1. MEFV mutation/variation frequency was reported as 20% in healthy Turkish population (Yilmaz et. al., 2001). According to Ozalkaya *et. al.*, the allele frequency of four mutations/variations in FMF patients are M694V (46%), M680I (15.6%), E148Q (12.8%) and V726A (1%) (Ozalkaya et. al., 2011).

Table 1.1 : Five Common Mutations/Variations on MEFV Gene (Bernot, 1998).

Exon	Mutation / Variation	Codon Change	Amino Acid
2	E148Q	GAG → CAG	Glu → Gln
10	V726A	GTT → GCT	Val → Ala
	M694V	ATG → GTG	Met → Val
	M694I	ATG → ATA	Met → Ile
	M680I	ATG → ATC	Met → Ile

Despite the fact that FMF is known as autosomal recessive disorder, there are some studies suggesting autosomal dominant inheritance or compound heterozygote transmission in FMF (Booth et. al., 2000; Aldea et. al., 2004; Stoffels et. Al., 2014), nevertheless there are some cases with no mutation/variation (Ozalkaya et. al., 2011).

There are some studies suggesting the relationship between symptoms of the disease and type of mutation/variation (Yalcinkaya et. al., 2000; Yilmaz et. al., 2001). M694V homozygote and M694V/M680I compound heterozygous mutation/variation were observed in patients having more severe disease with amyloidosis while not observed in healthy controls. Consequently, these mutations/variations were found to be linked with FMF phenotype (Ozalkaya et. al., 2011). In contrast, milder or asymptomatic FMF patients were observed having an E148Q homozygous mutation or more correctly variation as it is quite commonly observed in healthy individuals where the disease frequency is high (about 5%) (Ben-Chetrit et. al., 2000). In another study was reported that also homozygotes for the complex V726A–E148Q allele, by far outweighed that associated with the V726A allele, are as severely affected as

M694V homozygotes, suggesting the fact that E148Q variation is not a benign polymorphism (Gershoni et. al., 2002). They also point at the wide allelic variability and additional genetic and/or environmental modifiers' role in disease expression.

Up until today, the association between MEFV gene variations with other autoinflammatory disorders were studied, such as Behcet's disease (BD) (Touitou et. al., 2000; Rustemoglu et. al., 2013), Rheumatoid Arthritis (RA) (Koca et. al., 2010; Inanir et. al., 2013), inflammatory bowel disease (IBD) (Uslu et. al., 2010; Beser et. al., 2013), ankylosing spondylitis (AS) (Alayli et. al., 2009; Maras et. al., 2014), gouty arthritis (GA) (Sari et. al., 2014; Nonaka et. al., 2014) and adult-onset still's disease (AOSD) (Cosan et. al., 2013; Nonaka et. al., 2015) as summarized in Table 1.2.

Cosan *et. al.* reported that the MEFV mutation/variation rate, especially in exon 10, was increased in AOSD patients; yet the difference were not statistically significant (Cosan et. al., 2013). Nonaka *et. al.* supported the findings of Cosan, but their data were inconsistent with those of Kim *et. al.*, who found no association between AOSD and MEFV exon 2 variants in Korean patients (Nonaka et. al., 2015; Kim et al., 2013). Sari *et. al.* reported that the carriage rate of MEFV mutations/variations for GA patients and controls were 22.7% and 24%, respectively and there were no significant difference regarding the distribution of exon 2 and 10 mutations/variations (Sari et. al., 2014), however a case in Japanese patient of GA was reported to carry heterozygous E148Q mutation/variation (Nonaka et. al., 2014). Taken together, the MEFV gene specificity for FMF was suspected (Ben-Chetrit et. al., 2009).

Table 1.2 : Literature Summary of MEFV Mutations/Variations in Autoinflammatory Diseases.

Disease	No. of Patients / Controls	MEFV Mutation / Variation Allele Frequency Patient% / Control% p (OR, 95% CI)						Reference
		E148Q	V726A	M694V	M694I	M680I	Others	
BD	38 / 114	5.2% / 2.2% NS	2.6% / 0% NS	2.6% / 0% NS	-	-	-	Touitou, 2000
BD	207 / 200	7.25% / 2.25% 0.001 (3.39, 1.59-7.24)	3.14% / 1.50% NS	4.35% / 3% NS	-	4.59% / 2% 0.049 (2.36, 1.02-5.44)	P369S - 1.69% / 1.00% NS	Rustemoglu, 2013
RA	103 / 103	11.7% / 12.6% NS	5.8% / 1.9% NS	1.9% / 5.8% NS	2.9% / 1.9% NS	0.9% / 0% NS	P369S - 4.9% / 0.9% NS	Koca, 2010
RA	101 / 110	NS	NS	NS	-	NS	P369S - NS Total: 30.7% / 23.6%*	Inanir, 2013
IBD	33 / 0			0.03			Total in IBD: 25.7%	Uslu, 2010
IBD	12 / 0	8.3%*	-	41.7 %*	-	25 %*	K695R - 25%*	Beser, 2013
AS	80 / 85	NS	NS	NS	NS	NS	Total in AS: 30%* Total in HC: 20%*	Alayli, 2009
AS	112 / 100	AS - 30% NS	AS - 13% NS	AS - 30% NS	NS	NS	A744S in AS - 8% K695R in AS - 2% P369S in AS - 17% Total: 15.2% / 11%	Maras, 2014
GA	97 / 100	NS	NS	NS	NS	NS	Total Exon 2: 6.2% / 11% Total Exon 10: 5.7% / 3%	Sari, 2014
AOSD	20 / 103	NS	NS	NS	NS	NS	Total AOSD > HC	Cosan, 2013

Table 1.2 (continued): Literature Summary of MEFV Mutations/Variations in Autoinflammatory Diseases.

Disease	No. of Patients / Controls	MEFV Mutation / Variation Allele Frequency Patient% / Control% p (OR, 95% CI)						Reference
		E148Q	V726A	M694V	M694I	M680I	Others	
AO SD	96 / 165	45.8%* / 46.7%* 25% NS	-	-	-	-	P339S - 7.3 %* / 6.1%* 4% NS	Kim, 2013
AO SD	49 / 105	29.6% / 24.8% NS	NS	NS	2% / 0% NS	NS	G632S - 1% / 0% P369S - 6.1% / 6.2% R408Q - 5.1% / 5.7% L110P 8.2% / 7.1% R202Q - 4.1% / 2.9% G304R - 0% / 2.9% E84K - 2% / 1% NS	Nonaka, 2015
FMF	450 / 100	3.55 % / 12% P<0.005 (0.02 ± 0.09)	2.88% / 2% P<0.005 (0.003 ± 0.02)	51.55% / 3% P<0.005 (0.002 ± 0.03)	0.44% / 0% P<0.005 (0 ± 0.02)	9.22 % / 5% P<0.005 (0.003 ± 0.04)	-	Yilmaz, 2001
FMF	1090 / 0	-	8.6% p < 0.001	51.4% p < 0.001	-	14.4% p < 0.001	-	Tunca, 2005
FMF	308 / 164	12.8%	1%	46%	-	15.6%	-	Ozalkaya, 2011

*: Individual frequency

NS: Non-significant

1.2.2 MEFV expression

The association between MEFV mutations/variations and MEFV messenger RNA (mRNA) expression levels were reported recently. In 2002, Notarnicola *et. al.* found reduced MEFV mRNA expression levels in FMF patients compared to healthy controls and intermediate mRNA levels in healthy carriers. Also, they reported the relationship between MEFV mRNA expression and type of mutation/variation. The localization of MEFV mutations/variations close to 3' end, such as M694V, could change MEFV mRNA stability. M964V mutations/variations, especially homozygous type, were associated with lowest MEFV mRNA levels, while E148Q variation was associated with the highest MEFV mRNA levels. To sum up, MEFV genotype could directly or indirectly involved in the regulation of its own expression (Notarnicola *et. al.*, 2002).

These results were confirmed by another study of Ustek *et. al.* in 2007. They reported decreased mRNA levels of MEFV in FMF patients compared to healthy controls. In addition, much higher reduced level of MEFV mRNA expression was observed in FMF patients during attacks. Therefore, they indicated the lower mRNA levels of MEFV are related to inflammation (Ustek *et. al.*, 2007). Supportively, in a study by our group was indicated that MEFV mRNA expression decreased in FMF patients (N=51) compared to healthy controls (N=11) ($p=0.031$) (Kirectepe *et. al.*, 2011). In opposite of these three studies, Booty *et. al.* reported higher MEFV expression in FMF compared to healthy controls. They also examined P/M levels by Western blot analysis and found significant increase in P/M levels between FMF patients and healthy controls (Booty *et. al.*, 2009).

Grandemange *et. al* investigated the possible post-transcriptional regulation of the MEFV gene by nonsense-mediated decay (NMD), which acts like an mRNA quality control mechanism that detects and degrades mRNAs in order to prevent translation of unnecessary and aberrant transcripts or to modulate protein level in a cell specific manner. In addition, they investigated the different cellular localization of protein isoforms generated by alternative splicing. Some MEFV transcripts (full length (FL), 2a, 2D, 8ext, 2D/8ext, 9ext) can produce different functional P/M and its isoforms, whereas others (4a, 2D/9ext, del234) are degraded by NMD (Grandemange *et. al.*, 2009).

Two different FL transcript levels were found to be significantly decreased in FMF patients compared to controls (primers spanning exon 2-3 junction; $p = 0.017$ and primers spanning exon 4-5 junction; $p = 0.0052$), by our group. On the other hand, the second exon lacking MEFV transcripts' levels (2D) were found to be significantly higher in FMF patients compared to healthy controls (primers spanning exon 1-3 junction; $p = 0.026$), in samples derived from whole leukocyte (Tahir Turanli et. al., 2011).

1.2.3 MEFV gene product – Pyrin/Marenostrin and Inflammasome

MEFV transcripts encode a 781 amino acids protein called Pyrin (FMF consortium) or Marenostrin (French FMF consortium). Pyrin/Marenostrin (P/M) protein composes of five domains. These are N-terminal PYRIN domain (PYD, PAAD, DAPIN), bZIP transcription factor basic domain, B-Box zinc domain, α -helical (coiled-coil) domain (CC) and C-terminal B30.2 domain (PRYSPRY, SPRY) with interacting proteins of each domain (Figure 1.3).

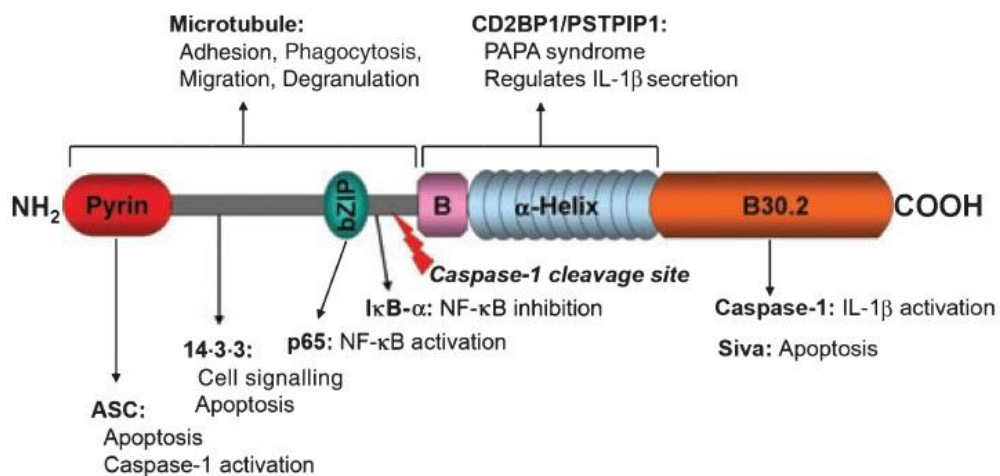


Figure 1.3 : Pyrin/Marenostrin Protein Structure (Chae et. al., 2009).

All of the domains play role in inflammatory pathways, such as apoptosis, cytokine secretion, and transcriptional regulation. The main function of P/M protein is the regulation of caspase-1 activation, relatively interleukin-1 β (IL-1 β) production through the interactions of PYRIN and PRYSPRY domains with an adaptor protein, apoptosis-associated speck-like protein with a CARD (the caspase-recruitment domain) (ASC) and caspase-1 respectively (Chae et. al., 2009). Schematic representation of the nucleotide oligomerization domain (NOD)-like receptor

containing a pyrin domain (NLRP3) inflammasome and general inflammatory pathway are shown in Figure 1.4 and 1.5.

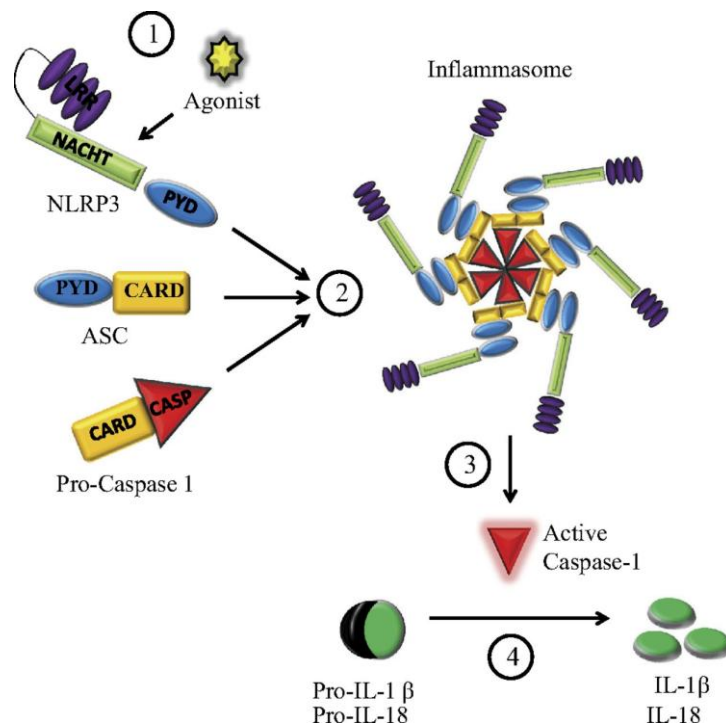


Figure 1.4 : NLRP3 Inflammasome Structure (Dos Santos et. al., 2012).

