ARMAH KORHENE WILSON

THE EVALUATION OF BETA-GLOBIN GENE VARIANTS IN FAMILIAL MEDITERRANEAN FEVER DISEASE

THE EVALUATION OF BETA-GLOBIN GENE VARIANTS IN

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MSc. THESIS

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Approval

We attest to having perused the thesis that has been presented for evaluation by ARMAH KORHENE WILSON titled **"The Evaluation of Beta-Globin Gene Mutations in Familial Mediterranean Fever Diseases Patients"** in our combined assessment, it is our considered opinion that the scope and quality of the subject matter is entirely satisfactory as a thesis for the degree of Master in Medical Biology and Genetics.

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Declaration

I hereby attest that all information, documentation, analysis, and findings contained in this thesis have been gathered and presented in alignment with established academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I hereby affirm that, in accordance with the prescribed regulations and protocols, I have duly acknowledged and attributed all external information and data sources utilized in this study.

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COMPLIANCE AND APPROVAL

This master thesis titled "THE EVALUATION OF BETA-GLOBIN GENE MUTATIONS IN FAMILIAL MEDITERRANEAN FEVER DISEASES PATIENTS" was written in accordance with the NEU Postgraduate proposal and thesis guidelines.

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DEDICATION

With a cheerful heart, I hereby dedicate this astonishing thesis to my father LombehFassia Wilson and my sister Mrs. Siemoa Wilson Piah and my mother Ireme Harmon.

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ARMAH KORHENE WILSON

Abstract

The Evaluation of Beta-Globin Gene Variants in Familial Mediterranean Fever Disease

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Individual from the Mediterranean region, such as Arabs, Armenians, Turks, Greeks, and Cypriots, are more prone to develop autoinflammatory disease known as familial Mediterranean Fever (FMF). FMF is an autosomal recessive, single-gene condition. Changes in the *MEFV* gene, which produces the pyrin protein responsible for the disease. The *MEFV* gene found on chromosome 16 synthesizes full-length transcript, which is 781-amino acid protein called pyrin. The study examined beta-globin gene variants in individuals with FMF at the Near East Hospital. Two patient groups were examined: the case group, consisting of 41 FMF patients and their parents, and the second group, which included 100 patients who had been hospitalized for pre-diagnosis but lacked any pathogenic mutations. This study aimed to investigate *MEFV* mutations that cause FMF. In this study, the *MEFV* genes of 41 patients diagnosed with FMF and their respective parents were subject to examination for mutations associated with FMF. The case group was found to exhibit multiple pathogenic mutations in the beta-globin gene that are related to FMF. In contrast, the beta-globin genes of the second group, consisting of 100 patients who were hospitalized pre-diagnosis for FMF, did not show any pathogenic alterations. Thus, this study aims to define the variances observed between the two groups. The analysis of beta-globin gene mutations in FMF patients was successfully carried out. These results highlight the importance of studying beta-globin gene mutations in FMF patients for accurate diagnosis and management of the disease.

Keywords: Familial Mediterranean Fever; Genotype-Phenotype Correlations; *MEFV*; National Genetic Consortium.

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ABBREVIATIONS

Kb: Kilobyte/ Kilobases

Hb: Human Hemoglobin

HbA: Adult Human Hemoglobin

HbF: Woman Fetal Hemoglobin

HbA_1Hemoglobin Subunit Alpha 1

HbA_2Hemoglobin Subunit Alpha 2

HbE: Embryonic Hemoglobin

***HBB*:** Human Subunit Beta Gene

NMDBs: National Mutation Databases

DNA: Deoxyribonucleic Acid

SCD: Sickle Cell Disease

P/M: Pyrin or Marenostrin

ASC: Apoptosis-Associated Speck-like Protein

RFLP: Restriction Fragment Length Polymorphism

MIM: Major Incident Management

MCSMultispecies conserved sequences

NLRP3: NOD-like Receptor Pyrin-Containing 3

PCR: Polymerase Chain Reaction

***MEFV*:**Mediterranean fever

SS: Sickle Cell (Homozygous)

UV: Ultraviolet

bp: Base Pair

FMF: Familial Mediterranean Fever

SNPs: Single Nucleotide Polymorphisms

HGBASE: human genetic biallelic sequences

mRNA: MessengerRibonucleic Acid

CNVs: Copy Number Variants

NCBI: National Center for Biotechnology Information

SNVs: Single-Nucleotide Variants

MIEs: Mendelian Inheritance Error/mistakes

SAA: Serum Amyloid A

AID: Autoinflammatory Diseases

IL-1: Interleukin-1

PRR: Pseudo-Response Regulators

TB: Beta Thalassemia

CAPS: Cryopyrin-Associated Periodic Syndromes

FDA: Food and Drug Administration

Mg: Milligram

TM: Thalassemia Major

TI: Thalassemia Intermedia

HBh*:* Human Hemoglobin

RBC: Red Blood Cells

TIF: Thalassemia International Federation

MCV: Mean Corpuscular Volume

MCH: Mean Corpuscular Hemoglobin

HbG2: Hemoglobin Subunit Gamma 2

HbG1: Gamma Globin Genes

HS: Hypersensitive Sites

LCR: Locus Control Region

NF-E2: Nuclear Factor, Erythroid 2

XP: EXPerience

ND: Nanodrop

CY5: Cyanine 5

HEX: Hexachloro-Fluorescein

MM: Master Mix

µL: Microliter

HWE: Hardy-Weinberg Equilibrium

CHAPTER I

Introduction

1.1 Introduction

A genetic inflammatory disorder called familial Mediterranean fever, is one of the most frequently observed diseases (Yao et al., 2016). The symptoms of Familial Mediterranean Fever (FMF) include recurrent fever attacks that are followed by peritonitis, pleurisy, arthritis, or an erythema resembling erysipelas. People with Mediterranean ancestry, including Arabs, Armenians, Turks, Greeks, and Cypriots, are more likely to develop this disease (Deltas et al., 2002).

As an autosomal recessive trait, FMF is a single-gene condition that is passed down through the generations. (Bilginer & Ozen, 2019). Changes in the *MEFV* gene, which produces the non-functional pyrin protein, is the underlying cause of FMF. Humans' *MEFV* gene, which has 10 exons, is found on chromosome 16's short arm (16p13.3). The full-length transcript of the *MEFV* gene is 3.5 kilo bases long. The final product of this pathway is pyrin, a protein composed of 781 amino acids. Pyrin is involved in the NOD-like Receptor Pyrin-Containing 3 (NLRP3) inflammasome complex.

Currently, there are 327 distinct mutations that can be divided into nucleotide alterations and small deletions. These mutations are likely to change the polypeptide chain's amino acid composition, which would alter the protein pyrin's typical function as a result of the gene's expression.In white blood cells, the *MEFV* gene is abundantly expressed.Changes in pyrin quantity or quality might have an effect on the regulation of inflammasome activity, which can lead to uncontrolled inflammation and alter inflammatory signaling pathways (De Nardo et al., 2015).

The Greek patients are predisposed to M694V (38.1%), M680I (19.7%), V726A (12.2%), and E148Q (10.9%) deletions which are all placed in exon 2. E148Q (10.9%), which are all found in exon 10. The observed prevalence rate of common *MEFV* mutations among healthy Greek controls is a mere 0.7% (Papadopoulos et al., 2007). FMF is characterized by a delay in diagnosis, which is itself a symptom. Here, we provide the case of a patient who experienced both FMF and intermediate beta-thalassemia. The study looks into the circumstances that contributed to the lengthy delay in a diagnosis for this specific patient. Additionally, the study investigated the issue of whether the two conditions coexist or if there is some type of relationship between them that presented a case of a 37-year-old male patient with intermediate beta-thalassemia concentration.

McDermott and colleagues (1999) were the firstscientists who used the terminology of "auto-inflammatory diseases" to characterize these conditions. In contrast to autoimmune diseases, due to a deficiency in acquired immunity, inflammatory syndromes have been associated with deficiencies in innate immunity. Clinically, recurrent episodes of inflammation could be used for the characterization of autoinflammatory disease (Yao & Furst, 2008).

To date, 70 *MEFV8*-related disease-causing mutations have been identified. According to Milhavet et al. (2008), the majority of the mutations were missense and found in exons 2 and 10. Hence, an increasing number of people with clinical FMF either have no mutations or just one mutation in the *MEFV* coding area. Other different research teams studied mutations in genes apart from *MEFV* to check the viability of the digenic model (Grandemange et al., 2009).

Almost every kind of cell expresses the *MEFV* gene, including neutrophils, monocytes, and dendritic cells, as well as a few cell lines and eosinophils. It gives code for the synthesis of a protein made up of 781 amino acids called pyrin or marenostrin. The process of converting procaspase-1 to caspase-1 through proteolytic activation is facilitated by the adaptor protein known as Apoptosis-Associated Speck-like Protein (ASC). This protein is among the cellular proteins that Pyrin/Marenostrin (P/M) interacts with.

These connections allow (P/M) to take part in a variety of distinct multi protein complexes, which are referred to as inflammasomes. Based on the severe inflammatory symptoms of FMF as well as current research indicates that (P/M) in the control of inflammasomes, Inflammation and innate immunity are regulated by (P/M).

The study was conducted at Near East University Hospital Genetic Diagnostic Laboratory. For this study, 100 blood samples were collected from patients with FMF. The aim of this study is to scrutinize the findings, to explore the correlation between inflammatory occurrences and the degree of mutations in individuals affected with FMF disease.The study also assesses the beta-globin mutation gene *HBB*, the most prevalent mutations in FMF patients presenting FMF clinic, but possessing heterozygous *MEFV* mutations, will be examined. The findings uncover potential gene-to-gene interactions between *HBB* and *MEFV* genes, thereby enhancing our comprehension of the molecular pathogenesis of FMF.

1.2Human Genome and Variation

In recent years, great progress has been made in technology which also advanced genomic studies. By using next generation sequencing technologies, scientists are trying to sequence most of the organism's genomes (Sherman et al., 2020). One of the main goals of human genetics is the identification of variants in DNA sequence that influence biological properties, particularly those implicated in the onset and progression of human diseases. In the last 25 years, huge progress has been made in the technology field that has also advanced fundamental genetic resources, analytical tools, and access to huge amounts of genotype and phenotypic data.

Genetic advances have substantially improved our knowledge of the mechanism behind many common and uncommon diseases and also have contributed to the development of innovative treatment and approaches. Importantly, one of the goals of the personalized medicine is to determine the right treatment according to the unique genetic makeup of a person (Clautssnitzer et al., 2020).

1.2.1 The Role of Genetic Variation in Diseases

Previously, genetic variations involved in the etiology of FMF have been identified in Turkey and Cyprus population. The patients were classifiedinto categories, namely homozygous, mixed heterozygous, or single heterozygous, based on the presence or absence of the M694V mutation. Through comparative analysis of data across these subgroups, logistic regression was used to identify risk variables associated with M694V homozygous or mixed heterozygous status (Güneş et al., 2021).

It can be difficult to understand the exact effects of genetics on human health and disease. Genes interact with one another as well as with the environment, which can influence all common complex diseases. In contemporary society, it remains essential to differentiate between the diverse components that contribute to an adaptive and dynamic state, namely the multitude of life forms on our planet, and to correlate this with an individual's level of well-being. One example of such endeavors is the pursuit of identifying causal genes for intricate phenotypes, encompassing numerous mental diseases, which have consistently failed to meet anticipated outcomes.

