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Genetic variations in Familial Mediterranean fever related *MIR197* gene in Turkish population

Türk popülasyonundaki Ailevi Akdeniz ateşi ilişkili *MIR197* genindeki genetik varyasyonlar

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Abstract

Objective: Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease caused by the gain of function mutations in the *MEFV* gene, which encodes pyrin protein. This study aimed to determine single nucleotide polymorphisms in the *MIR197* gene locus in FMF patients and healthy controls in Turkish population. We have previously identified miR-197-3p as a differentially expressed miRNA in FMF patients according to disease severity.

Methods: DNA was isolated from peripheral blood samples of six FMF patients and 12 healthy controls. The *MIR197* gene region was amplified by polymerase chain reaction and Sanger sequencing was performed. DNA sequence analysis results were analyzed with the Chromas (version 2.33). Additionally, open access GWAS data (Harvard Dataverse, V2; Turkish_A1_A2.txt) covering 1011 healthy Turkish people was analyzed using R.

Results: Genetic variants in the *MIR197* region were not detected in study group. Also, we couldn't find any genetic variants in the *MIR197* region through the analysis of a GWAS study (Harvard Dataverse, V2; Turkish_A1_A2.txt).

Conclusion: In this study, we demonstrated that the dysregulated miR-197-3p profile seen in FMF patients is not explained by variations in the *MIR197* gene locus. These results suggest that other factors such as promotor methylation, histone modifications, other non-coding RNAs and alterations in post-transcriptional processing may be involved in the changes in miRNA expression and should be investigated further.

Keywords: FMF, microRNA, genetic variations, SNPs, *MIR197* gene

Öz

Amaç: Ailevi Akdeniz ateşi (AAA), pyrin proteinini kodlayan *MEFV* genindeki fonksiyon kazanımı mutasyonlarının neden olduğu kalıtsal otoenflamatuvar bir hastalıktır. Bu çalışmanın amacı, Türk toplumundaki AAA hastalarında ve sağlıklı kontrollerde *MIR197* gen lokusundaki tek nükleotit polimorfizmlerini incelemektir. Grubumuzun daha önceki çalışmalarında, miR-197-3p'nin AAA hastalarında hastalık şiddetine göre değişken ifade gösteren bir miRNA olduğu tanımlanmıştır.

Yöntem: Altı AAA hastası ve 12 sağlıklı kontrolün periferik kan örneklerinden DNA izolasyonu yapılmıştır. *MIR197* gen bölgesi polimeraz zincir reaksiyonu ile çoğaltılmış ve Sanger dizilemesi yapılmıştır. DNA dizi analizi sonuçları Chromas (sürüm 2.33) ile analiz edilmiştir. Ayrıca Türk popülasyonundan 1011 sağlıklı katılımcıyı kapsayan açık erişimli GWAS verileri (Harvard Dataverse, V2; Turkish_A1_A2.txt) R programı kullanılarak analiz edilmiştir.

Bulgular: Çalışma gruplarında *MIR197* gen bölgesinde herhangi bir genetik varyasyon saptanamamıştır. Türk toplumundan 1011 sağlıklı kontrolü içeren GWAS çalışmasının (Harvard Dataverse, V2; Turkish_A1_A2.txt) analizi sonucunda da *MIR197* gen bölgesinde herhangi bir genetik varyant tespit edilememiştir.

Sonuç: Bu çalışmada, AAA hastalarında görülen miR-197-3p ifade değişikliğinin nedeninin *MIR197* gen lokusundaki varyasyonlarla açıklanmadığını belirlenmiştir. Bu sonuçlar, promotor metilasyonu, histon modifikasyonları, diğer kodlamayan RNA'lar ve transkripsiyon sonrası modifikasyonlar gibi diğer faktörlerin miRNA ekspresyonundaki değişikliklerde rol oynayabileceğini ve daha ileri araştırmaların yapılması gerektiğini göstermektedir.