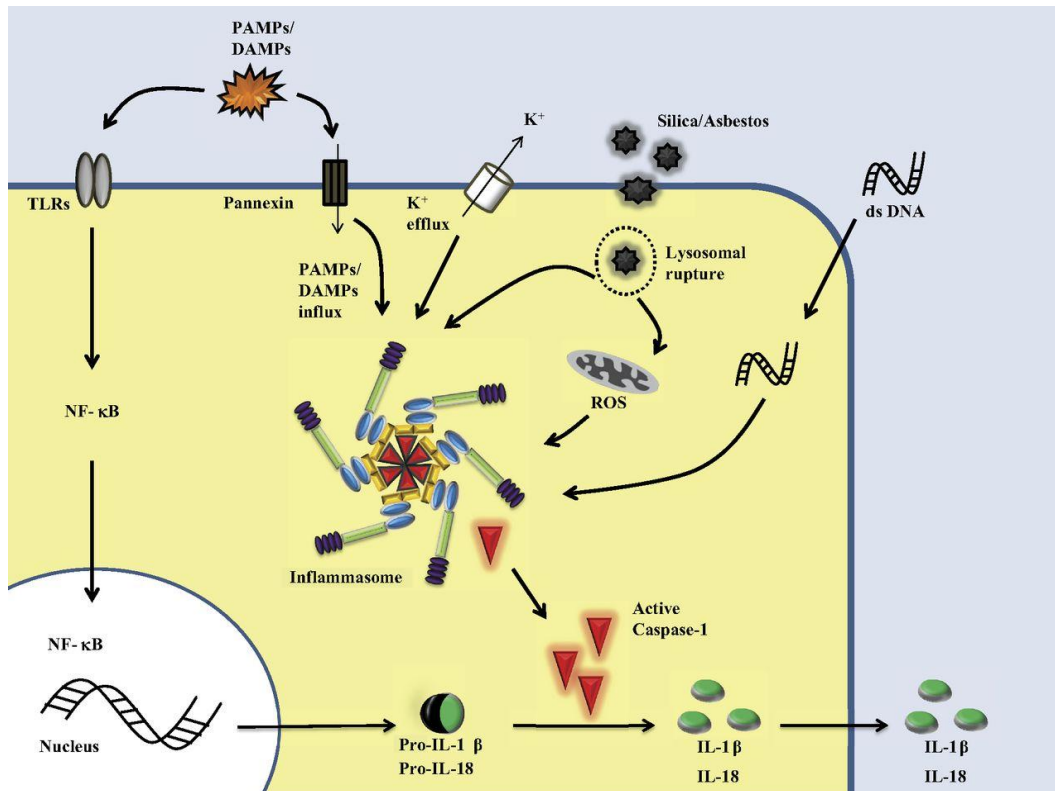


Figure 1.5 : General Inflammatory Pathway (Dos Santos et. al., 2012).

Plasma-membrane associated toll-like receptors (TLRs) and cytosolic Nod/NACHT-LRR (NLR) proteins are expressed in macrophages and monocytes which ensure the recognition of pathogen-associated molecular patterns (PAMPs) and non-pathogen-associated danger signals (Ting et. al., 2006). Macrophages and monocytes produce pro-IL-1B and pro-IL-18 in response to these signals. They also play role in the processing of inactive pro-IL-1B and pro-IL-18 and the production of active cytokines IL-1B and IL-18, respectively, by activating caspase-1 enzyme, which are mediators of inflammation (Dinarello et. al., 1998). Caspase-1 functions in regulating the cleavage and maturation of pro-IL-1 β , it involves in inflammatory responses, which requires formation of the enzyme within multiprotein complexes called “inflammasomes” for its activation following an inflammatory stress (Schroder and Tschopp, 2010). Recently, there are several inflammasome complexes identified including ICE-protease activating factor (IPAF), NALP1, NALP2, cryopyrin/NALP3, and pyrin inflammasomes. Cryopyrin/NALP3 is not associated directly with pro-caspase-1 and requires the adapter protein ASC to recruit and activate caspase-1 (Agustini et. al., 2004).

NALP protein family, such as NALP1, NALP2 or NALP3 is located in the center of inflammasome and associate with the adaptor protein ASC, which connects the NALPs with caspase-1 (Martinon et. al., 2006). ASC is an adapter protein, 22-kDa, with an N-terminal PYD and C-terminal CARD. ASC links the pro-caspase-1 to PYD-containing NLR family member via its PYD domain to interact with NLRs' PYD and CARD domain to interact with CARD domain of pro-caspase-1 (Srinivasula et. al., 2002).

P/M is another PYD-containing inflammasome regulator; however its regulation on inflammasome is still controversial. P/M can assemble an inflammasome with ASC and caspase-1 like NALP3/NLRP3 (Fernandes-Alnemri et. al., 2007). It was suggested that P/M plays role in innate immune system by effecting caspase-1-dependent IL-1 β maturation. Most of the disease causing mutations/variations were found in B30.2 domain (PRYSPRY) of P/M protein, which corresponds to the mutational hot spot region and tenth exon of MEFV gene. P/M is found to be interacting with cytoskeleton-organizing protein PSTPIP1. It unlocks PYD domain in N-terminal and enables the formation of Pyrin/ASC complex. The interaction of P/M

with actin filaments may explain the colchicine treatment mechanism (Weinert et. al., 2009).

Chae *et. al.*, reported that Pyrin deficiency causes increased endotoxin sensitivity and enhanced caspase-1 activation in mice (Chae et. al., 2003). It was also reported that P/M protein cleavage by caspase-1 leads the activation of NF- κ B (Chae et. al., 2008).

P/M isoforms of MEFV gene, which are generated by alternative splicing, were expressed and localized in cell specific manner. 8ext transcripts were degraded in monocytes, in contrast not degraded in neutrophils (Grandemange et. al., 2009). The ratio between 2D and FL transcripts were found to be three-fold in mononuclear cells compared to polynuclear cells (Papin et. al., 2000). On the other hand, in monocyte cells, 781 amino acids FL form of P/M protein located in cytoplasm and 570 amino acids spliced form located in nucleus. In contrast, opposite localization was reported in neutrophils (Diaz et. al., 2004).

Booty *et. al* reported significant increase in P/M levels between 8 FMF patients and healthy controls (Booty et. al., 2009), however these levels in Gout and AOSD patients were not shown.

1.3 Aim of the Study

The association between FMF and MEFV gene mutations/variations is well established in the previous studies, still the existence of patients without mutations/variations and P/M role were not clarified.

The aim of this study was to examine the specificity of MEFV gene to FMF or to general inflammatory pathway by comparing with other autoinflammatory diseases. MEFV exon 2 and 10 mutations/variations were explored in a group of Gout and AOSD disease patients and compared the frequencies between disease groups and healthy controls.

MEFV gene product P/M is indicated to have a role in inflammatory pathway. In the second part of the study, the aim was the quantification of P/M levels in FMF patients compared to healthy controls.

2. MATERIALS AND METHODS

2.1 Materials and Laboratory Equipments

2.1.1 Used equipments

The laboratory equipment used in this study is listed in Appendix A.

2.1.2 Used chemicals, enzymes, markers and kits

The chemicals, enzymes and markers used are given with their suppliers in Appendix B. The compositions and preparation of buffers and solutions are given in Appendix C. The kits used are given with their suppliers in Appendix D.

2.2 Sample Selection

The study group was composed of 75 FMF, 30 Gout and 28 AOSD patients who came to Romatology Polyclinic of Cerrahpasa Medical Faculty, Istanbul University. The control group was composed of 54 healthy patients chosen among whom came to the clinic either for a general checkup or diagnosed with a non – inflammatory disease. The age of the patients or healthy group is between 6 to 83. In the second part of the study, 22 FMF, one of the patients was in attack period and 9 healthy patients' samples were collected for protein study.

The Ethics Review Committee of Istanbul University Cerrahpasa Medical Faculty approved the study. The parents of children were informed and permission forms, shown in Appendix E, were fulfilled before the collection of the samples. Then, the blood samples were collected in tubes containing EDTA and were kept at 4 °C until isolation in maximum one week.

2.3 DNA Isolation from Whole Blood

The DNA isolation was done from whole blood by DNA Isolation Kit for Mammalian Blood (Roche). 6 ml Red Blood Cell Lysis (RBL) Buffer was added in to room temperature warmed 2 ml blood sample. The tube was placed on a shaker at

room temperature for 10 minutes then centrifuged at 875 X g for 10 minutes. The red supernatant was poured off carefully. This RBL step was repeated once if the pellet was not clearly white. The white pellet was homogenized by vortex. 1 ml White Blood Cell Lysis (WBCL) Buffer was added on white pellet and the tube was mixed thoroughly by vortexing. To facilitate lysis, the tube was incubated at 37 °C for 15 minutes. 560 µl Protein Precipitation Solution was added to each sample and mixed about 15 second by vortexing. The samples were transferred into sterile 2 ml eppendorf tubes. The samples were centrifuged at 12.000 X g for 10 minutes. DNA containing supernatant was transferred carefully into a new sterile 15 ml falcon tube. 2 volumes of absolute ethanol were added to the supernatant and was mixed by inversion until the liquid is no longer cloudy. Precipitated DNA was transferred by sterile pipette tip from absolute ethanol to 500 µl cold 70% ethanol. The sample was centrifuged at 875 X g for 5 minutes. The supernatant was discarded and DNA pellet was dried at room temperature. DNA pellet was resuspended in 100 µl TE buffer and incubated at 65 °C for about 30 minutes. The samples were kept at 4 °C for short term and -20 °C for long-term storage.

2.4 DNA Amount, Purity and Working Solution Calculations

The absorbance measurements of DNA at 260, 280 and 320 nm are used for the calculation of the amount and purity. The equation of DNA amount calculation is given in equation 2.1, which is the multiplication of the differences between absorbance at 260 and 320 nm by the dilution factor and the absorbance constant. It is assumed that 1 OD is equivalent to 50 µL of DNA.

$$DNA\ Concentration\ (ng / \mu L) = (A_{260} - A_{320}) \times Dilution\ Factor \times 50$$

(2.1)

DNA purity is calculated via the equation 2.2 :

$$DNA\ Purity = (A_{260} - A_{320}) \div (A_{280} - A_{320})$$

(2.2)

DNA concentrations were measured by NanoDrop (Thermo). The ratio between the absorbance value at 260 nm and 280 nm (A_{260}/A_{280}) is used to assess the purity of DNA. ~1.8 is generally accepted as “pure” for DNA. If the ratio is appreciably, it

may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. The ratio between the absorbance value at 260 nm and 230 nm (A_{260}/A_{230}) is used to assess nucleic acid purity. These measurement values of A_{260}/A_{230} for “pure” nucleic acid are often higher than the respective A_{260}/A_{280} values. They are commonly in the range of 1.8-2.2. If the ratio is appreciably lower, this may indicate the presence of co-purified contaminants. Nanodrop measurements are by mean of ng/ μ L.

All sample concentrations are adjusted to 50 ng/ μ L in 50 μ L by dilutions from stock DNA to be used as aliquots for preventing the contamination of the stock DNA.

2.5 MEFV Genotyping

2.5.1 Polymerase chain reaction (PCR)

The second and tenth exons of MEFV gene were amplified using isolated genomic DNAs as a template by Polymerase Chain Reaction (PCR). General PCR mix was prepared to amplify the target DNA sequence with relative primers and Q5 High-Fidelity DNA Polymerase (NEB). General PCR mix is given in Table 2.1.

Table 2.1 : General PCR Mix.

Ingredient	Stock Concentration	Volume	Final Concentration
Q5 Reaction Buffer	5X	10 μ l	1X
Q5 High GC Enhancer	5X	10 μ l	1X
dNTPs	10 mM	1 μ l	200 μ M
Forward Primer	10 pmol/ μ L	2.5 μ l	0.5 μ M
Reverse Primer	10 pmol/ μ L	2.5 μ l	0.5 μ M
Q5 High-Fidelity DNA Polymerase	2 U/ μ l	0.5 μ l	0.02 U/ μ l
Template DNA	50 ng/ μ L	3 μ l	150 ng
Deionized Water	-	20.5 μ l	-
TOTAL	50 μ l		

2.5.1.1 Oligonucleotide primers

The oligonucleotide primers used to amplify target sequences of MEFV were previously chosen and prepared by my lab coworkers. All of them were designed in Primer3 online tool, which calculates the efficiency of binding and the amplicon sizes. The hairpin, heterodimer and self-dimer analysis of the primer sets were

assessed with the OligoAnalyzer on the IDT DNA website. The sequences of primers are given in Table 2.2 and their location in gene in Appendix F.

Table 2.2 : Oligonucleotide Primers.

Exon	Primer Set	Primer Sequence	Amplicon Size
2	2.1	F-5'-CTCCTCTGCCCTGAATCTTG-3'	479 bp
		R-5'-AGGGCCTGCACTCCTTCT-3'	
	2.2	F-5'-GATGCGACCTAGAAGCCTTG-3'	343 bp
		R-5'-CCAGGCTGGTCTCAAAGTCT-3'	
10	10.1	F-5'-TACCCTGTCCCTGTTTCCTG-3'	660 bp
		R-5'-GATGCAGTGTTGGGCATTC-3'	

2.5.1.2 PCR conditions

PCR conditions were optimized by my lab coworkers for two different enzymes. The general PCR protocol is given in Table 2.3

Table 2.3 : General PCR Protocol.

Temperature	Time	Repeat	PCR Phase
98 °C	30 sec	1	Initial Denaturation
98 °C	10 sec	35	Denaturation
Variable	30 sec		Annealing
72 °C	30 sec		Extension
72 °C	2 min	1	Final Extension

All PCR conditions were similar, however only annealing temperatures were different because of using different primer sets shown in Table 2.4.

Table 2.4 : Annealing Temperatures of Primer Sets.

Primer Set	T _m (°C)
2.1	66
2.2	64
10.1	63

2.5.2 Agarose gel electrophoresis of PCR products

Agarose gel electrophoresis is performed to visualize the PCR products. It gives an opinion whether the PCR reaction was successful and the resulting product is the correct size.

In this study, the amplicon sizes were between 343 – 660 bp. These PCR products can be seen in 1% agarose gel. Since the percentage of the gel is important to observe the PCR bands, 1 % mini and midi gels were prepared. For mini gel, 0.5 g agarose was dissolved in 50 ml 1 X TAE buffer and mixed with 0.25 µl ethidium bromide (EtBr) just before pouring the gel into tray. For midi gel, 2 g agarose was dissolved in 200 ml 1 X TAE buffer and mixed with 0.75 µl EtBr.

In order to make the samples visible by naked eye and ease the precipitation, 5 µl of PCR products and 1 µl of 6 X loading dye (Fermentas) were mixed and loaded into wells. Only in the first well, DNA ladder low range (Fermentas) was loaded as standard, in order to assess the relative lengths of the PCR products. The gels were run at 120 V for about 40 min. and analyzed under UV light with transilluminator. The pictures of gels were taken with UV PhotoMW software.