This is primarily due to the complexity of researching such traits. (Bree, Owen, Inoue, and Lupski 2003). Most of the common complex diseases and traits have a polygenic nature. Scientists are studying how genes are interacting with one another and with the environment, by this way the etiology of several diseases will be understood in detail (Xu & Meyers 2001). Numerous factors, encompassing both hereditary and non-hereditary components, as well as social and cultural contexts, socioeconomic factors, after the formation and progression of common complex diseases. SNPs are the most common types of variation observed in human beings. SNPs are found both in the coding and noncoding regions of the genome.

Although many mutations are not pathogenic and have no effect on gene expression or protein function, some mutations can alter the function of proteins. An in-silico study was conducted by scientists to identify novel genetic variations in the genes' coding regions (Faire & Morgenstern, 2001; Edmonson & Cassidy, 1999). Polymorphisms have to be studied for their effects on promoter activity, protein function, mRNA stability, andsplicing regions.

In vitro studies should be conducted as well to understand the effect of genetic variants on phenotype. Measuring the concentrations and functions of human proteins is a crucial step to understand the effect of genetic variants on proteins. Furthermore, it is important to understand the effects of genetic variants on oxidative stress. It could be also useful to study the effect of genetic variants on antioxidant events.

1.2.2 Genetic Inheritance Patterns

Statistical analyses identified projections of transmission patterns and DNA haplotypes to accurately identify genomic features with unconventional inheritance patterns, including hemizygous lesions or copy number variations (CNVs), thereby establishing their significance. In a study a nanoarray-based short-read sequencing-by-ligation technique was used to sequence the DNA extracted from peripheral blood cells of each member of the family (Roach et al. in 1995).Drmanac (2010) found that at least one member of the family had a variant allele that is not present in the reference genome.

1.2.3 Familial Mediterranean Fever

Gain-of-function mutations in *MEFV*, which encodes the inflammasome protein pyrin, cause the autoinflammatory disease Familial Mediterranean fever. The frequency of heterozygous carriers of several *MEFV* mutations is rather high in certain Mediterranean populations (Park et al., 2020).

According to research by (Bernot et al., 1997), FMF is characterized by fever and serositis and it is inherited in an autosomal recessive pattern. One of the transcripts of *MEFV* results in the production of marenostrin, a new protein that shares similarities with the ret-finger protein and butyrophilin. The *MEFV* gene have four missense variants which co-segregate in patients with FMF.

FMF and other inflammatory diseases associated with FMF has been widely investigated. Colchicine is widely used to treat patients with FMF.In addition to colchicine interleukin(IL)-1 antagonists are also used for the treatment of FMF.
As a result, a substantial number of FMF patientsshowing resistant to colchicine IL-1 antagonists anakinra and canakinumab could be used for the treatment of FMF (Tufan&Lachmann, 2020).

1.2.4 Mutation in *MEFV* Gene and FMF Disease

FMF is an autosomal recessive auto-inflammatory disease caused by mutations in the *MEFV* gene, which is located on the short arm of chromosome 16. This gene has 781 codons, 10 exons, and two hotspots for mutation at exons 2 and 10. The *MEFV* gene encodes a protein called pyrin, which is a part of the pyrin inflammasome, a multi protein organelle in the cytoplasm that helps produce active IL-1. Mutated versions of pyrin impair the inflammasome's ability to assemble, which causes an abnormally high level of inflammatory cytokines, primarily IL-1, which is essential to FMF pathophysiology (El Roz et al., 2020).

Among racial groups including Turkish, Armenian, Arabic, and Jewish, Mutations in the *MEFV* gene, which codes for the Pyrin protein, are responsible for the clinical presentation of FMF, an autosomal recessive disease characterized by recurrent self-limiting fever, peritonitis, pleuritis, arthritis, and erysipelas-like erythemas (Arpac et al., 2021). Mutations in *MEFV* gene, is responsible from innate immune disorders and severe inflammation, and FMF (Tumgor et al., 2021).

In the study conducted by Saito (2020) Patients with the *MEFV* gene mutations and chronic gastrointestinal mucosal inflammation mimicking inflammatory bowel

disease (IBD) have been studied. The authors of this retrospective study analyzed the clinical characteristics of patients with IBD unclassified (IBDU) with the *MEFV* gene.

Salehzadeh, and Fathi (2015) revealed that FMF is characterized by short polyserositis and a fluctuating temperature as symptoms.
The most serious consequence of FMF which is amyloidosis, is defined as the major cause of mortality," and could be avoided by colchicine treatment (Padeh et al.,2012).

1.2.5 Potential Heterozygote Advantage of *MEFV* Mutations

The symptoms FMF disease vary from person to person, depending on the nature of the genetic mutation, permissive environmental conditions, and underlying genetic predisposition. Frequent attacks of fever accompanied by serous inflammation, arthralgia or arthritis, abdominal discomfort, and a localized erythematous rash are the most common symptoms. Normally, self-limiting episodes end within 48 to 72 hours. Patients who are heterozygous typically experience less negative side effects and shorter seizures. Increased inflammation can be observed in asymptomatic carriers (Tufan et al., 2013).

M694V mutation leads to formation and progression of the severe form of the disease. The severity of the disease can be significantly affected by the environment (Yasunami et al., 2015). Additional factors include patient age and orientation, microRNAs, HLA I quality A, and microbiota that affect the severity of the disease (Schnappauf et al., 2019). While evaluating the environmental factors, ethnicity should also be considered.

1.2.6 Diversity of *MEFV* Mutations

In Turkey, the majority (94%) of FMF diagnoses were found in patients from the central and western regions; however, over half of these patients come from the eastern provinces, indicating the migration of both mutations and diseases

throughout these areas (Sahin et al.,2021). According to Ella, (2017) migration is one of the reasons why the incidence of *MEFV* mutation is commonly observed in Turkey and the Jordanian population. Among patients with FMF of Arab descent, the M694V mutation appears to be notably widespread (Khayat et al., 2021).
North African Bedouins show a higher incidence of M694I alteration compared to other Middle Eastern populations, potentially resulting from intermarriage with indigenous individuals of the region. The M694V mutation is particularly widespread among Arab individuals affected by FMF (Atroshi et al., 2021).

As there are numerous different Jewish ethnic groups in Europe, the *MEFV* mutation pattern is unique to Jewish people. While the mutations above vary by country and ethnicity, the mutations mentioned above are commonly observed in Jewish FMF patients (Almalky et al., 2021).

The findings suggest that the presence of R202Q homozygosity may play a pathological role, potentially in addition to other patient-specific genetic and environmental risk factors. V726A, M694V, and E167D (c. F479L (c), 501G > C, and 1437C > G) are examples of mutations and most commonly observed in FMF disease. While the F479L mutation is rare in other populations, it is present in 20.6% of Greek Cypriots, which shows that it might have originated in Cyprus (Neocleous et al., 2009). DNA testing can be performed to analyze the *MEFV* gene mutations associated with FMF. To date, *MEFV* gene mutations have been associated with several autoimmune and chronic inflammatory diseases (Arakelyan et al., 2017).

1.2.7 Etiology and Pathophysiology of FMF

A group of conditions known as autoinflammatory diseases, or AIDs, are characterized by systemic inflammation and repeated fever attacks. T and B cells are not involved in the process in this situation. Research findings suggest that FMF is associated with a minimum of 12 distinct autoimmune disorders, all of which are genetic disorder that impair the innate defense mechanisms of the immune system. The innate immune system is the body's first line of defense against foreign invaders

and potentially harmful substances that enter the body from the outside environment (Frizinsky et al., 2019).

The *MEFV* gene is composed of ten exons. The protein pyrin exerts a significant influence on the expression of genes related to intranuclear peptides, which have been identified as key factors in various inflammatory processes. Many different types of cells, including neutrophils, eosinophils, dendritic cells, mature monocytes, fibroblasts from the serosa and synovium, as well as cells from the colon and prostate malignancies, are capable of producing pyrin in the nucleus.

Pyrin is an essential part of the innate immune system. According to Portincasa et al., (2013), pyrin may also be present during leucocyte infiltration in the spleen, the lung, and the muscle. Pyrin has been observed to show co-localization with microtubules, thereby suggesting that there may be some potential impact of colchicine or other microtubule-destabilizing medications. The conversion of pro-interleukin-1 into the active form of interleukin-1 requires the internal component known as the inflammasome. An active inflammasome has been associated with fever, inflammation, and the death of cells.

Functional pyrin interferes with the cleavage of pro-IL1 by activated caspase-1. Pyrin has been demonstrated to co-localize with microtubules, and that affects the response towards colchicine or other microtubule-destabilizing medications. Pro-interleukin-1 is converted into its mature form, interleukin-1, by an intracellular complex called the inflammasome. Pyrin is an inflammasome component with a regulatory function. An active inflammasome has a substantial relationship with fever, inflammation, and cell death. In accordance with the research conducted by Chae et al. in (2008), it has been found that the presence of "normal" Pyrin has a deleterious effect on the process of activated caspase-1.

There exists substantive evidence that links FMF to a minimum of twelve distinct AIDs. These AIDs are all familial disorders that disrupt the normal functions of the immune system. According to the research done by Hejrati et al. (2020), innate immune system its first line of defense.According to Hejrati et al. (2020) findings,

the innate immune system serves as the main defense against foreign agents and diseases that protect the body from the external environment.

1.2.8 Clinical Indications and Treatment FMF

In 90 percent of cases, the first FMF incidence happened in people under the age of 20, according to a study by Bakkaloglu published in 2003. It consists of recurrent, brief episodes of acute, one-third febrile pain that last one to three days and resolve on their own without the need for medical attention. One or more types of serositis, together with peritonitis, pleurisy, and synovitis, are responsible for the discomfort. It is challenging to identify the specific occurrence that results in FMF, and the frequency of attacks may vary from one instance to the next. In some situations, the only symptom is a fever, which may even reach a temperature of 38 to 40 degrees and appear before any other symptoms.

According to Fava's (1990) research, prodromic symptoms can include discomfort on the mental, emotional, and bodily levels. The onset of severe abdominal cramping is something which occurs most often, in around 95% of patients (Starkopf et al., 2015). It is conceivable for a localized pain to become systemic later. A dynamic ileus, as well as pain, guarding, and stiffness, are possible side effects. The patient may have symptoms comparable to an acute abdomen; during an episode, it could need a laparotomy and appendectomy that weren't required. In between episodes of their disease, patients are completely symptom-free.

Repeated peritoneal inflammation occurs during attacks, and it's likely that later on, visceral adhesion may develop (Lachmann et al., 2015). While late diarrhea affects 10–20% of FMF patients, constipation affects the majority of those with the condition. Arthritis is another frequent FMF symptom that nearly invariably appears as a monoarticular condition (Manna et al., 2019). The large joint in the lower extremities is the one most frequently affected. Despite the fact that symptoms of arthritis can last for longer than a month at a time, the condition is not frequently associated with joint degeneration.

When the fever first appears, FMF patients with arthritis are frequently younger, and they also have more rashes that resemble myalgia and erysipelas. According to Watts et al., (2016), people who simultaneously have arthritis are more likely to develop vasculitis. Unilateral pleuritis, which affects one-third of people, can produce acute febrile chest pain that can happen alone or in addition to attacks that affect the joints and belly. Pericarditis affects less than 1% of people at some point in their lifetime.

Additional clinical indicators include polyarteritis nodosa, chronic abdominal febrile myalgia, Henoch-Schonlein purpura, Behcet disease, and inflammation of the vaginal tunica, which is linked to painful scrotal enlargement imitating orchitis and testicular torsion. According to many studies, a condition called irritated tunica vaginalis is characterized by severe scrotal enlargement that mimics testicular torsion and orchitis may be fatal (Tang et al., 2020).