Anahtar Kelimeler: AAA, mikroRNA, genetik varyasyonlar, SNPs, *MIR197* geni

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Introduction

Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disease caused by the gain of function mutations in the *MEFV* gene, which encodes pyrin protein.[1] A variety of phenotypic differences between FMF patients with identical mutations and changing severity of the disease pathogenesis imply a role for epigenetic regulation in this disease.[2] There is growing evidence that microRNAs (miRNAs) as epigenetic regulators may serve as promising candidates for FMF.

miRNAs are a class of small non-coding RNAs that play crucial roles in various biological processes, including gene expression regulation and post-transcriptional gene silencing. miRNAs, typically composed of 18-25 nucleotides, are highly conserved across species and have been found to be involved in numerous cellular processes such as cell proliferation, differentiation, and apoptosis.[3] miRNA biogenesis comprises a multi-step process that ends with the production of a mature miRNA.[4] The mature miRNA is loaded onto the RNA-induced silencing complex. This complex guides the miRNA to its target mRNA sequence through base pairing, leading to gene silencing through translational repression or mRNA degradation.[4] Although the canonical function of miRNAs is defined by this silencing effect, studies show that it can have diverse effects on translational and transcriptional activity.[5] The dysregulation of miRNA expression can result from genetic variations, including single nucleotide polymorphisms, in the genes involved in miRNA biogenesis, regulation, and miRNA sequence itself.[6-8]

Single nucleotide polymorphisms (SNPs), or single nucleotide polymorphisms, are the most common type of genetic variation that can occur within miRNA genes or target sites in the human genome. They occur when a single nucleotide base in the DNA sequence is altered. These SNPs can affect miRNA biogenesis, function, and regulation, ultimately impacting gene expression and contributing to the development of various human complex diseases. According to the miRNASNP-v3, there are 46,826 SNPs and 4,712 DRVs (Disease Related Variants) located in pre-miRNA loci in the human genome.[9]

miRNA SNPs or polymorphisms may have varying downstream effects depending on the miRNA function and the region of SNP.[10] The regulatory functions of miRNAs exclusively depend on the sequence alignment of their seed region to their target mRNA. SNPs located in the seed region may disrupt this binding.[11] On the other hand, SNPs or mutations that result in perfect matching with the target site can result in the degradation of the mRNA, instead of transcriptional silencing.[12] A seed sequence SNP of miR-125a prevents Drosha binding to the other arm and inhibits its transcription.[13] Seed sequence mutations are important in this manner because they are directly related to the function of the miRNA causing a disease state in the organism.

miRNA gene loci also include sequences for directing the biogenesis and their regulation with other factors. SNPs and mutations in these regions can cause alterations in biogenesis, changes in miRNA level, altered precision of processing, biased 5p/3p strand selection, and malfunctioning in the target silencing. miRNA clusters and individual miRNA genes form hairpin structures during transcription that generate a 3D form for DROSHA and other proteins to recognize and process the pri- to pre-transformation. Nucleotide changes may generate unstable hairpins or changed distributions of these loops causing disruption in pri-miRNA processing. These changes in the stability of the 3D structure can alter the expression patterns of miRNA. SNPs may also affect specific modifications on RNA nucleotides, which is important for their further localization and processing.

Polymorphism studies on miRNAs showed that decreased seed sequence ratios are seen in the human population, but different populations have a high frequency of specific polymorphisms in *MIR-146A* gene.[11] miR-146a is mainly expressed in immune cells, a SNP from G to C change in this miRNA, disturbs DROSHA cleavage in biogenesis, and this polymorphism was linked to a higher risk for multiple sclerosis in women along with a lower risk of ankylosing spondylitis and psoriasis.[11,14,15] This polymorphism is one of the most studied ones and has many ties with different cancer types and inflammatory diseases. miRNA SNP research on Mendelian disorders highlighted the many miRNAs germ line mutation's role in familial diseases. Non-syndromic hearing loss was one of the first miRNA-related Mendelian disorder in which many candidate genes showed no mutation correlations but only mutant miR-96's role could explain the disease progression.[16] Different studies on atherosclerosis phenotype differences, SNPs effecting pri-miRNA formation and pre-miRNA processing in a variety of populations causing the phenotypic differences in stages of atherosclerosis.[17] A study on miR-146a polymorphism found that in Behçet's cases, decreased homozygous rs2910164 CC genotype and C allele effecting the miR-146 level reduction in Chinese BD patients.[18] A meta-analysis study confirms this polymorphism effect on BD susceptibility in Caucasians.[19] miR-499 is another well studied miRNA, especially in autoimmune diseases. One polymorphism