2.5.3 Sequencing

The second and tenth exon products of MEFV gene were Sanger sequenced and chromatograms were analyzed using Geneious softwares by two independent researchers.

2.5.4 Genotype analysis

According to target sequences, first part of the second exon, second part of the second exon and first part of the tenth exon were introduced as reference sequences to the program. The patients'/healthy controls' sequences were aligned to its related reference sequence and these two sequences were compared. All peaks and bases were searched for differences and single and/or double peaks. The analysis depends on the base change or peak numbers observed. A single peak corresponds to homozygote variation, whereas double peaks are observed in heterozygote mutation/variation. The genotype analysis were performed in two directions; forward and reverse. Moreover, the heterozygote variation can also be seen without the change in bases, but giving double peaks.

All the alleles and genotypes analyzes were counted in all groups and calculations were performed to test whether the Hardy-Weinberg requirements were fulfilled. Hardy-Weinberg equation (HWE) assumes that the genetic variation amount in a population will remain constant from one generation to the next in the absence of disturbing factors. An online tool by Tufts University, Court Lab, was used for HWE calculations.

The HWE is shown in equation 2.3 :

$$p^2 + 2pq + q^2 = 1$$

(2.3)

2.6 Protein Isolation from Whole Blood

The protein isolation starts with white blood cell obtain as the same protocol in DNA isolation from whole blood. The white pellet was washed with 1X PBS by vortexing to remove the remaining RBL residues from the sample. The tube was centrifuged at 1000 X g for 5 minutes, the supernatant was removed and the tube was placed on ice. The following steps were applied on white pellet. Isolated proteins were kept at -20 °C for short term and -80°C for long-term storage.

400 µl of Igepal-Protease Inhibitor-PBS mixture was added on white pellet, mixed by pipetting to avoid bubble formation and pulsed for 30 seconds. The supernatant was removed to a new sterile tube as cytoplasmic protein of the sample and kept on ice. Another 400 µl of buffer was added on pellet and vortexed well. The tube was pulsed for 15 seconds and the supernatant was removed. 100 µl of TNN buffer was added on top of the pellet, without mixing, the tube was incubated on ice for 30 minutes. Next, centrifugation at 13.000 rpm for 10 minutes was done. In this step, the supernatant was nuclear proteins. To measure both cytoplasmic and nuclear protein concentrations, some amount of both proteins were separated and then mixed as whole protein in a new sterile tube.

2.7 Bradford Assay

Bradford Assay was performed to determine the total protein concentrations of the samples. The assay is based on the absorbance for Coomassie Brilliant Blue Dye binding to proteins, which result in colour change from brown to blue form of the

dye. The optimal wavelength is 595 nm to measure the Coomassie Dye and protein complex. Bovine Serum Albumin (BSA) was used as protein standard, which is known to show a linear absorbance profile. The measurement was performed in 96 well plate at room temperature.

Bradford Reagent was prepared 1:5 in ratio by using distilled proteomic water as diluent. 195 μ l of diluted reagent was added into each well. 5 μ l of BSA standards or protein samples were added and the mixture homogenized by pipetting. After 2 minutes of incubation at room temperature the absorbance values were measured at 595 nm by Microplate reader (Biorad).

2.8 SDS-PAGE

SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis) is the first step of Western blotting, which separates the proteins based on their molecular weight and different migration rate through a gel under an applied electrical field. The acrylamide percentage in two types of SDS PAGE gels, separating and stacking gels, depends on the size of the target protein in the sample. The lower acrylamide percentage is needed for the higher molecular weight of protein, since it provides much bigger pores. For example, 7% acrylamide gel is used for proteins between 50 to 500 kDa, whereas 15% acrylamide gel is used between 3 to 100 kDa proteins.

SDS Gel Preparation Kit (Sigma-Aldrich) was used for SDS-PAGE gels' preparation. 12.5% separating gel was prepared in 50 ml centrifuge tube and transferred into the gap between gel cassettes via pipetting. Isopropanol was filled into the gap until an overflow to achieve the separating gel be horizontal. After 30 minutes of separating gel polymerization at room temperature, the isopropanol was discarded with a tissue paper and the gel cassette was filled with deionized water to clean the isopropanol completely. 5% stacking gel was prepared and added onto the polymerized separating gel until an overflow by pipetting, well-forming comb was inserted without trapping air under the teeth and was let polymerization for 45 minutes. Then, protein samples were prepared for loading. Total protein loaded was calculated via the measurements from Bradford Assay for each sample. ~15 μ g of protein samples were mixed with the same volume of sample buffer (loading buffer) and denatured at 95 °C for 5 minutes. ~20 μ l of each sample was loaded into wells. 10 μ l of protein ladder was loaded into the first lane. Electrophoresis was performed

in 1X Running Buffer at 90 V for 20 minutes and then the voltage was increased to 120 V for 90 min.

After electrophoresis, the SDS-PAGE gels were gently taken and placed into plastic box. Coomassie Blue Stain was added to cover the gel placed in a plastic box and incubated on shaker overnight at room temperature. The Coomassie Stain was discarded and the gel was washed with deionized water to remove the remaining staining solution from the container. Then, destain solution was added on gel and heated in microwave on high power for 45 seconds to 1 minute until the solution boiled. The solution was discarded and the gel was washed. Destaining steps were repeated twice, in the second time for 30 seconds of heating. Finally, folded paper towel was put in container with the gel and destaining solution and left for 2 to 4 hours on shaker. Destain solution was discarded and destained gels were kept in box full of deionized water at room temperature.

The analyzes of protein samples stained with Coomassie Brilliant Blue following Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed by densitometry scanning by using *ImageJ* software.

2.9 Western Blotting

In Western blotting method, the separated proteins were transferred from gel to a nitrocellulose membrane in order to visualize the target protein via using primary and secondary antibodies specific to the protein of interest.

The transfer was performed as wet electroblotting (tank transfer), which includes sponges, pads and filter papers in transfer sandwich. After SDS-PAGE is completed, the gel was placed in a plastic box with some amount of transfer buffer and shaken for 15 minutes at room temperature. Since high voltage (up to 200 V) or high current (up to 1.6 A) produce tremendous heat, the transfer was performed with an ice pack at 4°C room on a magnetic stirrer. The membrane was incubated with blocking buffer at room temperature in a plastic box for an hour and washed twice with TBS to discard the remaining blocking buffer residues. The membrane was incubated with primary antibody solution overnight at 4°C room in 50 ml centrifuge tube. The membrane was washed with TBS once for 10 minutes and TBST for four times for 5 minutes each to avoid non-specific binding. Then, the second incubation of HRP-

linked secondary antibody solution was done for half an hour at room temperature on a rotator in 50 ml centrifuge tube. The membrane was washed with TBS once for 10 minutes and TBST for six times for 5 minutes each to avoid non-specific binding.

All the buffers were kept at 4°C prior to use. The membranes were kept in TBS at 4°C until scanning was performed for a maximum of a week.

The membrane was incubated with HRP substrates solution in dark for 5 minutes until the protein bands were detectable. The membranes were analyzed by imaging system (ChemiDoc XRS+ Imaging System, *Image Lab* software).

2.10 Statistical Analysis

Statistical analyses were performed to assess the significance of MEFV allele frequencies between groups. Fisher's and chi-square test were performed to evaluate the distribution of genotypes of FMF, Gout and AOSD patients and healthy controls to find the existence of significant difference between the mutation/variation frequencies. T test was performed to examine the P/M level differences in FMF and HC group.

The p -value is calculated to determine the significance of the results. The p -value intervals are:

$p < 0.001$: extremely significant difference

$p < 0.01$: highly significant difference

$p < 0.05$: significant difference

$p > 0.05$: the absence of significant difference

Calculations of p -value, chi-square and Odds Ratio (OR) were done via the online tool of Graphpad.

3. RESULTS

3.1 Demographic Data of the Patients

Samples were collected from 75 FMF, 30 Gout, 28 AOSD patients and 54 healthy controls. The demographic data of the patients are given in the Table 3.1.

Table 3.1 : Demographic Data of the Patients.

Group	Gender	Number	Percentage (%)	Mean Age
FMF (N= 75)	Female	41	54,7	33,5 ± 12,4
	Male	34	45,3	
GOUT (N= 30)	Female	3	10	58,5 ± 8,2
	Male	27	90	
AOSD (N= 28)	Female	12	42,9	37,8 ± 6,7
	Male	16	57,1	
Healthy (N= 54)	Female	35	64,8	32,1 ± 8,2
	Male	19	35,2	

3.2 DNA Isolation Results

The average of DNA concentration was 552.59 ng/μL and A_{260}/A_{280} ratio was 1.87 (N=187).

3.3 Genotype Analysis Results

The obtained genotypes are shown in Table 3.2. The chromatogram analyses of MEFV exon 2 and 10 are shown in Appendix G. Sample PCR results are given in Appendix H.

Table 3.2 : Obtained Genotypes.

Mutation / Variation	Base Change	Genotype	Group			
			FMF (N=75)	GOUT (N=30)	AOSD (N=28)	HC (N=54)
A165A	C>A	CC	11	17	19	27
		CA	33	11	6	17
		AA	31	2	3	10
D102D	T>C	TT	18	13	17	27
		TC	27	15	8	17
		CC	30	2	3	10
D103D	T>C	TT	74	30	28	54
		TC	1	0	0	0
		CC	0	0	0	0
E148Q	G>C	GG	67	28	24	48
		GC	8	1	4	6
		CC	0	1	0	0
E167D	G>C	GG	72	30	28	54
		GC	3	0	0	0
		CC	0	0	0	0
G138G	A>G	AA	12	12	19	29
		AG	30	16	6	15
		GG	33	2	3	10
L110P	T>C	TT	73	30	27	53
		TC	2	0	1	1
		CC	0	0	0	0
R202Q	G>A	GG	29	16	17	37
		GA	29	13	11	13
		AA	17	1	0	4
R278P	G>C	GG	75	30	27	54
		GC	0	0	1	0
		CC	0	0	0	0
A744S	G>T	GG	74	30	28	54
		GT	1	0	0	0
		TT	0	0	0	0
K695R	A>G	AA	75	29	28	54
		AG	0	1	0	0
		GG	0	0	0	0
M680I (G/A)	G>A	GG	73	30	28	54
		GA	1	0	0	0
		AA	1	0	0	0

Table 3.2 (continued): Obtained Genotypes.

Mutation / Variation	Base Change	Genotype	Group			
			FMF (N=75)	GOUT (N=30)	AOSD (N=28)	HC (N=54)
M680I (G/C)	G>C	GG	62	30	28	54
		GC	11	0	0	0
		CC	2	0	0	0
M694I	G>A	GG	74	30	28	54
		GA	1	0	0	0
		AA	0	0	0	0
M694V	A>G	AA	30	26	27	51
		AG	23	4	1	3
		GG	22	0	0	0
P780T	C>A	CC	69	30	28	53
		CA	6	0	0	1
		AA	0	0	0	0
R761H	G>A	GG	70	30	28	52
		GA	5	0	0	2
		AA	0	0	0	0
S675N	G>A	GG	74	30	28	54
		GA	1	0	0	0
		AA	0	0	0	0
V726A	T>C	TT	68	30	27	53
		TC	5	0	1	1
		CC	2	0	0	0

In this study I692del, I772V, M680V and P769A on exon 10 were not observed.

MEFV mutations/variations were also examined in protein study group. Observed genotypes were shown in Table 3.3.

Table 3.3 : Obtained Genotypes in P/M Group.

Mutation / Variation	Base Change	Genotype	Group	
			FMF (N=22)	HC (N=9)
A165A	C>A	CC	6	2
		CA	9	5
		AA	7	2
D102D	T>C	TT	7	2
		TC	8	5
		CC	7	2

Table 3.3 (continued) : Obtained Genotypes in P/M Group.

Mutation / Variation	Base Change	Genotype	Group	
			FMF (N=22)	HC (N=9)
G138G	A>G	AA	6	2
		AG	9	5
		GG	7	2
E148Q	G>C	GG	20	8
		GC	2	1
		CC	0	0
L110P	T>C	TT	20	9
		TC	2	0
		CC	0	0
R202Q	G>A	GG	9	2
		GA	9	7
		AA	4	0
M680I (G/A)	G>A	GG	21	9
		GA	0	0
		AA	1	0
M680I (G/C)	G>C	GG	16	9
		GC	6	0
		CC	0	0
M694V	A>G	AA	10	8
		AG	7	1
		GG	4	0
P780T	C>A	CC	22	8
		CA	0	1
		AA	0	0
R761H	G>A	GG	19	8
		GA	3	1
		AA	0	0
V726A	T>C	TT	20	9
		TC	1	0
		CC	1	0

3.4 Statistical Mutation/Variation Analysis

The allele frequencies of MEFV mutations/variations between three patient groups are compared and given in Table 3.4. All of the genotypes were consistent with HWE (except D102D, M694V, V726A in FMF; A165A, D102D and G138G in HC).

Table 3.4 : Allele Frequencies of MEFV Mutations/Variations.

Mutation / Variation	Group			
	FMF (%) (N=150)	GOUT (%) (N=60)	AOSD (%) (N=54)	HC (%) (N=108)
A165A	63,3	25,0	21,4	34,3
D102D	58,0	31,7	25,0	34,3
D103D	0,7	0,0	0,0	0,0
E148Q	5,3	5,0	7,1	5,6
E167D	2,0	0,0	0,0	0,0
G138G	64,0	33,3	21,4	32,4
L110P	1,3	0,0	1,8	0,9
R202Q	42,0	25,0	19,6	19,4
R278P	0,0	0,0	1,8	0,0
A744S	0,7	0,0	0,0	0,0
K695R	0,0	1,7	0,0	0,0
M680I (G/A)	2,0	0,0	0,0	0,0
M680I (G/C)	10,0	0,0	0,0	0,0
M694I	0,7	0,0	0,0	0,0
M694V	44,7	6,7	1,8	2,8
P780T	4,0	0,0	0,0	0,9
R761H	3,3	0,0	0,0	1,9
S675N	0,7	0,0	0,0	0,0
V726A	6,0	0,0	1,8	0,9

19 types of MEFV gene mutations/variations were detected in 73 (97.3%) patients in FMF group, 21 (75%) in AOSD, 20 (66.6%) in Gout and 35 (64.8%) in healthy control group. MEFV mutations/variations were detected in 16.2% of alleles in FMF, 6.8% in Gout, 5.4% in AOSD patients and 7% in healthy controls. The difference in the frequency of the MEFV mutations/variations between the patient groups and control group was statistically significant ($p < 0.0001$).