1.3 Clinical Diagnosis of FMF

There is no one clinical finding or set of laboratory tests that can definitively diagnose FMF in an individual. FMF diagnostic criteria need that it is necessary to rule out other familial periodic fever syndromes, as well as clinical signs, family history, biochemical and genetic test data, and the patient's reaction to therapy. A genetic study, despite the fact that it lends support to the diagnosis, is not a definitive diagnostic criterion in the process of identifying FMF. If the patient is seen during an attack, the presence of associated inflammatory symptoms [increases in leukocytosis, C-reactive protein (CRP), fibrinogen, and/or erythrocyte sedimentation rate (ESR) and their decline to normal levels at the conclusion of the attack assists in the diagnosis. The clinical symptoms are used to make the diagnosis of FMF, and this is supported by the patient's ethnic background and family medical history.

According to research done by Lindgreen et al., (2017), a significant number of patients, including young children, show less severe symptoms during their episodes. In this condition, diagnosis of the disease might be challenging. For the identification of the *MEFV* gene mutations, molecular genetic testing has been widely used. Further tests are required for the correct diagnosis of the disease.

1.3.1 Treatment

Treating individuals who show symptoms of FMF is of utmost importance to prevent the occurrence of acute episodes and the emergence and progression of advanced secondary amyloidosis (AA). The study conducted by Goldfinger (1972) shows that Colchicine is a kind of tricyclic alkaloid, and its primary structural components are as follows: a tri methoxy phenylpropanoid tropolonic ring, a ring with seven members in which the seventh member is an acetamide. Colchicine is a medication that may treat both gout and cancer, and it works by inhibiting the movement of leukocytes and the phagocytosis process during inflammatory reactions (Goldfinger, 1972; Lidar et al., 2004). In 2009, the medicine was approved by the FDA which can be used for the treatment of FMF in the United States in 2013.

For a long time, the pathogenesis of cardiovascular diseases that affect the pericardium has not been understood clearly.However, recent advances in understanding the causes and mechanisms of recurrent pericarditis have shed light on first clinical trials demonstrating the effectiveness and safety of colchicine in combination with other anti-inflammatory therapies, as well as the use of anti-IL-1 agents (anakinra and rilonacept), in patients with pericarditis who have not responded to corticosteroids (Imazio et al., 2021). Therefore, it is crucial to remember that these treatments have not been scientifically approved and that each person will respond differently. Some of the commonly used methods include herbal supplements, dietary modifications, and lifestyle changes (Mischoulon et al., 2018).

Colchicine is widely used in the treatment of patients with FMF; nevertheless interleukin (IL)-1 antagonists are used for patients presenting resistance or intolerance to the standard therapy, and novel therapeutic interventions are currently under development. Thousands of colchicine-resistant or -intolerant FMF patients are now using IL-1 antagonists like anakinra and canakinumab, and there is growing evidence of their effectiveness and safety. However, the side effects of these treatments are still not very well-known (Tufan&Lachmann 2020).The study

conducted by Cohenetal., (2023) showed that people with FMF need to take colchicine for a long time to avoid amyloidosis formation.

1.4 Human Globin and Hemoglobinopathies

Hemoglobinopathies are example of autosomal recessive diseases. Thalassemia is an example of hemoglobinopathies that have an autosomal recessive inheritance (Jamet et al., 2006). To date, nearly 1,100 known hemoglobin variations have been identified (Giordano, 2013). The structural variation known as sickle hemoglobin (Hb S) is one of the most frequent hemoglobin variations. The genetic combinations that lead to clinically significant phenotypic expressions of varying severity, such as thalassemia major, thalassemia intermedia, sickle cell syndromes, and HBE syndromes, are of great interest due to their homozygous nature.

These diseases are inherited in an autosomal recessive pattern. Thalassemia refers to a group of disorders that possess wide-range of implications. Conversely, those who possess heterozygosity do not show any symptoms; however, they do showavariety of hematological characteristics that arecrucial in their identification.If these traits are inherited together, different types of thalassemia and variant hemoglobin genotypes can interact, resulting in intricate hematological characteristics that are frequently challenging to diagnose accurately. Therefore, additional examination through family research and DNA analysis is necessary.

The condition known as p3-thalassemia is a genetic disorder that affects the production of hemoglobin in individuals. It is caused by mutations in the *HBB* gene, which is responsible for encoding the β-globin protein. The p3 thalassemia is characterized by an abnormally low rate of development of 13-globin chains, which leads to a deficiency in the synthesis of hemoglobin.

A. Clegg and M. A. Naughton published their findings in 1965, along with Karon and Weissman in the same year. The production rates of normal human hemoglobin are predominantly associated with genetic variants that exhibit slower rates. Aberrant types, on the other hand, are frequently detected in the blood of heterozygotes at concentrations that are inferior to those of hemoglobin A. (Boyer et al.,1964).

In the context of thalassemia and hemoglobinopathies, the process of mRNA translation involves the sequential interlinking of amino acids within ribosomes, with the ultimate goal of generating polypeptides. This process has been hypothesized to potentially contribute to a delay in the formation of globin (Harte et al., 1967).

Research by Clegg, Weatherall, Na-Nakorn, and Wasi (1997) found that the translation rate of 18-globin m-RNA in the reticulocytes of thalassemic and non-thalassemic. This study provides an analysis of the translation of 13-globin mRNA in multiple individuals of Italian ancestry who presented with typical presentations of Cooley's anemia. Additionally, it examines a case study of an African American patient diagnosed with "thalassemia intermedia." The average translation times for the 13-polypeptide chain has been determined in nucleated red blood cells and reticulocytes of thalassemic and unrelated hemolytic anemic patients, as reported by Rieder et al., (1971). Furthermore, the rates of 1-chain translation have been investigated. In the reticulocytes of patients affected by sickle cell anemia, sickle thalassemia, or sickle hemoglobin C diseases.

1.4.1 Thalassemia

Thalassemia, a genetic disease, affects a considerable portion of the global population. Thalassemia is one of the most commonly observed hemoglobinopathies especially in the Mediterranean region (Shafique et al., 2021). Thalassemia's represent a collection of hereditary disorders characterized by the incapacity to synthesize one or more of the hemoglobin (Hb) chains that are typically present in erythrocytes.

Due to the accumulation of unstable non-thalassemic Hb chains, erythroid precursors within the body's organs undergo death, and RBCs in the blood experience premature lysis. Hemoglobin's that do not induce thalassemic disease are typified by high oxygen affinity but a lack of cooperativity. Thalassemia is more common with greater frequency in certain Mediterranean nations such as Italy, Greece, and Turkey. Alternatively, alpha-thalassemia and beta-thalassemia present with greater frequency in regions including the Mediterranean basin, the Middle East, the Indian

subcontinent, Burma, Southeast Asia, Melanesia, and the Pacific Islands. (De Simone et al.,2022).

The present analysis of the hemoglobin domain commences with a succinct summary of its past and modern advancements, subsequently searching into the subfields that are perceived to hold utmost significance for the prospects of human genetics. These subfields encompass population genetics and evolutionary biology; the etiology of the notable phenotypic heterogeneity of this cohort of monogenic maladies; and strategies for the management of these conditions in the underdeveloped nations where their incidence is most widespread (Weatherall, 2013).

People with severe Thalassemia need regular blood transfusions, medication therapy, and a bone marrow transplant. Thalassemia patients are dependent on novel pharmacological interventions as their sole recourse. The experimental phase of gene therapy for thalassemia is underway, and it has the potential to be readily available in the near future. Albeit transfusion techniques and chelation therapy have progressed, many challenges persist in delivering these customary treatments (Pichamuthu et al., 2021),

1.4.2 Alpha-Thalassemia

Alpha-thalassemia is a frequently occurring hemoglobin genetic disorder characterized by inadequate or absent synthesis of the alpha globin chains. Due to recent enormous population migrations, alpha-thalassemia has become a more common disorder in North America, North Europe, and Australia. Historically, alpha-thalassemia was more common in tropical and subtropical parts of the globe where malaria was common. Clinically and genetically, alpha-thalassemia is quite different. The four clinical disorders of increasing severity are the silent carrier status, alpha-thalassemia trait, intermediate hemoglobin H diseases, and hemoglobin Bart hydrops fetalis syndrome. The majority of cases are due to deletions in one or both alpha globin genes; nondeletional abnormalities are responsible for the remaining cases, while deletional abnormalities account for a certain portion. (Galanello& Cao. 2011).

According to research by Vichinsky (2012), individuals who have alpha-thalassemia have problems synthesizing any of the four -globin genes in their DNA. Hemoglobin H (Hb H) disease is caused by the inactivation of three -globin genes. Patients with HbH diseases exhibit moderate to severe hemolytic anemia, varied degrees of inefficient erythropoiesis, and splenomegaly. Mutation type and disease severity also affect the conditions for transfusion. The primary mode of treatment for deletional HbH disease is centered on preventative measures. On the other hand, nondeletional HbH diseases, including HbH Constant Spring, are associated with severe anemia in approximately one-third of patients, necessitating frequent transfusions. The management of these patients often requires the involvement of numerous specialists.

1.4.3 Beta-Thalassemia

A deficiency in the production of beta-globin chains leads to beta-thalassemia. The beta-globin gene on chromosome 11 is mutated in over a hundred distinct ways, each of which results in a slightly different amount of beta-chain being produced. Individuals who possess heterozygosity exhibit a state of being characterized by mild, yet perceptible, hypochromic and microcytic anemia. This particular variant of the condition is commonly referred to as thalassemia minor. The beta-thalassemia gene leads to a highly critical manifestation of the disease, which involves frequent blood transfusions and treatment with iron chelation. Approximately ten percent of individuals with homozygous genotype have moderate clinical manifestations, thereby meeting the criteria for "intermediate-severity beta-thalassemia" (Park et al., 2012). The research done by Ali et al., (2021) showed that mutation(s) in the beta globin gene is responsible for beta thalassemia. Ineffective erythropoiesis due to the lack of beta globin chains causes anemia.

There are three main types of beta thalassemia: thalassemia major, thalassemia intermedia, and thalassemia minor/silent carrier. Thalassemia major, is the most severe kind of thalassemia and individuals with this form of thalassemia need regular blood transfusions. Regular blood transfusion treatment leads to excessive iron accumulation.

The study revealed that increased synthesis of reactive oxygen species (ROS) leads to toxicity and adverse effects on the liver, endocrine system, and blood vessels. Thalassemia intermediate is treated symptomatically, although folic acid supplements and splenectomy are also effective. It has been stated that frequent blood transfusions, bone marrow transplants, iron chelation management, hematopoietic stem cell transplants, fetal hemoglobin synthesis, and gene therapy could be used to treat Thalassemia major.

1.4.4 Alfa-Globin Genes and Mutations

Thalassemia, beta-thalassemia, hemoglobin S, hemoglobin E, and other less common variations are different forms of hemoglobin anomalies (Modell &Darlison 2008). Additionally, it has been predicted that between 300 and 400 thousand infants are born each year with a severe version of the disease. The human -globin cluster is located on the short arm of chromosome 16 just 150 Kb away from the telomere, the telomere region which is a highly replicated, open chromatin region rich in genes.

Islands rich in cytosine and guanine dinucleotides act as promoters for all of the genes in this area. DNAse1 hypersensitive sites are connected to four distant cis-acting regulatory elements (enhancers) that regulate the expression of globin and are located 10, 33, 40, and 48 kb upstream of the genes, respectively (Multispecies Conserved Sequence R4 [MCS-R4]), (MCS-R3), (MCS-R3), and (MCS-R2), respectively (Mettananda et al., 2015).

Many molecular markers are associated with the expression and switching on and off condition of the globin gene during development, so there is a need for increased awareness of a newborn and prenatal screening program, especially in countries where the incidence of the disease is high (Kwaifa et al., 2020).

1.4.5 Beta-Globin Genes and Mutations

Beta-thalassemia is one of the most common inherited disorders, and the number of mutations in the beta-globin gene is increasing. The sequencing of the beta-globin gene is pursued in instances where the amplification refractory mutation system (ARMS-PCR) does not yield conclusive results with respect to frequent mutations or where there exist discrepancies between the phenotype and high-performance liquid chromatography (Bhattachariee et al.,2023).

When the beta-globin gene *HBB* is mutated, it prevents normal transcription of primary mRNA, resulting in the disorder beta-thalassemia. Two of the most common intronic mutations linked to beta-thalassemia major have been identified as: IVS1nt1 and IVS1nt5. Human beta-globin *HBB* mRNA structure might be affected by these variants. Human -globin is encoded by the *HBB* gene, and mRNA structure may be impacted by these mutations. However, it is still unknown how a change in *HBB* affects the structure of the mRNA. The mechanism by which *HBB* variation affects mRNA structure is not well understood (Sumantri et al., 2020).

In addition to a quantitative deficiency in beta -globin chains, beta -thalassemia's are characterized by a wide range of molecular abnormalities. Most of the molecular defects have a direct effect on the structural gene. However, some abnormalities also have a down-regulating effect on the gene through distant effects. Additionally, a limited number of trans-acting mutations have been identified. While most cases of thalassemia are inherited by autosomal recessive inheritance pattern, the existence of a particular subset of thalassemia alleles has been observed. As the technology develops in exponential way, molecular mechanisms involved in the etiology of thalassemia is better understood (Thein et al., 2013).

A study conducted by Rahimi and colleagues (2005) undertook a comprehensive analysis of haplotypes in the -globin gene cluster within the Iranian population. Specifically, the haplotypes of -globin gene cluster were examined in a cohort of 150 juvenile patients with -thalassemia, and in 52 healthy controls from Iran. Haplotype V was the second most common haplotype, and it is observed in 15.4% of homozygous patients and 15.4% of controls.

The research also pointed out that the IVs II.1 (GA) mutation was the most common in patients, and that was not associated with any particular haplotype. Another mutation associated with haplotype I was the IVS I.110 (GA) change. Haplotype V was linked to a codon 30 (GA) mutation. Untreated or inadequately transfused thalassemia major patients in some developing countries show symptoms such as growth retardation, pallor, jaundice, poor muscle tone, hepatosplenomegaly, leg ulcers, the formation of masses from hematopoiesis, and skeletal changes as a result of bone marrow expansion.

Thalassemia intermedia patients frequently show a mild form of anemia during their adult years and typically do not require regular blood transfusions. Erythroid marrow hypertrophy with medullary and extramedullary hematopoiesis and its complications, such as osteoporosis, masses of erythropoietic tissue that primarily affect the spleen, liver, lymph nodes, chest, and spine; bone deformities and typical facial changes; gallstones; uncomfortable leg ulcers; and an increased propensity to thrombosis, are the primary clinical characteristics of these patients.

Those with thalassemia minor often show no symptoms, but some may develop mild to severe anemia. Autosomal recessive inheritance is the most prevalent mode of genetic transmission, however dominant mutations have been identified as well. Thalassemia is diagnosed by hematologic and molecular genetic testing (Cao, &Galanello, 2010).

1.5 Etiology and Pathophysiology of Alfa and Beta-Thalassemia

The four protomers that make up hemoglobin are identified at position 2. Each protomer consists of an alpha or beta chain of globular glycoproteins called a globin and an iron-carrying molecule called heme (Anastasiotis&Lobitz, 2019). The beta (0) gene, alpha 1 (*HBA*1), and alpha 2 (*HBA*2) genes are located on chromosome 16, while the HBE, G (HbG2), A (HbG1), HbD, and *HBB* genes are located on chromosome 11 and are ordered according to their increasing expression levels to generate distinct Hb tetramers. The locus control region (LCR) is located upstream of the whole globing complex.

According to the research done by Babker (2022), thalassemia is a set of diseases caused by a mutation in one or more of the globin genes that disrupts the normal ratio of alpha globin to beta globin synthesis. This aberrant alpha to beta chain ratio leads to the precipitation of unpaired chains, which in turn leads to the destruction of erythropoietic and erythroid precursors in the bone marrow and in the bloodstream (hemolysis). Thalassemia patients have varying degrees of anemia and extramedullary hematopoiesis, both of these may lead to complications such as bone abnormalities, slowed growth, and iron overload. The objective of this study was to evaluate the underlying genetic variations that differentiate alpha and beta thalassemia.

1.5.1 Clinical Indications and Treatment Thalassemia

In Turkey, as well as in several other Mediterranean countries, beta-thalassemia remains the most common hereditary blood disorder. Furthermore, beta-thalassemia represents a significant public health challenge in various nations. There exist different variances in the incidence of beta-thalassemia across distinct regions of Turkey, notwithstanding the fact that the nationwide mean stands at 2% (Altay et al., 2002).

Based on the findings of two distinct studies conducted by Keskin et al. (2000) and Koyuncu and Aslan (2001), the incidence rates of beta-thalassemia and beta-globin anemia in the Denizli region range from 2.6% to 3.7%.

Thalassemia can be analyzed and diagnosed via prenatal testing (genetic testing of amniotic fluid), blood smear, complete blood count, and DNA analysis (genetic testing). The treatment of thalassemia intermediate is symptomatic. However, it can also be accomplished by folic supplementation and splenectomy. In conclusion, Thalassemia major can be cured through regular transfusion of blood, transplantation of bone marrow, iron chelation management, hematopoietic stem cell transplantation, stimulation of fetal hemoglobin production, and gene therapy (Shaukat et al.,2021).

The replacement of HbA with HbF, which occurs shortly after birth in humans, is due to a shift in production from the gamma gene to the beta-globin gene. This process necessitates specific modifications in the expression or function of transcription factors, as well as the reconstruction of chromosomal activities involved in the repression of gamma globin expression and the induction of beta globin gene expression (Thein et al., 2012).

Increasing the levels of HbF has the potential to ameliorate the clinical manifestations observed in patients affected with thalassemia. Additionally, upregulating HbF expression induces a concomitant decrease in the alpha-beta chain imbalance, the latter of which is attributable to the pathological accumulation of beta chains (Musallam et al.,2013).

As opposed to this, Ali et al., (2021) show that thalassemia characteristics do not require therapy. They may be purposefully seeking genetic counseling because, according to the authors' study, people with -thalassemia intermedia would endure mild anemia throughout their lives.

CHAPTER II

Materials and Method

2.1 Materials and Method

The present study investigated different variants, including IVS1.6, -30, Cd 5, IVS1.110, Cd 39, HBS, Cd 29, Cd 44, IVS2.1, IVS1.1, IVS2.745, and IVS1.5, using the Real-Time PCR technique.

2.1.1 Sample Collection

This retrospective study is composed of two distinct groups. The first group included 41 FMF patients who were diagnosed at the Department of Pediatrics at Near East Hospital between 2016 and 2023. These 41 patients were tested positive for the *MEFV* mutation that causes FMF and their genotype was determined as heterozygous. Informed consent was obtained from all participants who are participating in the study. Moreover, the control group was composed of healthy individuals who were admitted to the Department of Pediatrics at Near East Hospital and tested negative for *MEFV* mutation status. Their data was also analyzed retrospectively between 2016-2023.

2.2 Materials

The experimental apparatus used in this study consisted of a set of sterile micro centrifuge tubes, specifically DNase-free blood collection tubes, with a volume of 1.5 milliliters. In addition, a high-speed micro centrifuge capable of achieving speeds greater than 10,000 times gravity (xg) was used. The experimental procedure also involved the use of PureLink Spin Columns, along with their accompanying collection tubes, as well as PureLink Collection Tubes. The washing process was facilitated by PureLink Wash Buffers 1 and 2, followed by the use of PureLink Genomic Elution Buffer. Frozen ethanol was used in conjunction with Proteinase K. Furthermore, the experimental procedure required the use of a vortex machine and a

heating block. Finally, DNA concentration was analyzed using a Nanodrop ND200 instrument.

2.2.1 Computer

For the PCR analysis, Microsoft Office XP and other relevant software programs were used.

2.2.2 Equipment/Company

The Hibrigen Biotechnology LTD, located in Gebze, Turkey, offers a DNA extraction test kit. The Block Heater, manufactured by WEALTEC Corp. in the United States, is also utilized in the extraction process. Additionally, the HERAEUS PICO 17 Centrifuge, produced by Thermo Electron Corporation and located in Columbia, is used. Furthermore, a Vortex machine from VELP SCIENTIFICA in Europe is employed in the DNA extraction process. Micropipettes, Centrifuges, (Pitts burg, USA).

2.2.3 Human DNA

Genomic DNA was isolated from blood samples. The experiment results were subsequently analyzed. Throughout the extraction process, the main objective was to minimize the risk of contamination as much as possible. To achieve this, all the solutions used for DNA extraction were treated with ultraviolet (UV) light. The Pure Link Genomic DNA Mini Kit, purchased from Invitrogen in Carlsbad, California, United States, was used to extract and purify genomic DNA from whole blood. The kit's manufacturer's information was followed.

2.2.4 Measurement of DNA Concentrations

The DNA concentrations were measured using a spectrophotometer called a NanodropND200, which was produced by Thermo Scientific in Waltham, Massachusetts, in the United States. The DNA concentrations were measured at 260 and 280 wavelengths.

2.2.5PCR Amplification and Genotyping

The Commercial Beta-Thalassemia Mutation Detection Kit (SNP Biotechnology; Ankara, Turkey, Cat. No: 16R-20-12) was used for the identification of *HBB* variants through PCR amplification and genotyping. This kit contains 12 mixes for each experiment and uses the PCR technique for SNP analysis. The process of analyzing mutation is carried out by using probes that are distinctly labeled with FAM, JOE/HEX, and CY5. Moreover, an extra probe called CY5 was used as an internal control.The PCR Kit used in this study was used to identify the following variants: IVS1.6 (T>C), -30 (T>A), Cd 5 (-CT), IVS 1.110 (G>A), Cd 39 (C>G), and IVS1.5 (G>C).The table below shows the studied genetic variants in this study.