The common haplotype, A165A-D102D-G138G, was observed frequently in all groups, but the frequency was higher in FMF group (61.8%) compared to Gout (30%), AOSD (22.6%) and HC (33.6%) (FH, FG, FS: $p < 0.0001$, SH: $p = 0.0126$, others: NS).

One of the most common variations E148Q in exon 2 was observed at a frequency quite similarly between groups. The frequencies were 5.3%, 5.0%, 7.1%, 5.6% in FMF, Gout, AOSD patients and HC, respectively (non-significant). In exon 2, only

R202Q variation was found to be more frequent in FMF patients (42%) compared to other groups (19-25%) (FH: $p<0.0001$, FG: $p=0.0266$, FS: $p=0.0032$, others: NS). Out of 16.2% of all observed mutations/variations in FMF, R202Q was observed in 2.2%, whereas E148Q in 0.3%.

There was also significant difference in pathogenic mutations/variations between FMF and other groups. Out of 5.2% of all alleles, they were observed 3.5% in FMF, whereas 0,5-0,6% in other groups. Within these pathogenic mutations/variations, out of 19.7%, only E148Q (4.6%) and M694V (11.2%) were observed in all groups. The most prominent pathogenic mutation/variation was M694V. It was observed 8.9% in FMF, 1.3% in Gout, 0.4% in AOSD and 0.6% in healthy controls as being the most frequent mutation/variation ($p=<0.0001$).

Allele frequencies of P/M group were shown in Table 3.5.

Table 3.5 : Allele Frequencies in P/M Group.

Mutation / Variation	Group	
	FMF (%) (N=44)	HC (%) (N=18)
A165A	52,3	50,0
D102D	50,0	50,0
G138G	52,3	50,0
E148Q	4,5	5,6
L110P	4,5	0
R202Q	38,6	38,9
M680I (G/A)	4,5	0
M680I (G/C)	13,6	0
M694V	34,1	5,6
P780T	0,0	5,6
R761H	6,8	5,6
V726A	6,8	0

3.5 Protein Isolation Results

Protein samples' concentrations were measured by Bradford assay with five protein standards of BSA concentrations ranging from 0.125 mg/ml to 4 mg/ml. The average of protein concentration was 3.91 $\mu\text{g/ml}$ (N=31).

3.6 SDS-PAGE Results

The separation of protein bands in SDS-PAGE was optimized by using different concentration of gels and different concentration of proteins. The separating gel concentrations were 12.5%. Protein marker (Novex) was used to observe the localization of the interested proteins on gel.

Two gels were loaded for the same volumes of the same samples and run together in the same SDS-PAGE conditions. One of them was carried on with transferring while the other was not. When the transfer was completed, Coomassie staining was applied on both gels. The aim was to observe the exact protein locations on not-transferred gel and to confirm whether transfer was achieved or not on transferred-gel. Densitometric analyses are shown in Figure 3.1 (Figure 3.1a: Not-transferred SDS-PAGE gel, Figure 3.1b: transferred gel).

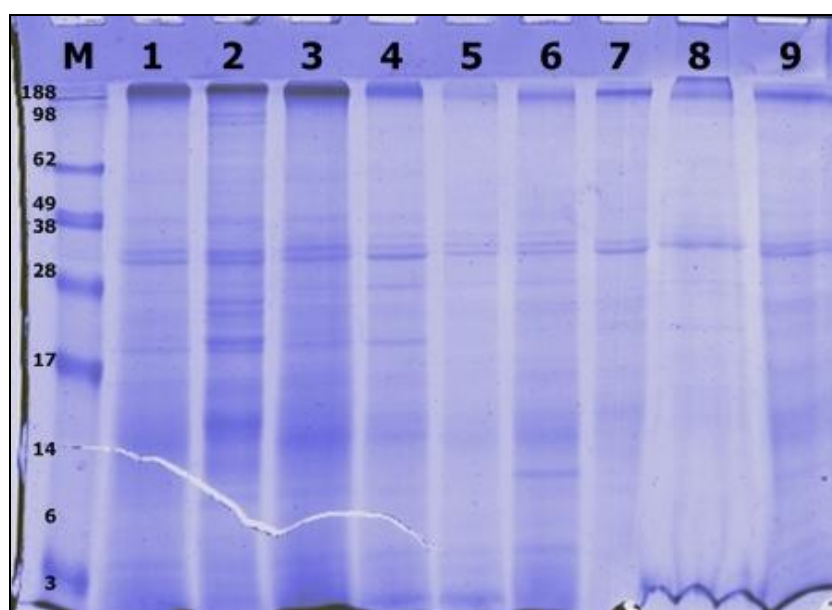


Figure 3.1a : Not-transferred SDS-PAGE Gel.

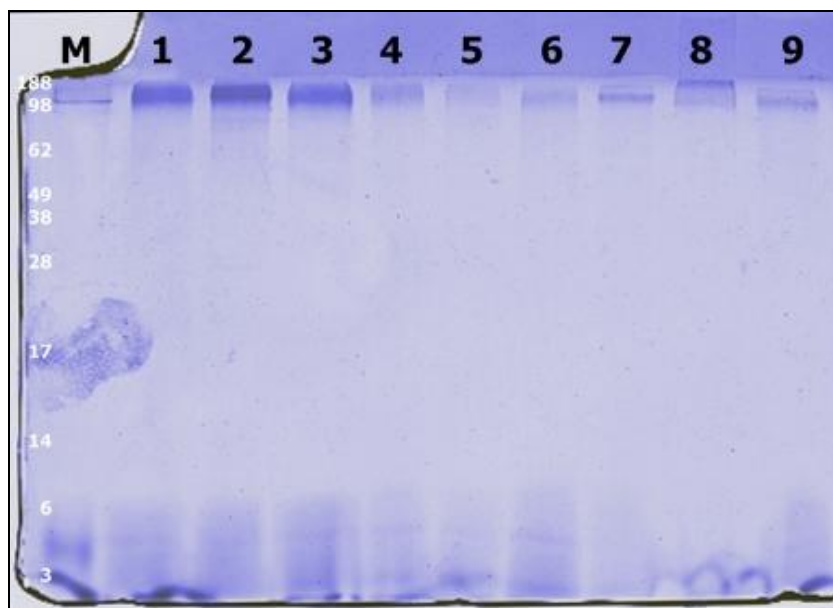


Figure 3.1b : Transferred Gel.

3.7 Western Blot Results

The Ponceu S staining was applied on membrane when the transfer was completed in order to confirm whether transfer was achieved or not. By the observation of protein bands, destaining solution was applied on the membrane to continue with Western blotting method. The transfer was performed by constant current of 60 mA an hour at 4°C with cold transfer buffer.

Application of compatible antibodies for proteins of interest and enzyme substrates enabled the visualization of target proteins. Analyses of the protein bands with *Image Lab* software gives numerical values linked to density and thickness of bands.

Histone H3 is a protein that is widely used as an internal control for nuclear fraction in Western blotting experiments. The figure below represents anti-pyrim and anti-histone studies, which suggest the possible optimized protocol for nuclear protein extraction and Western blotting experiments, as shown in Figure 3.2. Band report was given in Appendix I.

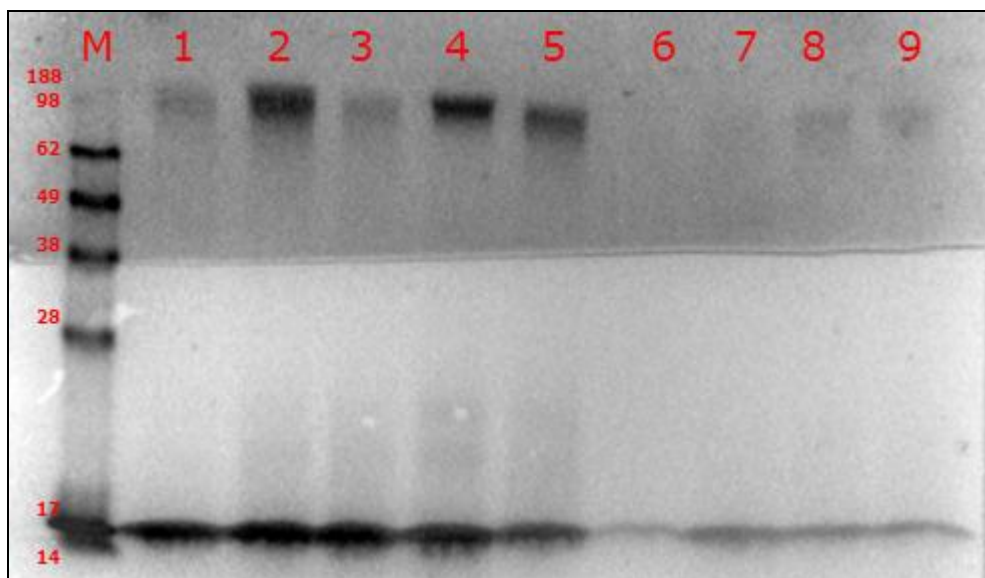


Figure 3.2 : Histone/Pyrin Western Blot Membrane.

3.8 Statistical P/M Level Analysis

To investigate the relationship between mutations/variations and P/M levels, MEFV mutation/variation analysis were also performed in protein study group. The P/M quantity and allele sum were further analysed as shown in Table 3.6.

Table 3.6 : Allele Sum vs. P/M Quantity.

Code	Allele Sum	P/M Quantity
F1	8	0,106
F2	7	0,841
F3	9	0,008
F4	6	0,058
F5	9	0,044
F6	5	0,09
F7	4	0,073
F8*	9	1,747
F9	10	0,022
F10	3	0,835
F11	2	0,016
F12	9	0,042
F13	7	1,239
F14	3	0,121
F15	4	0,189
F16	0	0,12

Table 3.6 (continued) : Allele Sum vs. P/M Quantity.

Code	Allele Sum	P/M Quantity
F17	1	0,168
F18	2	0,153
F19	0	0,062
F20	7	0,059
F21	8	0,186
F22	5	0,00012
H1	4	0,09
H2	4	0,169
H3	7	0,658
H4	0	0
H5	5	0,628
H6	4	0,926
H7	7	0,922
H8	0	0,162
H9	7	0,318

The allele frequencies of P/M group were shown in Table 3.7.

Table 3.7 : Allele Frequencies of P/M Group.

Mutation / Variation	Group	
	FMF (%) (N=44)	HC (%) (N=18)
A165A	52,3	50,0
D102D	50,0	50,0
G138G	52,3	50,0
E148Q	4,5	5,6
L110P	4,5	0
R202Q	38,6	38,9
M680I (G/A)	4,5	0
M680I (G/C)	13,6	0
M694V	34,1	5,6
P780T	0,0	5,6
R761H	6,8	5,6
V726A	6,8	0

The average of P/M quantities were 0.281 in FMF and 0.430 in HC. Anti-pyrimidine and anti-histone studies' results were analyzed as shown in Figure 3.3.

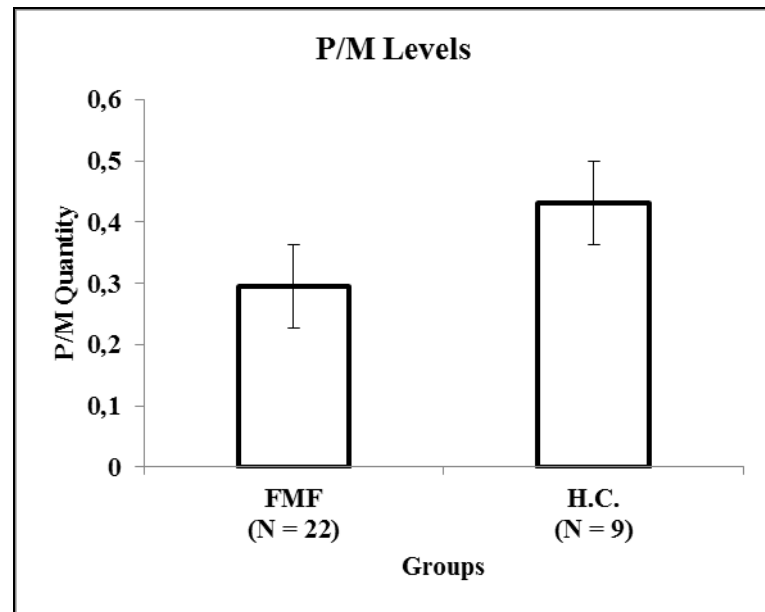


Figure 3.3 : P/M Levels of FMF and HC Group.

Although two fold decrease was observed in P/M levels in FMF patients compared to HC group, the t test result was non-significant ($p=0.3920$).

4. CONCLUSION AND RECOMMENDATIONS

MEFV gene encodes P/M protein, which is known to play role in inflammatory pathway and apoptosis. The association between FMF and MEFV gene mutations/variatiions is well established in the previous studies, still the existence of patients without mutations/variatiions and P/M role were not clarified. Recent studies have shown the MEFV mutations and variatiions existence in other inflammatory disorders other than FMF.

Touitou et. al. reported E148Q, V726A and M694V mutations/variatiions in Behcet's disease (BD) patients, the frequencies were 5.2%, 2.6%, 2.6%, respectively (non-significant). Rustemoglu et. al. reported E148Q, V726A, M694V and M680I mutations/variatiions in BD, the frequencies were 7.25% ($p=0.001$), 3.14% (NS), 4.35% (NS) and 4.59% ($p=0.049$), respectively. Koca et. al., E148Q, V726A, M694V, M94I and M680I mutations/variatiions in Rheumatoid Arthritis (RA) patients, the frequencies were 11.7%, 5.8%, 1.9%, 2.9% and 0.9%, respectively (NS). Beser et. al. reported E148Q, M694V, M680I and K695R mutations/variatiions in inflammatory bowel disease (IBD) patients.

In this study, we wanted to investigate the mutations/variatiions on exon 2 and 10 of MEFV gene in a group of FMF (N=75), Gout (N=30) and AOSD (N=28) patients and compare the frequencies between disease groups and healthy controls (N=54).

We first compared all mutations/variatiions between the patient and HC groups. MEFV gene mutations/variatiions were detected in 73 (97.3%) patients in FMF group, 21 (75%) in AOSD, 20 (66.6%) in Gout and 35 (64.8%) in HC group. MEFV mutations/variatiions were detected in 16.2% of alleles in FMF, 6.8% in Gout, 5.4% in AOSD patients and 7% in HC. The difference in the frequency of the MEFV mutations/variatiions between the patient groups and control group was statistically significant ($p<0.0001$).

In previous studies, there are cases reported as being clinically FMF without any MEFV mutation/variation (7%) (Ozalkaya et. al., 2011). In our study, FMF patients without MEFV mutation/variation were 2.7% of the group.

The common haplotype A165A-D102D-G138G was observed frequently in all groups, but the frequency was higher in FMF group (61.8%) compared to Gout (30%), AOSD (22.6%) and HC (33.6%) (non-significant).

Sari et. al. reported E148Q, V726A, M694V, M694I and M680I mutations in gouty arthritis (GA) patients (NS). They also reported total exon 2 and 10 mutation rate, 6.2% and 11%, respectively. Nonaka et. al. reported E148Q, V726A, M694V, M694I, M680I and also G632S, L110P, R202Q, G304R mutations/variations in AOSD patients (NS).

One of the most common variations E148Q in exon 2 was observed at a frequency quite similarly between groups. The frequencies were 5.3%, 5.0%, 7.1%, 5.6% in FMF, AOSD, Gout patients and healthy controls, respectively (non-significant). In exon 2, only R202Q variation was found to be more frequent in FMF patients (42%) compared to other groups (19-25%) (FH: $p < 0.0001$, FG: $p = 0.0266$, FS: $p = 0.0032$). Out of 16.2% of all observed mutations/variations in FMF, R202Q was observed in 2.2%, whereas E148Q in 0.3%.

There was also significant difference in pathogenic mutations/variations between FMF and other groups. Out of 5.2% of all alleles, they were observed 3.5% in FMF, whereas 0.5-0.6% in other groups. Within these pathogenic mutations/variations, out of 19.7%, only E148Q (4.6%) and M694V (11.2%) were observed in all groups. The most prominent pathogenic mutation/variation was M694V. It was observed 8.9% in FMF, 1.3% in Gout, 0.4% in AOSD and 0.6% in healthy controls as being the most frequent mutations/variations ($p < 0.0001$).

As a further aspect, increased M694V frequency in Gout and AOSD patients compared to HC requires further analysis.

Second part of this study, we wanted to investigate the quantification of P/M levels in FMF (N=22) patients compared to healthy controls (N=9). In previous studies, Booty et. al. reported higher MEFV expression in FMF compared to healthy controls. They also examined P/M levels by Western blot analysis and found significant

increase in P/M levels between FMF patients and healthy controls (Booty et. al., 2009).

Our preliminary results indicate approximately two fold decrease of P/M levels in FMF patients compared to HC group ($p=0.3920$), which is the opposite of the previous results. To investigate the relationship between mutations/variations and P/M levels, MEFV mutation/variation analysis were also performed in protein study group. The allele frequencies of M694V in FMF and HC were 34.1% and 5.6%, respectively ($p=0.0247$). E148Q, R202Q and R761H were also observed in both groups, however NS.

The average P/M quantity of the patients carrying M694V mutation/variation was 0.23, whereas non-carriers had 0.33. One of the FMF patients was in her/his attack period when the sample was collected and the P/M level was highest even though he/she was not M694V mutation/variation carrier. We have observed much higher P/M level and total mutation/variation carrying allele in attack patient compared to attack free patients. The protein study requires optimization with enlarged sample sizes and replicated for more accurate statistical results.

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APPENDICES

APPENDIX A: Laboratory Equipment

APPENDIX B: Chemicals, Enzymes and Markers

APPENDIX C: Buffers and Solutions

APPENDIX D: Used Kits

APPENDIX E: Permission Form

APPENDIX F: Primer Sets Locations on MEFV Exons

APPENDIX G: Chromatogram Analyses of MEFV Exon 2 and 10

APPENDIX H: Sample PCR Results

APPENDIX I: *Image Lab* Reports

APPENDIX A

LABORATORY EQUIPMENT

96 well plate	Greiner Bio-One
Autoclave	Tomy SX-700E
Balances	Precisa XB-620C Precisa XB-220A
Block Heater	Techne Dri-Block DB-2D
Centrifuge Tubes	Greiner Bio-One Cellstar Tubes
Centrifuges	Allegra 25R Centrifuge Beckman Coulter
ChemiDoc XRS+ Imaging System	Bio-Rad
Densitometry	Bio-Rad
Electrophoresis Equipments	Wealtec Elite 300 Power Supply
Fridge and Freezers (+4°C, -20°C, -80°C)	Vestel Arçelik Nüve DF490
Gel Documentation System	Biolab
Ice Machine	Scotsman AF580
Laminar Flow Hood	Biolab
Magnetic Stirrer	Velp Scientifica Age
Microplate Reader	Bio-Rad Benchmark Plus
Minicentrifuge	Thermo Scientific MicroCLL-17 Microcentrifuge
Mini-Vertical Gel System	Bio-Rad Mini-Protean 3 Cell

Nanodrop	Thermo Scientific Nanodrop 2000 Spectrophotometer
Orbital Shaker	Heidolph Duomax 1030
Parafilm	PM996
pH meter	Hanna Instruments pH211, Microprocessor pH meter
Pipette Tips	Axygen Scientific
Pipettes	Thermo Scientific
Sonicator	Bandelin Sonoplus
Thermo cycler	Bio-Rad T100 Thermal Cycler Techne TC-412 Palm Cycler
Transfer system	Bio-Rad Transblot Cell
Transilluminator	Biometra UV Star
Vortex	Labnet Vortex Mixer Heidolph Reax Top
Water Distillation System	TKA Pacific
Whatman Filter Paper	Schleicher & Schuel 1001 125

APPENDIX B

CHEMICALS

β-Actin	Cell Signaling
6X Loading Dye	Fermentas
Acetone (C₃H₆O)	Merck
Agarose	Applchem Invitrogen
Ammonium Chloride (NH₄Cl)	Sigma-Aldrich
Ammonium Persulfate (APS)	Sigma-Aldrich
Anti-Goat	Thermo Scientific
Anti-Mouse	Cell Signaling
Anti-Pyrim	Lifespan Biosciences
Anti-Rabbit	Cell Signaling
Bovine Serum Albumine (BSA)	Sigma-Aldrich
Bradford Reagent	Bio-Rad
Coomassie Brilliant Blue (CBB)	Sigma-Aldrich
dNTP, 10 mM	Fermentas
DreamTaq PCR Buffer	Thermo Scientific
Ethylene Diamine Tetraacetic Acid (EDTA)	Applchem
Ethidium Bromide (EtBr)	Amresco
Ethanol	Merck

Glacial Acetic Acid	Merck
Glycine	Applichem
Histone H3	Cell Signaling
HRP Substrates	Advansta Western Bright ECL Advansta Western Bright Sirius Bio-Rad Clarity Western ECL Substrate
Igepal	Sigma-Aldrich
Isopropanol	Applichem
Methanol	Merck Sigma-Aldrich
Magnesium Chloride (MgCl₂)	Fermentas
Sodium Chloride (NaCl)	Merck
Sodium Hydroxide (NaOH)	Riedel-de Haën
Nitrocellulose Membrane	Santa Cruz
Non Fat Dry Milk	Cell Signaling
Phosphate Buffered Saline (PBS) Tablet	Sigma-Aldrich
Ponceau S	Sigma-Aldrich
Potassium Bicarbonate (KHCO₃)	Sigma-Aldrich
Primers	Alpha DNA
Protease Inhibitor Coctail Tablet	Roche
Proteomic Water	Applichem Merck

Q5 PCR Buffer	New England Biolabs
RNA Polymerase	Millipore
Sodium Chloride (NaCl)	Merck
Sodium Dodecyl Sulfate (SDS)	Sigma-Aldrich
Trichloroacetic Acid (TCA)	Riedel-de Haen Applichem
Tris	Applichem
Trizma Base	Sigma-Aldrich
Tween-20	Applichem
Urea	Applichem
Vinculin	Cell Signaling

ENZYMES

DreamTaq DNA Polymerase

Thermo Scientific

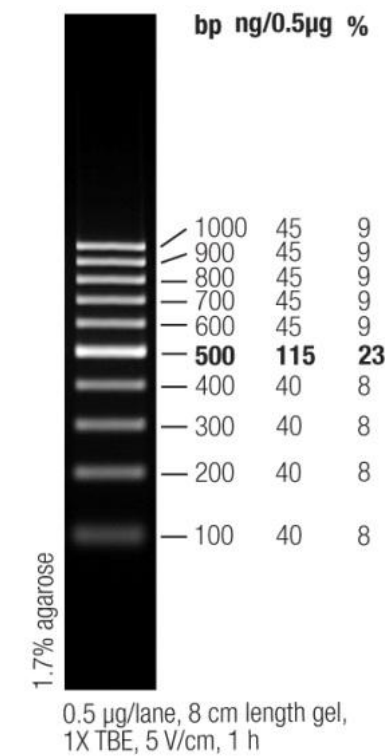
Q5® High-Fidelity DNA Polymerase

New England Biolabs

MARKERS

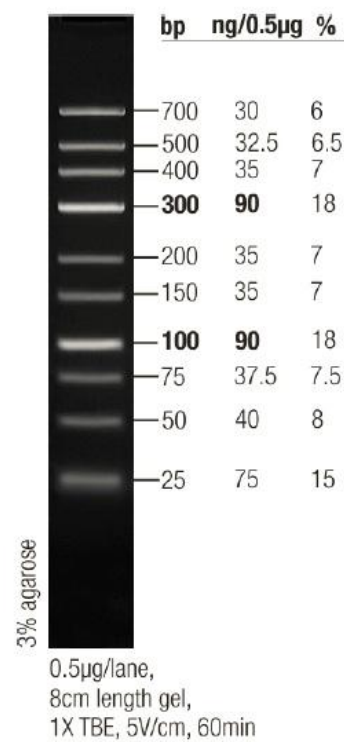
GeneRuler 100 bp DNA Ladder

Fermentas



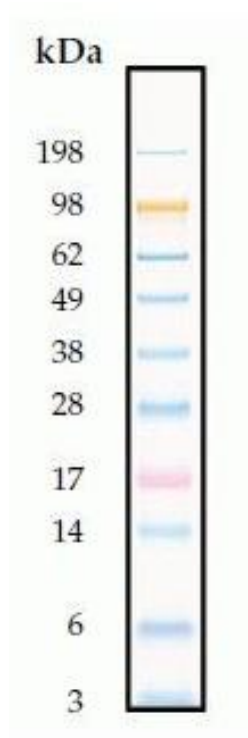
GeneRuler Low Range DNA Ladder

Fermentas



SeeBlue® Plus2 Pre-Stained Protein Standard

Invitrogen



APPENDIX C

BUFFERS AND SOLUTIONS

Ethylene Diamine Tetraacetic Acid (EDTA) Solution (0.5 M)

EDTA	7.30 g
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Add dH₂O up to 50 ml and adjust the pH to 8.0 .

Sodium Chloride (NaCl) Solution (5 M)

NaCl	292.2 g
------	---------

Add dH₂O up to 1 lt.

Sodium Hydroxide Solution (NaOH) (10 M)

NaOH	200 g
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Add dH₂O up to 500 ml.

Tris-HCl Solution (1 M)

Tris-HCl	121.1 g
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Add dH₂O up to 1 lt and adjust the pH to 7.0.

Red Blood Cell Lysis (RBL) Buffer (10X)

Ammonium Chloride (NH ₄ Cl)	87.4 g
--	--------

Potassium Bicarbonate (KHCO ₃)	10 g
--	------

0.5 M EDTA	2 ml
------------	------

Add dH₂O up to 1 lt .

RBL Buffer (1X)

Red Blood Cell Lysis (RBL) Buffer (10X)	100 ml
---	--------

Add dH₂O up to 1 lt .

Tris-EDTA (TE) Buffer (10X)

Tris	10 mM
------	-------

EDTA	1 mM
------	------

Add dH₂O up to 1 lt and adjust the pH to 8.0.

Tris-Acetic Acid-EDTA (TAE) Buffer (50X)

Trizma Base	242 g
Glacial Acetic Acid	57.1 ml
0.5 M EDTA	100 ml

Add dH₂O up to 1 lt .

Ethidium Bromide (EtBr) Solution

EtBr	500 mg
------	--------

Add dH₂O up to 50 ml.

Mini Agarose Gel (1%)

Agarose	0.5 g
TAE Buffer (1X)	50 ml
EtBr	2.5 µl

Add EtBr before pouring the gel into tray.

Midi Agarose Gel (1%)

Agarose	1.5 g
TAE Buffer (1X)	150 ml
EtBr	7.5 µl

Add EtBr before pouring the gel into tray.

Urea Solution (8M)

Urea	24.02 g
------	---------

Add dH₂O up to 50 ml.

Trichloroacetic Acid (TCA) Solution

TCA	220 g
-----	-------

Add dH₂O up to 100 ml.

Protease Inhibitor (PI) Coctail (10X)

Add 1 ml dH₂O per one Protease Inhibitor Coctail Tablet.

PI Solution (1X)

Protease Inhibitor (PI) Cocktail (10X)	100 µl
--	--------

Add dH₂O up to 1 ml.

Sodium Dodecyl Sulphate (SDS) Solution (10%)

SDS	100 g
-----	-------

Add dH₂O up to 1 lt .

Ammonium Persulfate (APS) Solution

APS	100 mg
-----	--------

Add dH₂O up to 1 ml.

Phosphate Buffered Saline (PBS) Solution (1X)

Add 200 ml dH₂O per one PBS tablet.

PBS-Tween (PBST) Buffer

Tween-20	200 µl
----------	--------

PBS Solution (1X)	200 ml
-------------------	--------

Tris Buffered Saline (TBS) Solution (10X)

Trizma Base	60.6 g
-------------	--------

NaCl	87.6 g
------	--------

Add dH₂O up to 1 lt .

TBS-Tween (TBST) Buffer (1X)

Tween-20	1 ml
----------	------

TBS Solution (10X)	100 ml
--------------------	--------

Add dH₂O up to 1 lt .

Lysis Buffer

Igepal	1%
--------	----

PI Solution (1X)	1%
------------------	----

Add PBS up to 20 ml.

TNN (Tris-HCl NaCl) Buffer

1 M Tris-HCl	10 ml
--------------	-------

5 M NaCl	10 ml
----------	-------

0.5 M EDTA	2 ml
------------	------

Igepal	10%
--------	-----

Add dH₂O up to 200 ml and adjust the pH to 8.0 .