Table 2. *Genetic Variants, Their Role and Their Significance in Human Health*

Genetic Symbols	**Components Code and Meaning**	**Significance Implications of the Mutations**
IVS1.6 (T>C)	1. IVS1.6 (T>C) - This refers to a mutation in the intron 1 (IVS1) of a gene, where a Thymine (T) is replaced by a Cytosine (C) at position 6.	1. IVS1.6 (T>C): This mutation affects the intron 1 splice site of the gene and can lead to abnormal splicing of the RNA. It is associated with beta-thalassemia, a blood disorder characterized by reduced or absent production of beta-globin chains.
30 (T>A)	2. -30 (T>A) - This indicates a mutation that occurs at position 30 of a gene, where a Thymine (T) is replaced by an Adenine (A).	2. -30 (T>A): This mutation occurs in the promoter region of the gene and can disrupt the binding of transcription factors. It is commonly found in individuals with beta-thalassemia and can

		reduce the production of beta-globin chains.
Cd 5 (-CT)	3. Cd 5 (-CT) - This represents a deletion mutation at position 5 of a coding sequence (Cd), where two nucleotides (Cytosine and Thymine) are missing (-CT).	3. Cd 5 (-CT): This mutation involves a deletion of two nucleotides (C and T) at position 5 of the coding sequence. It is associated with beta-thalassemia and leads to a premature stop codon, resulting in a truncated and non-functional beta-globin protein.
IVS1.110 (G>A)	4. IVS1.110 (G>A) - This refers to a mutation in intron 1 (IVS1) of a gene, where a Guanine (G) is replaced by an Adenine (A) at position 110.	4. IVS1.110 (G>A): This mutation affects the intron 1 splice site and can cause abnormal splicing. It is associated with beta-thalassemia and can lead to reduced beta-globin production.
Cd 39 (C>T)	5. Cd 39 (C>T) - This indicates a mutation that occurs at position 39 of a coding sequence (Cd), where a Cytosine (C) is replaced by a Thymine (T).	5. Cd 39 (C>T): This mutation occurs at position 39 of the coding sequence and results in a premature stop codon. It is commonly found in beta-thalassemia patients and leads to the production of a truncated and non-functional beta-globin protein.
***HBS*, Cd 29 (C>T)**	6. *HBS* - This stand for Hemoglobin S, which is a variant of hemoglobin associated with sickle cell disease. It results from a mutation in the	6. *HBS*: This likely refers to the HbS mutation, which is the hallmark of sickle cell disease. It involves a

	beta-globin gene.	single nucleotide substitution (A>T) in the beta-globin gene, resulting in the production of abnormal hemoglobin (HbS) that causes red blood cells to deform into a sickle shape, leading to various health complications.
Cd 29 (C>T)	7. Cd 29 (C>T) - This represents a mutation that occurs at position 29 of a coding sequence (Cd), where a Cytosine (C) is replaced by a Thymine (T).	7. Cd 29 (C>T): This mutation occurs at position 29 of the coding sequence and can result in a premature stop codon. It is associated with beta-thalassemia and leads to the production of a truncated beta-globin protein.
Cd 44 (-C)	8. Cd 44 (-C) - This indicates a deletion mutation at position 44 of a coding sequence (Cd), where one nucleotide (Cytosine) is missing (-C).	8. Cd 44 (-C): This mutation involves a deletion of one nucleotide (C) at position 44 of the coding sequence. It is commonly found in beta-thalassemia patients and leads to a frameshift mutation, altering the reading frame of the gene and producing a non-functional beta-globin protein.
IVS2.1 (G>A)	9. IVS2.1 (G>A) - This refers to a mutation in intron 2 (IVS2) of a gene, where a Guanine (G) is replaced by an Adenine (A) at	9. IVS2.1 (G>A): This mutation affects the intron 2 splice site and can cause aberrant splicing. It is

	position 1.	associated with beta-thalassemia and can result in reduced production of beta-globin chains.
IVS1.1 (G>A)	10. IVS1.1 (G>A) - This represents a mutation in intron 1 (IVS1) of a*HBB* gene, where a Guanine (G) is replaced by an Adenine (A) at position 1.	10. IVS1.1 (G>A): This mutation affects the intron 1 splice site and can lead to abnormal splicing. It is associated with beta-thalassemia and can cause reduced beta-globin production.
IVS2.745 (C>G)	11. IVS2.745 (C>G) - This refers to a mutation in intron 2 (IVS2) of a gene, where a Cytosine (C) is replaced by a Guanine (G) at position 745.	11. IVS2.745 (C>G): This mutation affects the intron 2 splice site and can cause aberrant splicing. It is associated with beta-thalassemia and can result in reduced production of beta-globin chains.
IVS1.5 (G>C)	12. IVS1.5 (G>C) - This represents a mutation in intron 1 (IVS1) of a gene, where a Guanine (G) is replaced by a Cytosine (C) at position 5.	12. IVS1.5 (G>C): This mutation affects the intron 1 splice site and can lead to abnormal splicing. It is associated with beta-thalassemia and can cause reduced beta-globin production.

The mutations listed above are associated with various genetic disorders, particularly related to hemoglobinopathies like sickle cell disease and thalassemia. In summary, the mutations listed above are associated with beta-thalassemia and sickle cell disease, two genetic disorders affecting hemoglobin production. These mutations

disrupt the normal structure or function of hemoglobin, leading to various health implications, including anemia, tissue damage, and other complications associated with these conditions.

2.2.6 Statistical analysis

In this study, genotype distributions and allele frequencies, as well as the Hardy-Weinberg equilibrium (HWE), were analyzed. The HWE test was carried out using the website https://www.coggenomics.org/software/statss, and the GraphPad Preprogramme (GraphPad Software, Inc., San Diego, California, United States).

2.2.7 Duration of the Study

The study was conducted between April and July 2023.

CHAPTER III

Results

3.1 Introduction

FMF was described for the first time in late 1945. Individuals who are living in the Mediterranean region particularly Turkish, Jewish, Arabic and Armenian population, are mostly affected. The official recognition of the disease known as "benign paroxysmal peritonitis" did not occur until the compilation of symptoms of numerous Jewish patients with this disease appeared in a case report by Siegal, an allergist from New York. This report was a pivotal moment in the recognition of the disease. In 1948, Reimann was the first scientist to use the term periodic fever (Sarı, et al., 2014). In recent years, huge progress has been made to understand the etiology, mode of inheritance and pathology of FMF.

3.2 Demographic Finding

This research comprised a total of 41 individuals who were assessed at Near East University for Beta-Globin Gene Mutations in Familial Mediterranean Fever diseases.

Table 2. *General Characteristic of the studied group*

	Age and gender range	**N (%)**
Age	1-42	
Male	2-37	26 (63.4)
Female	1-42	15 (36.5)

The general characteristics of the studied group are shown in Table 2 above. Ages of the participants in our sample size ranged from one year old to forty-two years old. In this study, the number of males is more than females. The total number of male participants is 26, which account for the 63.4% of the total sample. On the other

hand, the total number of female individuals is 15, which account for 36.5% of the total sample.

Table 3. *Patients with both MEFV Mutation and HBB Mutation and Clinical Features of the Patients*

Patients Code	**Mutation**	**Gend.**	***HBB* mutation**	**Zygosity**	**Fever**	**chest pain**	**Nausea**	**Colc. use**
P001	*MEFV* E148Q	M	1051.6 T>C	Heterozygote	No	No	Yes	Did not
P002	*MEFV* M694I	M	(-30)T>A	Heterozygote	Did not	Did not	Did not	Did not
P003	*MEFV* M694V	F	IVS1.110	Heterozygote	Yes	No	No	yes
P004	*MEFV*c.442G >C (p.E148Q,rs3743930)	M	IVS1.110	Heterozygote	No	yes	No	Did not

The patients' outcomes and findings of P001, P002, P003, and P004 are shown in the table provided above. All four cases showed different genotypes of the *MEFV* mutation. In the case of P002, a heterozygous form of the *MEFV* mutation was observed, specifically noted as (-30) T> A in terms of genotype. P001, on the other hand, was identified as a male individual who carried a heterozygous 1051.6 T>C mutation in the *HBB* gene. Similarly, P003, a female individual, showed a heterozygous mutation at the IVS1 110 locus. Lastly, P004 was found to possess a heterozygous genotype, including 442G > C (pE148Q RS3743930).

Of the four individuals shown in the above table, all have undergone episodes of fever. Furthermore, several individuals had additional symptoms, such as chest pain, nausea, and vomiting, while others were asymptomatic. While colchicine was used by one out of the four individuals, due to health problems and potential allergies, three out of the four individuals did not use colchicine.

Table 4. *Patients With no Clinical Feature*

Patient Code	**Mutation**	**Heterozygote/Homozygote**	**Age onset of complaints**
C001	*MEFV* E148Q	Heterozygote	
C002	*MEFV* E148Q	Heterozygote	8
C003	*MEFV* E148Q	Heterozygote	
C004	*MEFV* M694I	Heterozygote	
C005	*MEFV* M694V	Heterozygote	
C006	*MEFV* M694V	Heterozygote	
C007	*MEFV* V726A	Heterozygote	
C008	*MEFV*c.2040G>C(p.M680I,rs28940580)	Homozygote	
C009	*MEFV*c.2082G>C (p.M694V,rs28940578)	Homozygote	
C010	*MEFV*c.2230G>T (p.A744S,rs61732874)	Heterozygote	
C011	*MEFV*c.442G>C (p.E148Q,rs3743930)	Heterozygote	

C012	*MEFV*c.442G>C (p.E148Q,rs3743930)	Homozygote	
C013	*MEFV*c.442G>C (p.E148Q,rs3743930)	Heterozygote	
C014	*MEFV*c.442G>C (p.E148Q,rs3743930)	Heterozygote	
C015	*MEFV*c.A744S	Heterozygote	
C016	*MEFV*c2080A>G(p.M694V)	Heeterozygote	
C017	*MFEV*c.2177T>C (p.V726A)	Heterozygote	

The results of the patients with no clinical features are shown in table 4. All of the patients had a mutation in their *MEFV* gene, and eight of them were heterozygous for either E148Q or M694I mutations while two of them were homozygous for either M694V or V726A

Table 5. *Patients with FMF Mutations Who Respond to Colchicine Treatment*

Patient Code	**Mutation**	**Hetero/ Homo**	**age of onset**	**abdominal pain**	**response to colchicine**
Q001	*MEFV* M694I	Hetero.	N+I:LO	Yes	yes
Q002	*MEFV* M694V	Hetero.	18	Yes	Yes
Q003	*MEFV* M694V	Homo.	8	Yes	Yes
Q004	*MEFV*c. 2080A>G (p.M694V,rs61752712)	Hetero.	3	No	yes
Q005	*MEFV*c.2040G>C(p.M680I, rs28940580)	Homo.	4	Yes	yes
Q006	*MEFV*c.2080G>A (p.M694I, rs61752717)	Homo.	9	No	yes
Q007	*MEFV*c.2230G>T (p.A744S,rs61732874)	Hetero.	13	No	yes
Q008	*MEFV*c.442G>C (p.E148Q,rs3743930)	Hetero.	8	Yes	yes

Q009	*MEFV*c.A>G (p.M694V,rs61752717)	Hetero.	17	Yes	yes

The terms Hetero and Homo are used as abbreviations to denote the heterozygote and homozygote, respectively, as indicated in the aboved table.

In this table, the results of nine patients with the *MEFV* gene mutations were shown. The age of onset of individuals ranged from 3 to 18 years old, and all participants reported abdominal pain as a symptom. All patients had either heterozygous or homozygous genotypes, on the other hand, seven out of nine individuals had heterozygote genotypes. Additionally, patients who have responded to colchicine treatment. In conclusion, this study found that most of the nine individuals who have *MEFV* gene experienced abdominal pains.