Bradford Standards

S0	30 ml dH ₂ O
-----------	-------------------------

S1	0.125 mg/ml BSA + 30 ml dH ₂ O
-----------	---

S2	0.25 mg/ml BSA + 30 ml dH ₂ O
-----------	--

S3	0.5 mg/ml BSA + 30 ml dH ₂ O
-----------	---

S4	1 mg/ml BSA + 30 ml dH ₂ O
-----------	---------------------------------------

S5	2 mg/ml BSA + 30 ml dH ₂ O
-----------	---------------------------------------

S6	4 mg/ml BSA + 30 ml dH ₂ O
-----------	---------------------------------------

SDS-PAGE Stock Buffer (10X)

Trizma Base	30.3 g
-------------	--------

Glycine	144 g
---------	-------

Add dH₂O up to 1 lt and adjust the pH to 8.0.

SDS-PAGE Running Buffer (1X)

SDS-PAGE Stock Buffer (10X)	90 ml
-----------------------------	-------

10% SDS	10 ml
---------	-------

Add dH₂O up to 1 lt and adjust the pH to 8.0.

Transfer Buffer (1X)

SDS-PAGE Stock Buffer (10X)	100 ml
-----------------------------	--------

Methanol	200 ml
----------	--------

Add dH₂O up to 1 lt and adjust the pH to 8.0.

Seperating Gel (2V)

	7.5%	10.0%	12.5%
PAA Stock Solution	3.75 ml	5.00 ml	6.25 ml
Separation Gel Buffer	5.00 ml	5.00 ml	5.00 ml
Proteomic Water	6.00 ml	4.75 ml	3.50 ml
Tetramethylen Diamine (TEMED) Solution	0.25 ml	0.25 ml	0.25 ml
APS	0.10 ml	0.10 ml	0.10 ml

Stacking Gel (2V)

	5.0%
PAA Stock Solution	0.88 ml
Stacking Gel Buffer	1.00 ml
Proteomic Water	3.00 ml
TEMED	0.10 ml
APS	0.03 ml

Ponceau S Stain Dye

Ponceau S	0.1% (w/v)
-----------	------------

Acetic Acid	1%
-------------	----

Add dH₂O up to 1 lt .

Ponceau S Destain Solution

Acetic Acid	1%
-------------	----

Add dH₂O up to 1 lt .

Blocking Buffer

(5% Non-Fat Dry Milk in PBST)

Non-Fat Dry Milk	2.5 g
------------------	-------

PBST	50 ml
------	-------

(5% BSA in TBST)

Bovine Serum Albumine (BSA)	2.5 g
-----------------------------	-------

TBST	50 ml
------	-------

(3% BSA and 2% Non-Fat Dry Milk in TBST)

Non-Fat Dry Milk	1 g
------------------	-----

BSA	1.5 g
-----	-------

TBST	50 ml
------	-------

Coomassie Brilliant Blue (CBB) Stain Dye

CBB	0.1% (w/v)
-----	------------

Methanol	20%
----------	-----

Acetic Acid	0.5%
-------------	------

Add dH₂O up to 1 lt.

CBB Destain Solution

Methanol	30%
----------	-----

Acetic Acid	0.5%
-------------	------

Add dH₂O up to 1 lt.

HRP Substrate Solution

Substrate 1	300 µl
-------------	--------

Substrate 2	300 µl
-------------	--------

Add dH₂O up to 1 ml.

APPENDIX D

USED KITS

DNA Isolation	DNA Isolation Kit for Mammalian Blood (Roche)
SDS-PAGE	SDS-PAGE Preparation Kit (Sigma-Aldrich)
	Mini-Protean Tetra Cell Handcasting Accessory Kit (Bio-Rad)

APPENDIX E

PERMISSION FORM

ASGARİ BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU

1. Davet edildiğiniz “Ailevi Akdeniz Ateşi, Gut ve Still Hastalarından Alınan Örneklerde Farklı MEFV Transkriptlerinin, Total Pryn Proteininin ve İzoformlarının Miktarlarının Belirlenmesi” isimli çalışma bir araştırma projesidir.
2. Çalışmanın amacı ülkemizde yaygın olarak görülen Ailevi Akdeniz Ateşi, Gut ve Still hastalıklarında rol oynayan MEFV transkriptlerinin, Pryn proteininin ve izoformlarının miktarlarının belirlenmesidir.
3. Araştırmada gönüllülere uygulanacak herhangi bir tedavi bulunmamaktadır.
4. Araştırmaya katılmayı kabul ederseniz görevlendirilmiş bir hekim tarafından muayene edileceksiniz ve klinik bulgularınız kaydedilecektir. Muayene sonucunda doktorunuz uygun görürse çalışmaya alınacaksınız. Katılan gönüllülerin kollarından 10 ml (1 tüp) kan alınacaktır.
5. Sizden alınan kanlardan nükleik asitler ayrılacaktır. Elde edilen nükleik asitlerden RFLP, qRT-PCR, ELISA, İki Boyutlu (2D) Jel Elektroforezi ve Kütle Spektrometresi yöntemleri kullanılarak Ailevi Akdeniz Ateşi, Gut ve Still gibi otoenflamatuvar hastalıklarda görülen MEFV transkriptlerinin, Pryn proteininin ve izoformlarının miktarları belirlenecektir.
6. Kan alınması sırasında oluşabilecek riskler: 1) İğne batmasına bağlı olarak acı duyabilirsiniz. 2) Düşük bir ihtimal olarak iğne batması sonucu kanamanın uzaması ve enfeksiyon riski bulunmaktadır.
7. Araştırmada gönüllü açısından hedeflenen herhangi bir klinik yarar bulunmamaktadır.
8. Bu çalışmaya katılmanız için sizden herhangi bir ücret talep edilmemektedir. Bunun yanı sıra size bir ödeme yapılmayacaktır.
9. Bağlı bulunduğunuz Sosyal Güvenlik Kurumuna (SGK) herhangi bir ücret ödemeniz gerekmemektedir.
10. Araştırmaya katılımınız sizin isteğinize bağlıdır ve istediğiniz zaman araştırmaya katılmayı reddedebilir veya araştırmadan çekilebilirsiniz.

11. Sizinle ilgili tüm kimlik bilgileri, klinik bulgular ve elde edilecek deneysel bulgular gizli kalacak ve kimseyle paylaşılmayacaktır. Araştırma konularının yayınlanmasında dahi kimlik bilgileriniz kimseyle paylaşılmayacaktır.

12. Araştırma konusuyla ilgili ve araştırmaya katılmaya devam etme isteğiniz etkileyebilecek yeni bilgiler edinildiğinde gönüllü veya yasal temsilcisi bilgilendirilecektir.

13. Sizden bir defaya mahsus olarak kan alınması planlanmaktadır.

14. Araştırmaya 100'er hasta ve sağlıklı kontrol katılması planlanmaktadır.

15. Elde edilen kan örnekleri Ailevi Akdeniz Ateşi, Gut ve Still hastalıklarında rol oynayan MEFV transkriptlerinin, Pyrin proteininin ve izoformlarının miktarlarını belirlenme amacı ile kullanılacaktır.

16. Çalışmada bulunan analizle İstanbul Teknik Üniversitesi Moleküler Biyoloji ve Genetik Bölümü laboratuvarlarında gerçekleştirilecektir. Örnekler hiçbir şekilde yurtdışına gönderilmeyecektir.

Yukarıda gönüllü kişiye araştırmaya katılmadan önce verilmesi gereken bilgileri gösteren metni okudum. Yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklamalar yapıldı. Karar vermem için yeterli süre tanındı. Bu koşullarla söz konusu araştırmaya kendi rızamla katılmayı kabul ediyorum. Araştırma kapsamında alınan kan örneklerinin,

- o Sadece yukarıda bahsi geçen araştırmada kullanılmasına izin veriyorum.
- o İleride yalpası planlanan tüm araştırmalarda kullanılmasına izin veriyorum.
- o Hiçbir koşulda kullanılmasına izin vermiyorum.

Bu formun imzalı bir kopyası bana verilecektir.

Gönüllünün,

Adı-Soyadı:

Adresi:

Tel-Faks:

Tarih ve İmza:

Velayet veya vesayet altında bulunanlar için veli veya vasinin,

Adı-Soyadı:

Adresi:

Tel-Faks:

Tarih ve İmza:

Açıklamaları yapan araştırmacının,

Adı-Soyadı:

Görevi:

Adresi:

Tel-Faks:

Tarih ve İmza:

APPENDIX F

PRIMER SETS LOCATIONS ON MEFV EXONS

Second Exon of MEFV and 2.1 Primer Sets Locations

CCGCGCCCGGCCCCGTTGTTTTCTCAATTTCTAAACTTTAATATCCAAGGGGATTCTCT**CTCCTCTGC**
CCTGAATCTTGGGCCCTAAACGTGGGACAGCTTCATCATTTTGCATCTGGTTGTCCTTCCAGAATATT
CCACACAAGAAAACGGCACAGATGATTCCGCAGCGTCCAGCTCCCTGGGGGAGAACAAGCCAGGAGC
CTGAAGACTCCAGACCACCCCGAGGGGAACGAGGGGAACGGCCCTCGGCCGTACGGGGGCGGAGCTGC
CAGCCTGCGGTGCAGCCAGCCCGAGGCCGGGAGGGGGCTGTGAGGAAGCCCCGTAGCAAACGCAGAG
AGAAGGCCTCGGAGGGCCTGGACGCGCAGGGCAAGCCTCGGACCCGAGCCCGGCCCTGCCGGGCGGG
AGAAGCCCCGGCCCCCTGCAGGGCGCTAGAGGGGGGCCAGGCCGAGGTCCGGCTGCGCAGAAACGCCAG
CTCCGCGGGGAGGCTGCAGGGGCTGGCGGGGGCGCCCCGGGGC**AGAAGGAGTGCAGGCCCT**TCGAAG
TGTACCTGCCCTCGGGAAAGATGCGACCTAGAAGCCTTGAGGTCACCATTTCTACAGGGGAGAAGGCG
CCCGCAAATCCAGAAATTCTCCTGACTCTAGAGGAAAAGACAGCTGCGAATCTGGACTCGGCAACAGA
ACCCCGGGCAAGGCCCACTCCGGATGGAGGGGCATCTGCGGACCTGAAGGAAGGCCCTGGAAATCCAG
AACATTTCGGTACCCGGTAAATTGTGTTCTTTCTACTTTATATCGGCTGCAGAGAAAGAATGGCTGGC
CGGGCACGATAGCTCATGCCTGTAATCCCAGCGCTTTGGGAGGCCAGGGCGGGAGGATTGCTGGAGGC
CAAGACTTTGAGACCAGCCTGGTGAA

Second Exon of MEFV and 2.2 Primer Sets Locations

CCGCGCCCGGCCCCGTTGTTTTCTCAATTTCTAAACTTTAATATCCAAGGGGATTCTCTCTCCTCTGC
CCTGAATCTTGGGCCCTAAACGTGGGACAGCTTCATCATTTTGCATCTGGTTGTCCTTCCAGAATATT
CCACACAAGAAAACGGCACAGATGATTCCGCAGCGTCCAGCTCCCTGGGGGAGAACAAGCCAGGAGC
CTGAAGACTCCAGACCACCCCGAGGGGAACGAGGGGAACGGCCCTCGGCCGTACGGGGGCGGAGCTGC
CAGCCTGCGGTGCAGCCAGCCCGAGGCCGGGAGGGGGCTGTGAGGAAGCCCCGTAGCAAACGCAGAG
AGAAGGCCTCGGAGGGCCTGGACGCGCAGGGCAAGCCTCGGACCCGAGCCCGGCCCTGCCGGGCGGG
AGAAGCCCCGGCCCCCTGCAGGGCGCTAGAGGGGGGCCAGGCCGAGGTCCGGCTGCGCAGAAACGCCAG
CTCCGCGGGGAGGCTGCAGGGGCTGGCGGGGGCGCCCCGGGGCAGAAGGAGTGCAGGCCCTTCGAAG
TGTACCTGCCCTCGGGAAA**GATGCGACCTAGAAGCCTTG**AGGTCACCATTTCTACAGGGGAGAAGGCG
CCCGCAAATCCAGAAATTCTCCTGACTCTAGAGGAAAAGACAGCTGCGAATCTGGACTCGGCAACAGA
ACCCCGGGCAAGGCCCACTCCGGATGGAGGGGCATCTGCGGACCTGAAGGAAGGCCCTGGAAATCCAG
AACATTTCGGTACCCGGTAAATTGTGTTCTTTCTACTTTATATCGGCTGCAGAGAAAGAATGGCTGGC
CGGGCACGATAGCTCATGCCTGTAATCCCAGCGCTTTGGGAGGCCAGGGCGGGAGGATTGCTGGAGGC
CA**AGACTTTGAGACCAGCCTGG**TGAA

Tenth Exon of MEFV and 10.1 Primer Sets Locations

GAAGAAC**TACCCTGTCCCTGTTTCTTG**CAGGTGGGGAGAACCCTGTAGGGATGTTGCCCATGGACCC
TACCTAGGTATTCAAATTTTCTTTGCAGTTAATGTGATTCTGGATGCAGAAACCGCTTACCCCAACCT
CATCTTCTCTGATGATCTGAAGAGTGTTAGACTTGGAACAAGTGGGAGAGGCTGCCTGATGGCCCGC
AAAGATTTGACAGCTGTATCATTGTTCTGGGCTCTCCGAGTTTCTCTCTG GCCCGCCGTTACTGGGAG
GTGGAGGTTGGAGACAAGACAGCATGGATCCTGGGAGCCTGCAAGACATCCATAAGCAGGAAAGGGAA
CATGACTCTGTGCCCAGAGAATGGCTACTGGGTGGTGATAATGATGAAGGAAAATGAGTACCAGGCGT
CCAGCGTTCCCCCGACCCGCTGCTAATAAAGGAGCCTCCCAAGCGTGTGGGCATCTTCGTGGACTAC
AGAGTTGGAAGCATCTCCTTTTACAATGTGACAGCCAGATCCCACATCTATACATTGCCCAGCTGCTC
TTTCTCTGGGCCCCCTTCAACCTATCTTCAGCCCTGGGACACGTGATGGAGGGAAGAACACAGCTCCTC
TGACTATCTGTCCAGTGGGTGGTCAGGGGCCTGACT**GAATGCCCAACACTGCATC**TCTCTTCCTGCTT
CTGGCCTTGTATCTTGCATTACACTCAATAGTCACGGAATGCCGACTAGGTGCTAGCTGCTATGGGA
AATGCAAAAATAACAAAATAGTTACTGTGCCACGGAGCCTACCCGATTATAGCAGAGGTAAGTTAGG
AACGAACATGTTAGTCAATCCGGGTGAAGACATGTACTGATGACACACCATGGATTTTCAGAGGAGGAA
GTACGGAGTCGTTGCATAATCCGCCCCCTGGTGGGTGGCACTCTCAGGTGCTCCTGAACAGAAGATTTG
GCCCTCATTTTCCCTCAGAACCCACGGCAAGGATATATGTCCCTTGTTCTCTCTGCTTCTGTCTTG
AGGATATGGGAAGCCTAGAGAAACGCAAGCAGACTGGATTGGGATAGAAGTATTTGTGTACCTGGATT
AATGAACATATGATTTTTTTTTTTTTTTTTTGGAGACCAATCTTGCTCTGTGGCCAGGCTGGAGTGCA
GTGGCACGATCTCAGCTCACTGCAACCTCCACCTCCAGGTTCAAGCGATTCTCTGCCTCAGCCTCC