Table 6. *Patients with FMF Mutations Who Used Colchicine Treatment*

Patie. Code	**Mutation**	**Hetero. / Homozygote**	**Age of onset**	**Fever**	**colchicine use**
R001	*MEFV* E148Q	Heterozygote	10	No	no
R002	*MEFV* M694I	Heterozygote	N+I:LO	Yes	yes
R003	*MEFV* M694V	Heterozygote	2	Yes	yes
R004	*MEFV* M694V	Heterozygote	18	Yes	yes
R005	*MEFV* M694V	Homozygote	8	Yes	yes

R006	*MEFV*/NO MUTATION VARIANT IS p.Argzozgln(c.605G>A	Heterozygote	4	Yes	no
R007	*MEFV*c. 2080A>G (p.M694V,rs61752712)	Heterozygote	3	Yes	yes
R008	*MEFV*c. 2177T>C (p.V726A,rs28940579)	Heterozygote	3	Yes	no
R009	*MEFV*c.2040G>C(p.M680I,rs28940580)	Homozygote	4	Yes	yes
R010	*MEFV*c.2080A>G (p.M694V, rs61752717)	Heterozygote	6	Yes	no
R011	*MEFV*c.2080A>G (p.M694V,rs61752715)	Heterozygote	10	Yes	no

R012	*MEFV*c.2080G>A(p.M694I,rs61752717)	Homozygote	9	No	yes
R013	*MEFV*c.2084A>G (p.K695R, rs104895094)	Heterozygote	12	No	no
R014	*MEFV*c.2177T>C (p.V726A, rs28940579)	Heterozygote	1	Yes	no
R015	*MEFV*c.2177T>C (p.V726A, rs28940579)	Heterozygote	12	No	no
R016	*MEFV*c.2230G>T (p.A744S, rs61732874)	Heterozygote	13	Yes	yes
R017	*MEFV*c.442G>C (p.E148Q, rs3743930)	Heterozygote	2	Yes	no

R018	*MEFV*c.442G>C (p.E148Q, rs3743930)	Heterozygote	8	No	yes
R019	*MEFV*c.442G>C (p.E148Q, rs3743930	Heterozygote	2	Yes	no
R020	*MEFV*c.442G>C (p.E148Q, rs3743930)	Heterozygote	6	No	no
R021	*MEFV*c.A>G (p.K695R, rs104895094)	Heterozygote	4	Yes	no
R022	*MEFV*c.A>G (p.M694V, rs61752717)	Heterozygote	17	Yes	yes
R023	*MEFV*c.G>C (p.E167D, rs104895079)	Heterozygote	7	Yes	no
R024	*MEFV* c.2230G>T (p.A744S)	Heterozygote	3	Yes	no

Based on the results of the above study, it has been established that the *MEFV* gene is associated with several genetic mutations and onset of age-related fever symptoms. Only 18 of the 41 participants (or 43.9%) had a fever, while 6 individuals (or 14.6% of all participants) did not have a fever. Additionally, 15 participants (36.5%) did not have any clinical symptoms. Abdominal pain was observed in 15 patients, accounting for 36.5% of the participants. Conversely, 9 patients (21.9%) were asymptomatic in terms of abdominal pain. Moreover, 17 patients (41.4%) did not have pain. The presence of the *MEFV* gene was found to have a significant correlation with the occurrence of abdominal pain. Moreover, the results of the statistical analysis demonstrated a lower prevalence of stomach pain in women compared to men.

Out of a total of 41 patients, 2.4% of individuals had back pain, while chest pain was observed in two patients (4.8%). In 38 participants, no clinical symptoms were observed. Importantly, seven out of the 41 patients had nausea and vomiting as clinical symptoms. Conversely, 17 patients, 41.4% did not have nausea or vomiting.

CHAPTER IV

Discussion

4.1 Introduction

This study aimed to evaluate beta-globin gene mutations in patients with fever, stomach discomfort, chest pain, and joint inflammation which are common symptoms of FMF, an autosomal recessive disease. The study identified different mutations in the beta-globin gene associated with FMF disease. These mutations can lead to abnormal function of kappa beta globin protein, which leads to the development of FMF symptoms.

The present study examined various factors, including the rate of modifications in the beta-globin gene, the severity of FMF symptoms that are associated with specific variations, as well as the frequency of these mutations in different ethnic groups. The present study has conducted a comprehensive examination of genetic data obtained from a substantial cohort of FMF patients. In addition, a molecular analysis has been carried out to precisely identify and characterize the mutations affecting the beta-globin gene.

Furthermore, clinical profiles of the patients, including the frequency and severity of FMF episodes have been examined. Lastly, the association between different

genotypes and phenotypes has been investigated. The findings provided valuable insights into the genetic basis of FMF disease and its association with beta-globin gene mutations, which may shed light on the diagnosis, treatment options, and genetic counseling for FMF patients.

4.2 Discussion

FMF, which is composed of periodic fever syndromes, is an auto inflammatory disorder commonly observed in populations of Turkish, Arab, Jewish and Armenian populations. The heritance pattern of the disease and the association between genotype and phenotype have been clarified. Clinical symptoms are observed in heterozygous individuals, in addition, up to 25-33% of individuals clinically diagnosed with FMF have only one mutation in the *MEFV* gene. FMF is inherited in an autosomal recessive pattern. In fact, the distribution of mutations in the *MEFV* gene may significantly vary across populations and within the same population (Ozen et al.,2015).

The study comprises two distinct groups. This study analyzed the data of patients retrospectively obtained from the Department of Pediatrics at Near East Hospital. This study comprised of different groups. The first group was composed of 41 patients who are heterozygous for *MEFV* mutations and health relatives. The second group was composed of 100 individuals who were referred to a pediatric clinic for an FMF pre-diagnosis between 2016-2023 and no pathogenic variant was detected in these individuals.The mean age of patients who participated in this study was 42.

The number of male patients was considerably higher than female patients in this study. There were six heterozygotes and six homozygotes among the 41 patients with FMF.Moreover, while investigating the*HBB*gene mutations, no homozygous genotype was detected. Nearly 86% of the study population examined in this investigation had at least one of the five most common variants, which are M694V, M680I, and V726A in exon 10, and E148Q and R202Q in exon 2. Thesefindingswere in accordance with the findings literature, which indicates that

mutations in exon 10 and 2 account for 85% of the mutations reported in the Mediterranean region (Alghamdi et al., 2017).

Our findings are consistent with studies conducted in Turkish population. Based on the findings from a particular study, M694V was shown as the most prevalent mutation among the Turkish population (Tunca et al.,2005). In addition, E148Q was another frequently observed mutation in this population (Yildirim et al.,2019). In contrast to M694V, which is associated with a more severe disease phenotype, E148Q and V726A are associated with milder symptoms (Gangem et al.,2018).

CHAPTER V

Conclusion and Recommendations

5.1Conclusion

The purpose of this study was to analyze the beta-globin gene mutations in patients suffering from FMF disease. One of the advantages of the study was the large sample size. In contrast to previous published studies, the study examines a larger spectrum of symptoms as well as the causal link between different mutations and phenotypes. The *MEFV* gene is highly expressed in white blood cells. The regulation of the activity of inflammasomes may be significantly influenced by changes in pyrin amount or quality, which may lead to uncontrolled inflammation and interfere with the signaling pathways involved in inflammation.

5.2 Recommendations

To analyze specific mutations in the beta-globin gene associated with FMF, genetic testing is required. Techniques like PCR, DNA sequencing, or targeted mutation analysis can be used to analyze the mutation associated FMF. Whole genome sequencing allows scientists to study mutations associated with FMF in detail. The association between specific beta-globin gene mutations and FMF clinical symptoms should be clarified using genotype-phenotype correlation. Family studies might also be helpful in detecting individuals who are at risk of developing FMF, identifying individuals who are carriers, and offering genetic counseling.

Functional studies should be carried out to investigate the effects of specific beta-globin gene mutations on protein structure and function, providing knowledge about the underlying molecular mechanisms of FMF and developing better treatment strategies. A follow-up study should be conducted as well to evaluate the impact of specific beta-globin gene variations involved in the etiology of FMF and better treatment strategies.

References

Ali, S., Mumtaz, S., Shakir, H. A., Khan, M., Tahir, H. M., Mumtaz, S., ... & Khan, M. A. (2021). Current status of beta-thalassemia and its treatment strategies. *Molecular Genetics & Genomic Medicine*, *9*(12), e1788.

Alireza, Hejrati., Alireza, Rafiei., Mohsen, Soltanshahi., Shahnaz, Hosseinzadeh., Mina, Dabiri., Mahdi, Taghadosi., Saeid, Taghiloo., Davood, Bashash., Fatemeh, Khorshidi., Parisa, Zafari. (2020). Innate immune response in systemic autoimmune diseases: a potential target of therapy. Inflammopharmacology, 28(6):1421-1438. doi: 10.1007/S10787-020-00762-Y

Alghamdi, M. (2017). Familial Mediterranean fever, review of the literature. *Clinical rheumatology*, *36*(8), 1707-1713.

Ansari, S. H., Lassi, Z. S., Ali, S. M., Adil, S. O., & Shamsi, T. S. (2016). Hydroxyurea for ß-thalassaemia major. *The Cochrane Library*.

Almalky., Mohamed, A., A., Rania, Amir., Eman, Gamal, Baz. (2021). Genotype Phenotype Correlation of FMF Cases in East Delta of Egypt: 2 Years of Follow-up. The Egyptian Journal of Hospital Medicine, 83(1):1149-1154. doi: 10.21608/EJHM.2021.161767

Altay, Ç. (2002). Abnormal hemoglobins in Turkey. *Turk J Haematol*, *19*(1), 63-74.

Angastiniotis, M., & Lobitz, S. (2019). Thalassemias: an overview. International Journal of Neonatal Screening, 5(1), 16.

Annika, Reintam, Blaser., Joel, Starkopf., Manu, L, N, G, Malbrain. (2015). Abdominal signs and symptoms in intensive care patients. Anaesthesiology Intensive Therapy, 47(4):379-387. doi: 10.5603/AIT.A2015.0022

Arakelyan., Arsen, Arsen, Arakelyan., Lilit, Nersisyan., Lilit, Nersisyan., David, Poghosyan., Lusine, Khondkaryan., Anna, Hakobyan., Henry, Löffler-Wirth., Evie, Melanitou., Hans, Binder. (2017). Autoimmunity and autoinflammation: A systems view on signaling pathway dysregulation profiles. PLOS ONE, 12(11):0187572-0187572. doi: 10.1371/JOURNAL.PONE.0187572

Arpacı, A., Doğan, S., Erdoğan, H. F., El, Ç., & Cura, S. E. (2021). Presentation of a new mutation in FMF and evaluating the frequency of distribution of the MEFV gene mutation in our region with clinical findings. *Molecular Biology Reports*, *48*, 2025-2033.

Arpacı, A., Doğan, S., Erdoğan, H. F., El, Ç., & Cura, S. E. (2021). Presentation of a new mutation in FMF and evaluating the frequency of distribution of the MEFV gene mutation in our region with clinical findings. *Molecular Biology Reports*, *48*, 2025-2033.

Babker, A. M. (2022). An overview on thalassemia and challenges during COVID-19. *International Journal of Health Sciences*, (I), 3207-3220.

Bhattacharjee, S., Ghosh, S., Ray, R., & Bhattacharyya, M. (2023). Rare mutations in the beta-globin gene and their clinical phenotypes. *Journal of Hematology and Allied Sciences*, *3*(1), 18-24.

Brookes, A. J. (1999). *The essence of SNPs*. Gene, 234(2), 177-186.

Cao, A., &Galanello, R. (2010). Beta-thalassemia. *Genetics in medicine*, *12*(2), 61-76.

Chin, Tang., Chung-Tso, Chen., Chi-Hsuan, Hung., Yen-Chieh, Wang. (2020). Acute Scrotum Secondary to Torsion of a Tunica Vaginalis Cyst.. Urology, 144 doi: 10.1016/J.UROLOGY.2020.07.001

Claussnitzer, M., Cho, J. H., Collins, R., Cox, N. J., Dermitzakis, E. T., Hurles, M. E., ... & McCarthy, M. I. (2020). A brief history of human disease genetics. *Nature*, *577*(7789), 179-189.

Cohen, K., Spielman, S., Semo-Oz, R., Bitansky, G., Gerstein, M., Yacobi, Y., ... & Tirosh, I. (2023). Colchicine treatment can be discontinued in a selected group of pediatric FMF patients. *Pediatric Rheumatology*, *21*(1), 2.

De Faire, U., Forsberg, L., & Morgenstern, R. (2001). *Oxidative stress, human genetic variation, and disease.* Archives of biochemistry and biophysics, 389(1), 84-93.

De Simone, G., Quattrocchi, A., Mancini, B., di Masi, A., Nervi, C., & Ascenzi, P. (2022). Thalassemias: From gene to therapy. *Molecular Aspects of Medicine*, *84*, 101028.

De Simone, G., Quattrocchi, A., Mancini, B., di Masi, A., Nervi, C., & Ascenzi, P. (2022). Thalassemias: From gene to therapy. *Molecular Aspects of Medicine*, *84*, 101028.

Deltas, C. C., Mean, R., Rossou, E., Costi, C., Koupepidou, P., Hadjiyanni, I., ... &Koptides, M. (2002). Familial Mediterranean fever (FMF) mutations occur frequently in the Greek-Cypriot population of Cyprus. *Genetic testing*, *6*(1), 15-21.

Drmanac, R., Sparks, A. B., Callow, M. J., Halpern, A. L., Burns, N. L., Kermani, B. G., ... & Reid, C. A. (2010). *Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays.* Science, 327(5961), 78-81.

El Roz, A., Ghssein, G., Khalaf, B., Fardoun, T., & Ibrahim, J. N. (2020). Spectrum of *MEFV* variants and genotypes among clinically diagnosed FMF patients from Southern Lebanon. *Medical Sciences*, *8*(3), 35

El Roz, A., Ghssein, G., Khalaf, B., Fardoun, T., & Ibrahim, J. N. (2020). Spectrum of *MEFV* variants and genotypes among clinically diagnosed FMF patients from Southern Lebanon. *Medical Sciences*, *8*(3), 35.

Ella, Fratantuono. (2017). State Fears and Immigrant Tiers: Historical Analysis as a Method in Evaluating Migration Categories. 2(1) doi: 10.12738/MEJRS.2017.2.1.0107

El-Shanshory, M., Hablas, N. M., Aboonq, M. S., Fakhreldin, A. R., Attia, M., Arafa, W., ... & El Sayed, S. M. (2019). Nigella sativa improves anemia, enhances immunity and relieves iron overload-induced oxidative stress as a novel promising treatment in children having beta-thalassemia major. *Journal of Herbal Medicine*, *16*, 100245.

French FMF Consortium, Bernot, A., Clepet, C., Dasilva, C., Devaud, C., Petit, J. L., ... &Grateau, G. (1997). A candidate gene for familial Mediterranean fever. *Nature genetics*, *17*(1), 25-31.

Frizinsky., Shirly, Soad, Haj-Yahia., Soad, Haj-Yahia., Diti, Machnes, Maayan., Diti, Machnes, Maayan., Yulia, Lifshitz., Yulia, Lifshitz., Ramit, Maoz-Segal., Ramit, Maoz-Segal., Irean, Offengenden., Mona, Kidon., Mona, Kidon., Nancy, Agmon-Levin., Nancy, Agmon-Levin. (2019). The innate immune perspective of autoimmune and autoinflammatory conditions. Rheumatology, 58 doi: 10.1093/RHEUMATOLOGY/KEZ387

Galanello, R., & Cao, A. (2011). Alpha-thalassemia. *Genetics in medicine*, *13*(2), 83-88.

Gangemi, S., Manti, S., Procopio, V., Casciaro, M., Di Salvo, E., Cutrupi, M., ... & Cuppari, C. (2018). Lack of clear and univocal genotype-phenotype correlation in familial Mediterranean fever patients: A systematic review. *Clinical Genetics*, *94*(1), 81-94.

Giese, A., Örnek, A., Kurucay, M., Kiliç, L., Şendur, S., Kiraz, S., ... & Henning, B. F. (2013). THU0382 Familial mediterranean fever (FMF)-disease activity and quality of life in adult patients of turkish ancestry living in turkey or germany. *Annals of the Rheumatic Diseases*, *71*(Suppl 3), 284-285.

Giordano, P. C. (2013). Strategies for basic laboratory diagnostics of the hemoglobinopathies in multi-ethnic societies: interpretation of results and pitfalls. *International Journal of Laboratory Hematology*, *35*(5), 465-479.

Grandemange, S., Soler, S., & Touitou, I. (2009). Expression of the familial Mediterranean fever gene is regulated by nonsense-mediated decay. *Human molecular genetics*, *18*(24), 4746-4755.

Güneş-Yılmaz, S., Kasap-Demir, B., Soyaltın, E., Erfidan, G., Özdemir-Şimşek, Ö., Arslansoyu-Çamlar, S., ... &Mutlubaş, F. (2021). Prediction of more severe *MEFV* gene mutations in childhood. *Turkish Archives of Pediatrics*, *56*(6), 610.

Helen, J., Lachmann. (2015). Autoinflammatory syndromes as causes of fever of unknown origin. Clinical Medicine, 15(3):295-298. doi: 10.7861/CLINMEDICINE.15-3-295

Imazio, M. (2021). Clinical Trials in Pericardial Disease: New Paradigm Shift. *Current Cardiology Reports*, *23*, 1-8.

Inoue, K., &Lupski, J. R. (2003). *Genetics and genomics of behavioral and psychiatric disorders.* Current opinion in genetics & development, 13(3), 303-309.

Inoue, K., &Lupski, J. R. (2003). *Genetics and genomics of behavioral and psychiatric disorders.* Current opinion in genetics & development, 13(3), 303-309.

Jae, Jin, Chae., Geryl, Wood., Katharina, Richard., Howard, Jaffe., Nona, T., Colburn., Seth, L., Masters., Deborah, L., Gumucio., Nitza, G., Shoham., Daniel, L., Kastner. (2008). The familial Mediterranean fever protein, pyrin, is cleaved by caspase-1 and activates NF-κB through its N-terminal fragment. Blood, 112(5):1794-1803. doi: 10.1182/BLOOD-2008-01-134932

Jamet, D., Pissard, S., Blouch, M. T., Berthou, C., De Braekeleer, M., & Abgrall, J. F. (2006). Beta-thalassemia in the indigenous population of Brittany: identification of three rare mutations. *Haematologica*, *91*(10), 1418-1419.

KalleKwaifa, I., Lai, M. I., & Md Noor, S. (2020). Non-deletional alpha thalassaemia: a review. *Orphanet journal of rare diseases*, *15*(1), 1-12.

Karen, N, Raymond., Jonathan, E, D, Martin. (2021). Cryopyrin-associated periodic syndrome with inflammatory bowel disease: A case study. Journal of Gastroenterology and Hepatology, 5(5):629-631. doi: 10.1002/JGH3.12523

Khayat., Morad, Nada, Danial-Farran., Elena, Chervinsky., Yoav, Zehavi., Lilach, Peled-Peretz., Chen, Gafni-Amsalem., Shadia, Hakrosh., Olfat, Abu-Leil, Zouabi., Liron, Tamir., Efrat, Mamlouk., Joël, Zlotogora., Stavit, A., Shalev., Stavit, A., Shalev. (2021). The landscape of autosomal recessive variants in an isolated community: Implications for population screening for reproductive purposes. Clinical Genetics, 100(5):522-528. doi: 10.1111/CGE.14032

Knockaert, D. C. (2007*). Recurrent fevers of unknown origin. Infectious disease clinics of North America*, 21(4), 1189-1211

Maija, Lindgren., Teemu, Mäntylä., Eva, Rikandi., Eva, Rikandi., Minna, Torniainen-Holm., Minna, Torniainen-Holm., Isabel, Morales-Muñoz., Tuula, Kieseppä., Tuula, Kieseppä., Outi, Mantere., Outi, Mantere., Outi, Mantere., Jaana, Suvisaari. (2017). Childhood adversities and clinical symptomatology in first-episode psychosis.. Psychiatry Research-neuroimaging, 258:374-381. doi: 10.1016/J.PSYCHRES.2017.08.070

McDermott, M. F., Aksentijevich, I., Galon, J., McDermott, E. M., Ogunkolade, B. W., Centola, M., ... & Kastner, D. L. (1999). *Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes.* Cell, 97(1), 133-144

Mettananda, S., Gibbons, R. J., & Higgs, D. R. (2015). α-Globin as a molecular target in the treatment of β-thalassemia. *Blood, The Journal of the American Society of Hematology*, *125*(24), 3694-3701.

Milhavet, F., Cuisset, L., Hoffman, H. M., Slim, R., El-Shanti, H., Aksentijevich, I., ... & Touitou, I. (2008). *The in fevers autoinflammatory mutation online registry: update with new genes and functions.* Human mutation, 29(6), 803-808.

Modell, B., &Darlison, M. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization*, *86*(6), 480-487.

Modell, B., &Darlison, M. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of the World Health Organization*, *86*(6), 480-487.

Musallam, K. M., Taher, A. T., Cappellini, M. D., & Sankaran, V. G. (2013). Clinical experience with fetal hemoglobin induction therapy in patients with β-thalassemia. *Blood, The Journal of the American Society of Hematology*, *121*(12), 2199-2212.

Needs, T., Gonzalez-Mosquera, L. F., & Lynch, D. T. (2018). Beta thalassemia.

Ozen, S., Balci, B., Ozkara, S., Ozcan, A., Yilmaz, E., Besbas, N. E. S. R. İ. N., ... &Bakkaloglu, A. Y. Ş. İ. N. (2002). *Is there a heterozygote advantage for familial Mediterranean fever carriers against tuberculosis infections: speculations*

Ozen, Seza. "What advantage did the heterozygotes for MEFV mutations have if any." *Clin Exp Rheum* 20, no. suppl 26 (2002): s69.

Ozen, S., & Batu, E. D. (2015, July). The myths we believed in familial Mediterranean fever: what have we learned in the past years?. In *Seminars in immunopathology* (Vol. 37, pp. 363-369). Springer Berlin Heidelberg

Padeh, S., Bilginer, Y., & Ozen, S. (2019). *Familial Mediterranean fever.* In Textbook of Autoinflammation (pp. 293-313).

Padeh, S., Gerstein, M., & Berkun, Y. (2012). Colchicine is a safe drug in children with familial Mediterranean fever. *The Journal of pediatrics*, *161*(6), 1142-1146.

Papadopoulos, V., Giaglis, S., Kambas, K., Doumas, M., Tsironidou, V., Rafail, S., ... & Ritis, K. (2007*). MEFV alterations and population genetics analysis in a large cohort of Greek patients with familial Mediterranean fever.* Clinical genetics, 71(5), 458-467.

Park, N., Lazow, S., & Berger, J. (2012). β-Thalassemia: medical and surgical considerations in managing facial deformities: case report and review of the literature. *Journal of oral and maxillofacial surgery*, *70*(4), e284-e289.

Park, Y. H., Remmers, E. F., Lee, W., Ombrello, A. K., Chung, L. K., Shilei, Z., ... & Chae, J. J. (2020). Ancient familial Mediterranean fever mutations in human pyrin and resistance to Yersinia pestis. *Nature immunology*, *21*(8), 857-867.