TGAGCAGCTGGGATTACAGGTGCGTGCCACCACACCAGGCTGGTTTTCTTGATTTTTTAGTAGAGACG
GGGGTTTCACCATGTTAGCCAGGCTGGTCTCGAACTCCTGACCTCAGGTGATCCACCCGCCTCAGCCT
CCCAAAGTGCTGGGATTACAGGCATGAGCCACTGTGCCCGGCCTATGATTCTTTTTTTTTTTTTTTTT
TGAGACAAAGTTTTGCTCTTGTCACCCAGGCTGGAGTGCAATCTTGGCTCGCAACCTCCG
CCTCCCAGGTTCAAGAGATTCTCCTGCCTCAGCCTCCGAAGTAGCTGGGATTACAGGCGCCCGCCACC
ATGCCCCGGCTAATTTTTTGCATTTTTTAGTAGACATGAGGTTTCATCATGTTGGCCAGGCCGGTCTCAA
ACTCCTGACCTCAGGTGATGCACCCACCTCAGCCTCCCAAAGTGCAGGGATTACAGGCATGAGCCACC
ATGCCGGGCCATGATTCTTAAGAGAATTGACTGGGCCTCATGAATAAAAAAATTAGAAAATCTGGTCA
TTTGCATTTGTCACTCAATCACTGTGGAATCCCATTTCCCGACTGCATTTCA

APPENDIX G

CHROMATOGRAM ANALYSES OF MEFV EXON 2 AND 10

Chromatograms of Exon 2

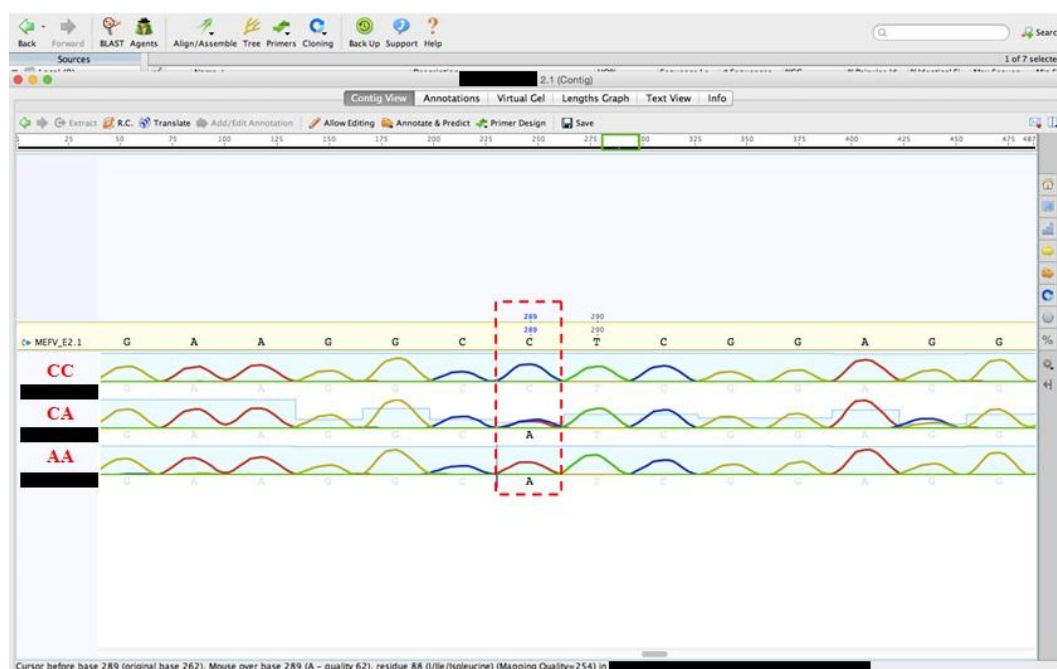


Figure G.1 : A165A.

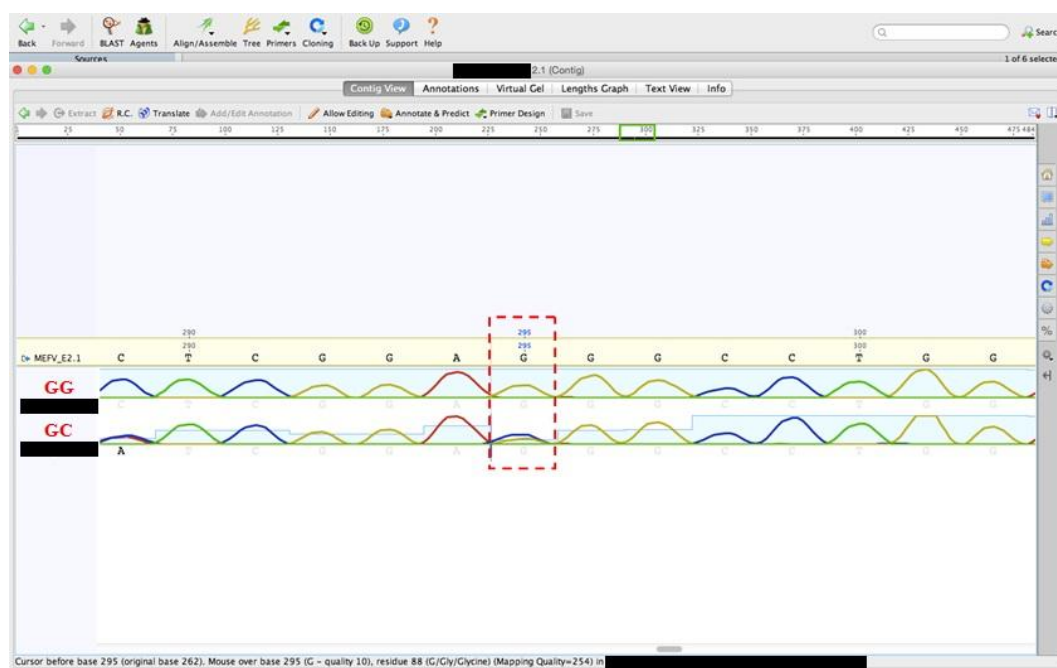


Figure G.2 : E167D.

Chromatograms of Exon 10

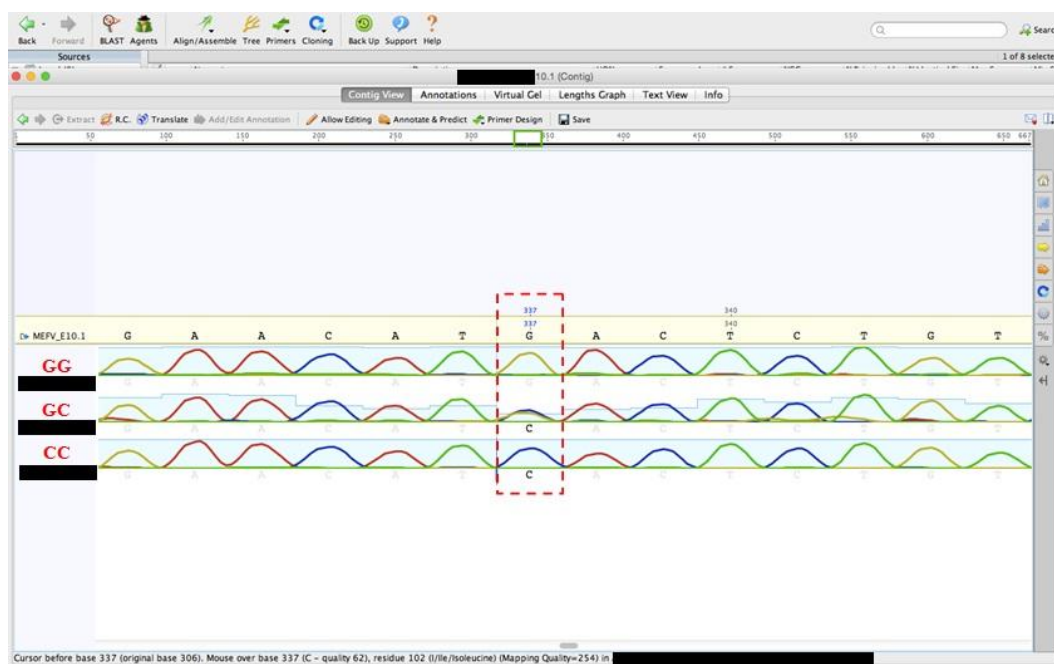


Figure G.3 : M680I (G/C).

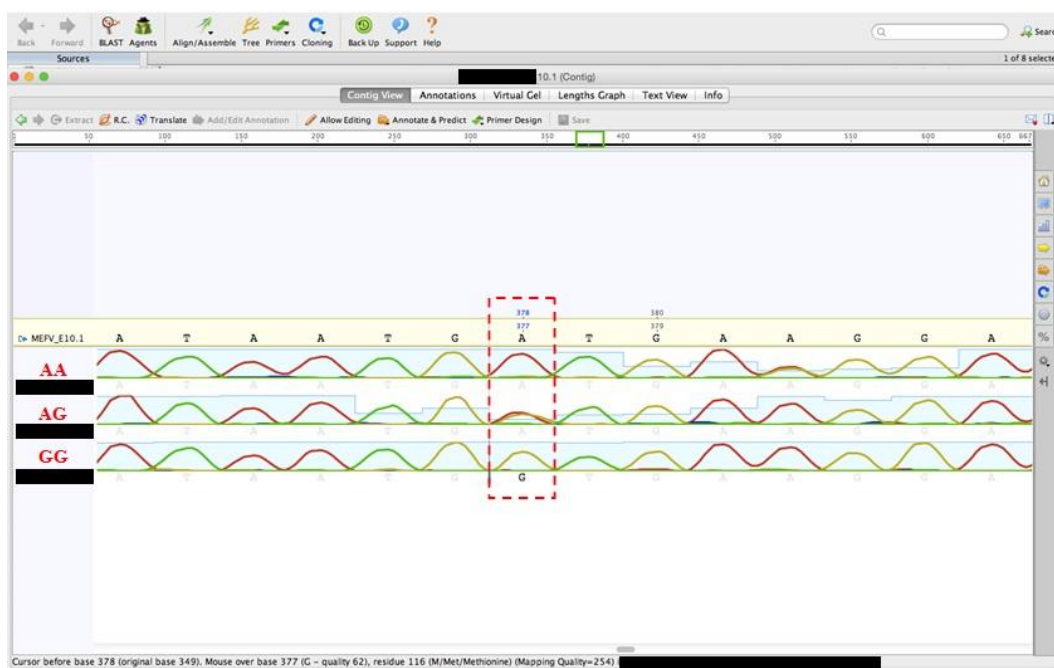


Figure G.4 : M694V.

APPENDIX H

SAMPLE PCR RESULTS

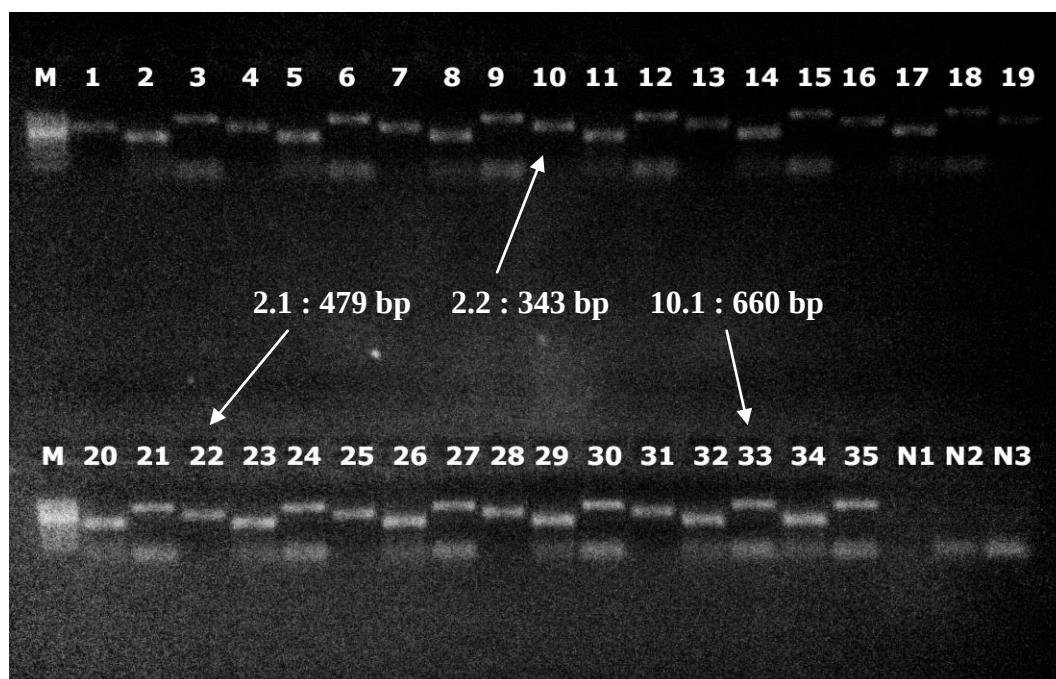


Figure J.1 : MEFV Exon 2 and 10 PCR Results.