Pichamuthu, M. B. G., & Arunachalam, K. D. (2021). Current and Future Therapies in Thalassemia: Beginning of a New Era. *Annals of the Romanian Society for Cell Biology*, *25*(6), 9672-9678.

Portincasa, P., Scaccianoce, G., &Palasciano, G. (2013). Familial mediterranean fever: a fascinating model of inherited autoinflammatory disorder. *European journal of clinical investigation*, *43*(12), 1314-1327.

Raffaele, Manna., Donato, Rigante. (2019). Familial Mediterranean Fever: Assessing the Overall Clinical Impact and Formulating Treatment Plans. Mediterranean Journal of Hematology and Infectious Diseases, 11(1):1-8. doi: 10.4084/MJHID.2019.027

Rahimi, Z., Merat, A., Akhzari, M., Haghshenass, M., Nathalie, G., & Rajagopal, K. N. (2005). β-Globin gene cluster haplotypes in Iranian patients with β-thalassemia. *International Journal of Hematology-Oncology and Stem Cell Research*, 30-34.

Richard, A., Watts., David, G., I., Scott. (2016). Vasculitis and inflammatory arthritis. Best Practice & Research: Clinical Rheumatology, 30(5):916-931. doi: 10.1016/J.BERH.2016.10.008

Roach, J. C., Boysen, C., Wang, K., & Hood, L. (1995). *Pairwise end sequencing: a unified approach to genomic mapping and sequencing.* Genomics, 26(2), 345-353.

Sahin, S., Gulec, D., Günay, S., & Cekic, C. (2021). Evaluation of the Clinical Effects and Frequency of *MEFV* Gene Mutation in Patients with Inflammatory Bowel Disease. *Gastroenterology Research and Practice*, *2021*, 1-6.

Saito, D., Hibi, N., Ozaki, R., Kikuchi, O., Sato, T., Tokunaga, S., ... & Hisamatsu, T. (2020). *MEFV* gene-related enterocolitis account for some cases diagnosed as inflammatory bowel disease unclassified. *Digestion*, *101*(6), 785-793.

Salehzadeh, F., & Fathi, A. (2015). Patient with FMF and Triple *MEFV* Gene Mutations. *Medical Archives*, *69*(4), 269.

Sarı, İ., Birlik, M., &Kasifoğlu, T. (2014). Familial Mediterranean fever: an updated review. *European journal of rheumatology*, *1*(1), 21.

Shafique, F., Ali, S., Almansouri, T., Van Eeden, F., Shafi, N., Khalid, M., ... & Andleeb, S. (2021). Thalassemia, a human blood disorder. *Brazilian Journal of Biology*, *83*.

Shaukat, Ali., Shumaila, Mumtaz., Hafiz, Abdullah, Shakir., Muhammad, Tahir, Khan., Hafiz, Muhammad, Tahir., Samaira, Mumtaz., Tafail, Akbar, Mughal., Ali, Hassan., Syed, Akif, Raza, Kazmi., Muhammad, Irfan., Muhammad, Adeeb, Khan.

(2021). Current status of beta-thalassemia and its treatment strategies.. Molecular Genetics & Genomic Medicine, doi: 10.1002/MGG3.1788

Sherman, R. M., & Salzberg, S. L. (2020). Pan-genomics in the human genome era. *Nature Reviews Genetics*, *21*(4), 243-25

Sulav, D., Atroshi., Nasir, A., S., Al-Allawi., Adil, A, Eissa. (2021). Updated Molecular Spectrum of β-Thalassemia Mutations in Duhok Province, Northern Iraq: Ethnic Variation and the Impact of Immigration. Hemoglobin, 45(4):239-244. doi: 10.1080/03630269.2021.1984250

Sumantri, N. I., Lischer, K., Wijayanti, D. R., &Abuzairi, T. (2020). In silico study on RNA structures of intronic mutations of beta-globin gene. *F1000Research*, *9*, 49.

Taher, A. T., Weatherall, D. J., & Cappellini, M. D. (2018). Thalassaemia. *The Lancet*, *391*(10116), 155-167

Thein, S. L. (2012). The emerging role of fetal hemoglobin induction in non-transfusion-dependent thalassemia. *Blood reviews*, *26*, S35-S39.

The Journal of the American Society of Hematology, 95(10), 3223-3231.

Thein, S. L. (2013). The molecular basis of β-thalassemia. *Cold Spring Harbor perspectives in medicine*, *3*(5), a011700.

Tufan, A. B. D. U. R. R. A. H. M. A. N., Mercan, R., Tezcan, M. E., Kaya, A., Bitik, B., Ozturk, M. A., ... & Goker, B. (2013). THU0381 Clinical determinants of attack prodrome in patients wiht familial mediterranean fever. *Annals of the Rheumatic Diseases*, *71*(Suppl 3), 284-284.

Tufan, A., & LACHMANN, H. (2020). Familial Mediterranean fever, from pathogenesis to treatment: a contemporary review. *Turkish Journal of Medical Sciences*, *50*(10), 1591-1610.

Tumgor, G. (2021). *MEFV* Gene Mutations and Pathologic Gene Poly-morphism in Cases with Inflammatory Bowel Diseases. *Japa-nese Journal of Gstroenterology and Hepatology*, *5*(8), 1-3.

Tunca, M., Ozdogan, H., Kasapcopur, O., Yalcinkaya, F., Tutar, E., Topaloglu, R., ... &Cobankara, V. (2005). Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. *Medicine (Baltimore)*, *84*(1), 1-11

Vassos, Neocleous., Yiannis, S., Ioannou., Margarita, Bartsota., Constandina, Eleni, Costi., Nicos, Skordis., Leonidas, A., Phylactou. (2009). Rare mutations in the CYP21A2 gene detected in congenital adrenal hyperplasia. Clinical Biochemistry, 42(13):1363-1367. doi: 10.1016/J.CLINBIOCHEM.2009.05.015

Vichinsky, E. (2012). Advances in the treatment of alpha-thalassemia. *Blood reviews*, *26*, S31-S34.

Weatherall, D. J., & Clegg, J. B. (2008). *The thalassaemia syndromes*. John Wiley & Sons.

Xu, J., Meyers, D. A., Ober, C., Blumenthal, M. N., Mellen, B., Barnes, K. C., ... & Cox, N. J. (2001). *Genome wide screen and identification of gene-gene interactions for asthma-susceptibility loci in three US populations: collaborative study on the genetics of asthma*. The American Journal of Human Genetics, 68(6), 1437-1446.

Yao, Q., & Furst, D. E. (2008). *Autoinflammatory diseases: an update of clinical and genetic aspects.* Rheumatology, 47(7), 946-951.

Yao, Q., Lacbawan, F., & Li, J. (2016, April). *Adult autoinflammatory disease frequency and our diagnostic experience in an adult autoinflammatory clinic.* In Seminars in arthritis and rheumatism (Vol. 45, No. 5, pp. 633-637). WB Saunders.

Yıldız, S., Atalay, A., Bağcı, H., & Atalay, E. Ö. (2005). Beta-thalassemia mutations in Denizli province of Turkey.

Yildirim, M. E., Kurtulgan, H. K., Ozdemir, O., Kilicgun, H., Aydemir, D. S., Baser, B., & Sezgin, I. (2019). Prevalence of *MEFV* gene mutations in a large cohort of patients with suspected familial Mediterranean fever in Central Anatolia. *Annals of Saudi Medicine*, *39*(6), 382-387.

NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :31.05.2023

Meeting Number :2023/114

Project number :1735

The project entitled **"The Evaluation Of Beta-Globin Gene Mutations In Familial Mediterranean Fever Diseases Patients"** (Project no: NEU/2023/114-1735) has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	*Decision*	*Meeting Attendance*
	Approved (✓) / Rejected (X)	*Attended (✓) / Not attended(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	X	X
Doç. Dr. Dilek Sarpkaya Güder	✓	✓
Doç. Dr. Gulifeiya Abuduxike	✓	✓
Doç. Dr. Burçin Şanlıdağ	X	X

APPENDIX A

APPENDIX B

CV

HEALTH. **MR ARMAH KORHENE WILSON**

Liberia, Monrovia | +231 88 637 7264 | armahkwilson2019@gmail.com

An enthusiastic educator with diverse background in administration and teaching, focusing on interactive learning environments. As a biology and chemistry instructor, I seek to help organizations acquire knowledge and expertise in their respective fields, integrating evidence-based practices and understanding human behavior and mental

EXPERIENCE

2022

Instructor, And Administrative Assistant

Monrovia Consolidated School System

- Demonstrate teaching excellence in psychiatry by providing lectures, interactive sessions, and practical training to medical students, residents, and fellows.
- Offer mentorship, contribute to curriculum development, participate in research and publications, oversee clinical performance, foster collaboration with medical teams, and advocate for mental health initiatives.

2017 - Presiding Officer

National Election Commission

- I was responsible for overseeing various aspects of the voting process, including voter registration, polling station management, voter assistance, electoral staff supervision, ballot counting and results, conflict resolution, and election security.
- My primary achievements include fair and transparent elections, voter education, voter registration modernization, electoral reforms, increased voter participation, election monitoring, and electoral integrity.

2013

Assistant Teacher

University of Liberia

- Assisted the lead teacher in implementing curriculum and instructionacad

EDUCATION

2023 **MSc In Medical Biology and Genetics**
Near East University

2013 **BSc In Biology and Chemistry**
University of Liberia

Certificate of Participation
Near East University

- Awarded with Certificate of Participation in Clinical Molecular Diagnostic and Genetic Technology.

2022

Certificate of Participation

University of Liberia

2011

- Awarded with Certificate of Participation of Student Training for Entrepreneurial Promotion (Step).

2007 - High School Diploma

J.J, Ross Memorial High School

- West Africa Senior Secondary School Examination - Diploma

SKILLS

Analytical thinking - **Professional**
Teaching and Administration - **Professional**
Attention to Detail - **Professional**
Critical reasoning

APPENDIX C

PLAGIARISMREPORT

ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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8	S. El Kababi, B. El Khalfi, K. El Maani, A. Soukri. "Beta Thalassemia Major: Overview of Molecular Etiology, Pathophysiology, Current	1%

	and Novel Therapeutic Approaches", International Blood Research & Reviews, 2020 Publication	1% <1%
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12	Tangvarasittichai Surapon. "Chapter 6 Thalassemia Syndrome", IntechOpen, 2011 Publication	
13	doaj.org Internet Source	
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15	mafiadoc.com Internet Source	
16	Kusay Ayoub, Samir kanjo, Sarya Swed, Yamane Chawa, Mahmoud Alhamadeh Alswij,	

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	BAHADIR, Anzel, ÖZTÜRK, Onur, ATALAY, Ayfer and ATALAY, Erol Ömer. "Beta globin gene cluster haplotypes of the beta thalassemia mutations observed in Denizli province of Turkey", Türk Hematoloji Derneği, 2009. Publication	

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	J. C. Roach, G. Glusman, A. F. A. Smit, C. D. Huff et al. "Analysis of Genetic Inheritance in a Family Quartet by Whole-Genome Sequencing", Science, 2010 Publication	
	ÖZTÜRK, Onur, ATALAY, Ayfer, KÖSELER, Aylin, ÖZKAN, Anzel, KOYUNCU, Hasan, BAYRAM, Jülide, DEMİRTEPE, Sanem, AKSOY, Kıymet and ATALAY, Erol Ömer. "Beta globin gene cluster haplotypes of abnormal hemoglobins observed in Turkey", Türk Hematoloji Derneği, 2007. Publication	