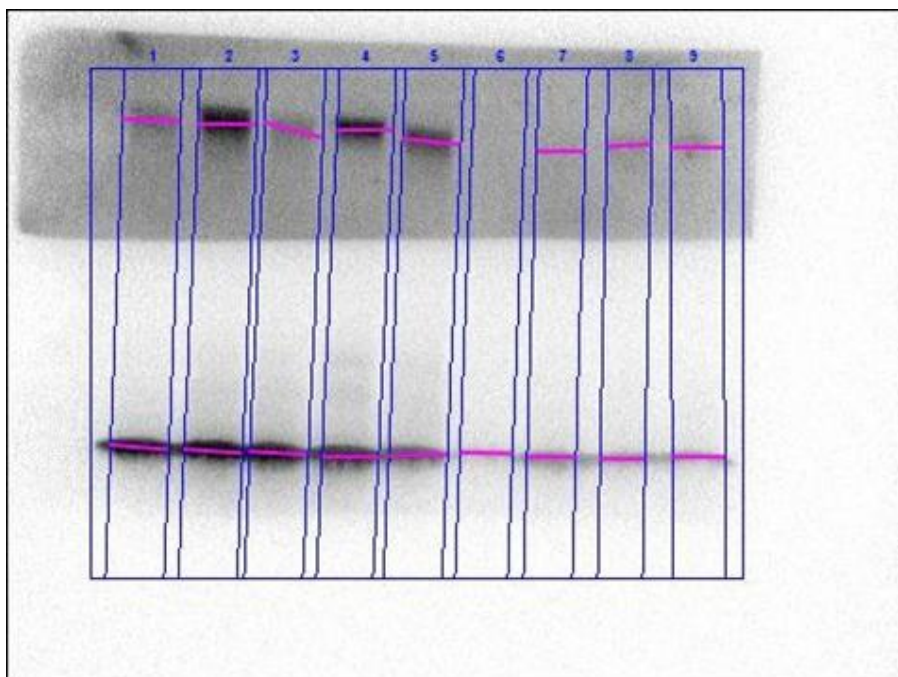
N1: Negative Control - 2.1

N2: Negative Control - 2.2

N3: Negative Control - 10.1

APPENDIX I

IMAGE LAB REPORTS



Acquisition Information

Imager	ChemiDoc™ MP
Exposure Time (sec)	14.000 (Signal Accumulation)
Flat Field	Applied (Lens)
Serial Number	731BR01993
Software Version	5.0
Application	Chemi
Excitation Source	No Illumination
Emission Filter	No Filter
Binning	3x3

Image Information

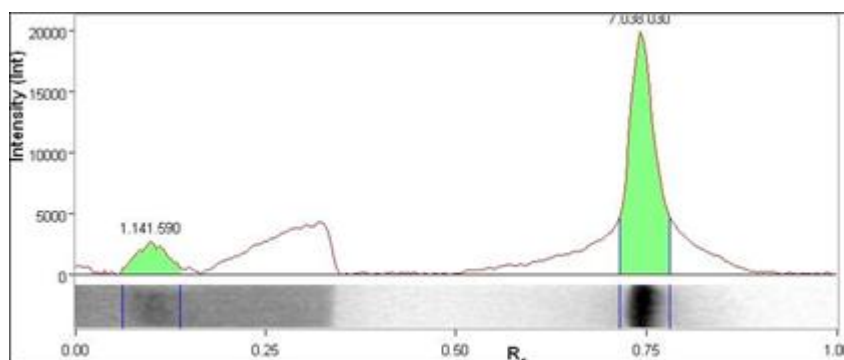
Acquisition Date	14.04.2015 18:46
User Name	Lenevo
Image Area (mm)	X: 105.0 Y: 78.3
Pixel Size (um)	X: 226.3 Y: 226.3
Data Range (Int)	0 - 27524

Analysis Settings

Detection Lane detection:
Automatically detected lanes with manual
adjustments
Band detection:
Automatically detected bands with sensitivity:
High
Lane Background Subtraction:
Lane background subtracted with disk size: 10
Lane width: Variable

Lane and Band Analysis

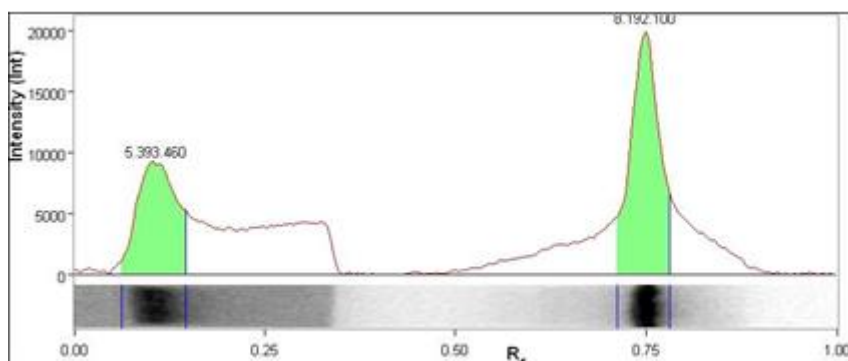
Lane 1



Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,103	1.141.590	N/A	N/A	14	6,7
2	N/A	0,744	7.038.030	N/A	N/A	86	41,5

Band Detection Automatically detected bands with sensitivity: High
Lane Background Lane background subtracted with disk size: 10
Lane Width 6.79 mm

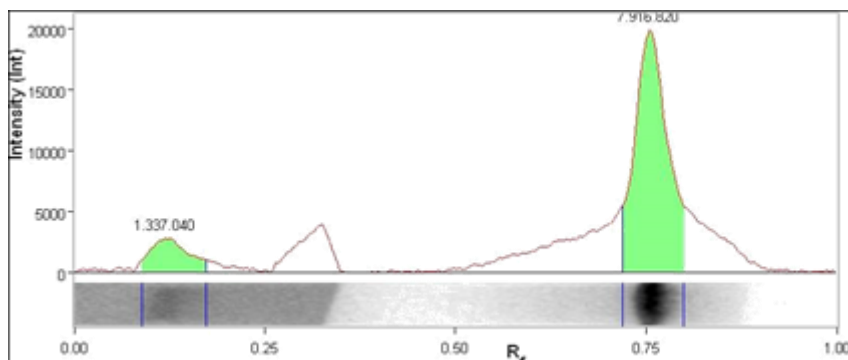
Lane 2



Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,111	5.393.460	N/A	N/A	39,7	19,4
2	N/A	0,752	8.192.100	N/A	N/A	60,3	29,5

Band Detection Automatically detected bands with sensitivity: High
Lane Background Lane background subtracted with disk size: 10
Lane Width 6.79 mm

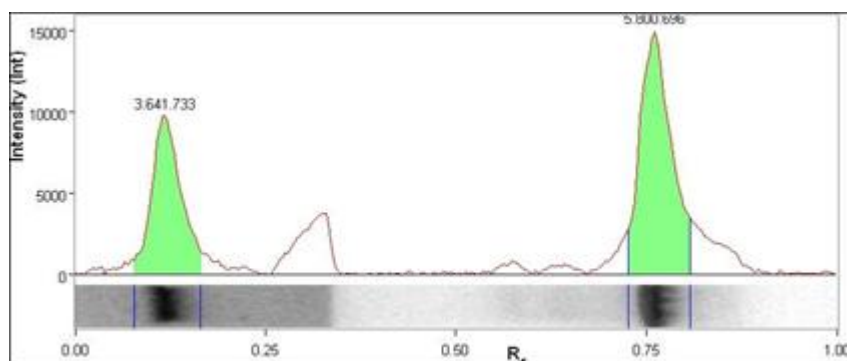
Lane 3



Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,122	1.337.040	N/A	N/A	14,4	7,6
2	N/A	0,756	7.916.820	N/A	N/A	85,6	45,2

Band Detection Automatically detected bands with sensitivity: High
Lane Background Lane background subtracted with disk size: 10
Lane Width 6.79 mm

Lane 4



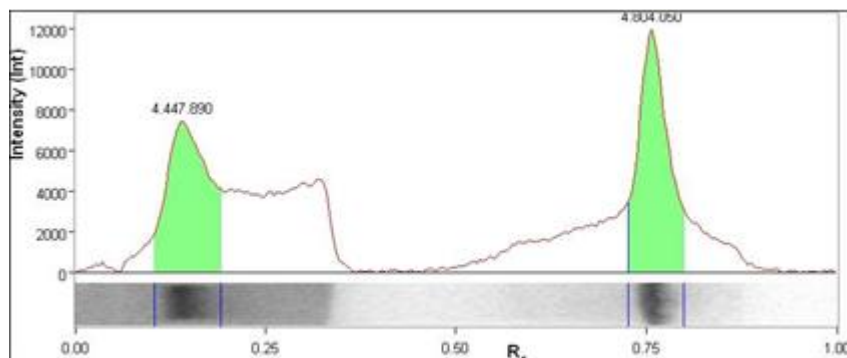
Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,122	3.641.733	N/A	N/A	38,6	26,4
2	N/A	0,763	5.800.696	N/A	N/A	61,4	42,1

Band Detection Automatically detected bands with sensitivity: High

Lane Background Lane background subtracted with disk size: 10

Lane Width 6.56 mm

Lane 5



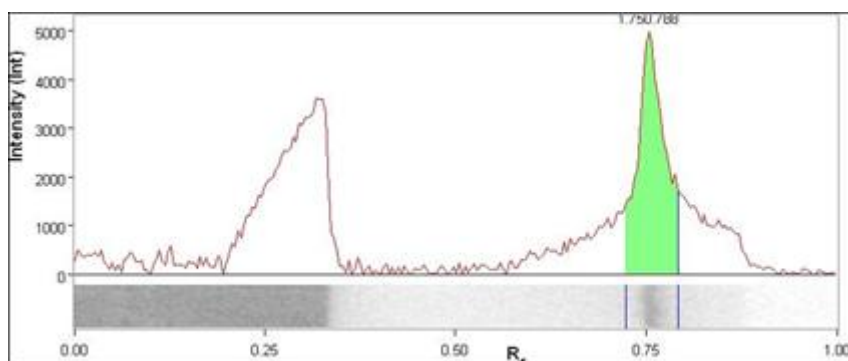
Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,145	4.447.890	N/A	N/A	48,1	22,2
2	N/A	0,76	4.804.050	N/A	N/A	51,9	24

Band Detection Automatically detected bands with sensitivity: High

Lane Background Lane background subtracted with disk size: 10

Lane Width 6.79 mm

Lane 6



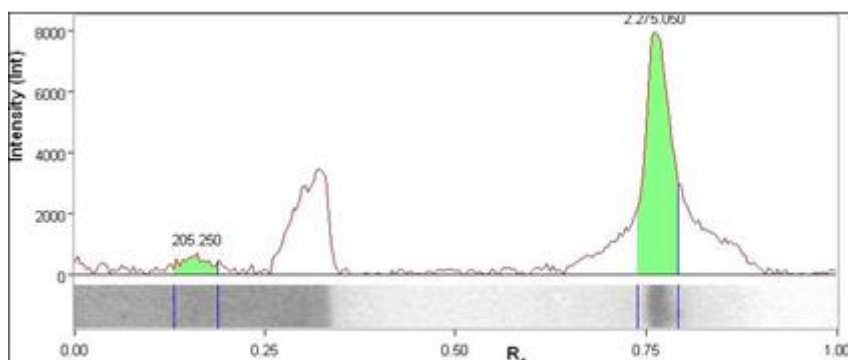
Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	-	-	-	-	-	-	-
2	N/A	0,756	1.750.788	N/A	N/A	100	26,4

Band Detection Automatically detected bands with sensitivity: High

Lane Background Lane background subtracted with disk size: 10

Lane Width 6.11 mm

Lane 7



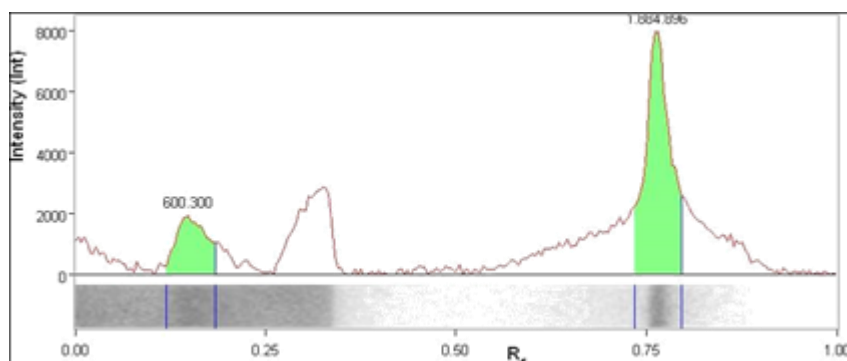
Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,164	205.250	N/A	N/A	8,3	3,5
2	N/A	0,763	2.275.050	N/A	N/A	91,7	38,9

Band Detection Automatically detected bands with sensitivity: High

Lane Background Lane background subtracted with disk size: 10

Lane Width 5.66 mm

Lane 8



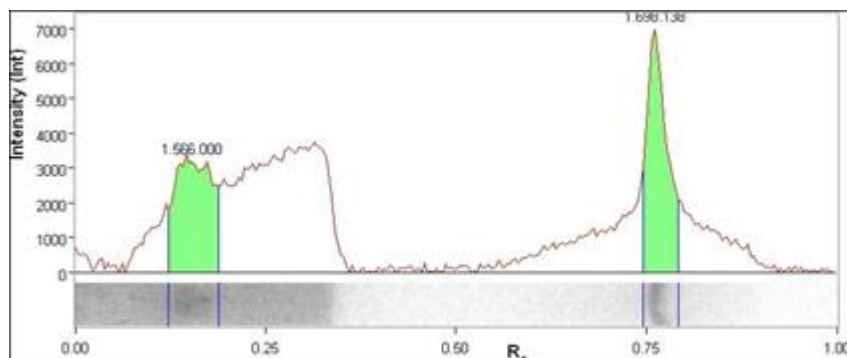
Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,153	600.300	N/A	N/A	24,2	9,6
2	N/A	0,767	1.884.896	N/A	N/A	75,8	30,1

Band Detection Automatically detected bands with sensitivity: High

Lane Background Lane background subtracted with disk size: 10

Lane Width 5.20 mm

Lane 9



Band No.	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1	N/A	0,156	1.566.000	N/A	N/A	48	15,2
2	N/A	0,763	1.698.138	N/A	N/A	52	16,5

Band Detection Automatically detected bands with sensitivity: High

Lane Background Lane background subtracted with disk size: 10

Lane Width 6.11 mm

CURRICULUM VITAE



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2009-2012 Istanbul University - Molecular Biology and Genetics, Turkey

2010-2011 (Erasmus) University of Gothenburg – Cellular and Molecular Biology, Sweden

M.Sc.:

2012 - ... Istanbul Technical University - Molecular Biology-Genetics & Biotechnology, Turkey

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS

Tahir Turanli E, Benyakar SG, Celikyapi GE, 2015: MEFV Gene Variation Analysis in a Group of Patients with FMF, Gout and Adult-Onset Still's Diseases. *International Congress - 8th International Congress of Familial Mediterranean Fever and Systemic Autoinflammatory Diseases*, September 30 – October 3, 2015 Dresden, Germany.