in this miRNA, rs3746444, is associated with an elevated risk of autoimmune diseases in Caucasian and Asian populations.[20]

miR-197-3p is encoded from the *MIR197* gene from chromosome one and its dysregulation profile is mostly observed in different cancer studies.[21-23] New research broadens the miR-197's role in inflammatory processes[24,25] such as miR-197-3p's effect on endothelial cells in Takayasu disease.[26] In our previous study, miR-197-3p was found to be decreased (FCH: -2,22) in M694V/M694V homozygotes FMF patients compared to healthy controls.[27] Our group also showed that miR-197-3p, which directly targets the interleukin-1beta (IL-1β) receptor type I gene (*IL1R1*), is differentially expressed in FMF patients.[28] In this paper, possible genetic variations of *MIR197* gene were searched in FMF patients to explain the altered miR-197-3p expressions between patients and controls.

Materials and Methods

Study Group

The study consisted of six FMF patients who were homozygous for the M694V mutation (M694V/M694V)[27] who showed a severe phenotype and 12 healthy individuals as a control group. The study group consisted of adults between 19-48 years of age. All patients displayed a typical phenotype with attacks of 12-72 hours duration. 10 mL of blood was taken from the individuals into DNA blood tubes. Blood samples were taken for sedimentation and determination of C-reactive protein and complete blood count levels from all individuals in the study group. Since the homozygote patients had a severe disease phenotype, five of them were also receiving anti-IL-1β therapy. Median ages were 28 years for homozygote patients and 34 years for healthy controls. Written consent was acquired from the patients and controls involved in the study and approved by national ethics committee (23.01.2013 date, GO13/54-07 number - Hacettepe University Non-invasive Clinical Research Ethics Committee).

Polymerase Chain Reaction

Polymerase chain reaction (PCR) primers used to amplify the *MIR197* gene region are provided in Table 1. Reactions were carried out in the Gene Amplification PCR System 9700 (Applied Biosystem). The sizes of the amplification products and the specificity of the amplification were determined by agarose gel electrophoresis.

DNA Sequencing

After the successful amplification of *MIR197* gene region, DNA sequencing studies were carried out. The amplicons were purified using Promega Wizard™ SV Gel and PCR Cleanup System (Fisher Scientific, CA) according to the manufacturer's instructions. Sequencing was performed using the BigDye Terminator kit v. 3.1 and cleaned up with BigDye XTerminator v. 3.1 (Applied Biosystems, Foster City, CA). The purified products of the cycle sequencing were analysed on the ABI 3130 Genetic Analyser (Applied Biosystems). DNA sequence analysis results were analyzed with the Chromas (version 2.33) program in Applied Biosystems 3130 Genetic Analyzer.

Analysis of GWAS Data

The GWAS data (Harvard Dataverse, V2; TurkishA1A2.txt)[29] was analyzed and *MIR197* locus was screened by using R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Results

To investigate the possibility that the decreased expression of miR-197-3p identified in our cohort of severe phenotype patients were due to variations the in *MIR197* locus, sequence analysis was performed for the *MIR197* gene in six *M694V/M694V* patients and 12 control individuals.

According to the results of sequence analysis of miR-197-3p gene of homozygous (*M694V/M694V*) individuals; no genetic variants were observed in either group (Figure 1).[30]

To expand our search for *MIR197* SNPs to a larger population, we analyzed open access GWAS data on 1011 healthy Turkish people *MIR197* SNPs, and consistent with our study results, no SNPs were observed.[29]

Discussion

In this study, *MIR197* gene variations were analyzed in severe FMF patients with low expression levels of miR-197-3p. Since variations in miRNA genes can affect the functionality or expression levels of miRNA, the fact that no difference was observed between patient and control

Table 1. Primer sequences of *MIR197* gene region for PCR

Exon	Forward (F) primer	Reverse (R) primer	PCR product (bp)
MIR197	5'GCCCAACACCGAAATCCTT 3'	5'ACGGTGAGACATAACAGCA 3'	181

bp: Base pair, PCR: Polymerase chain reaction

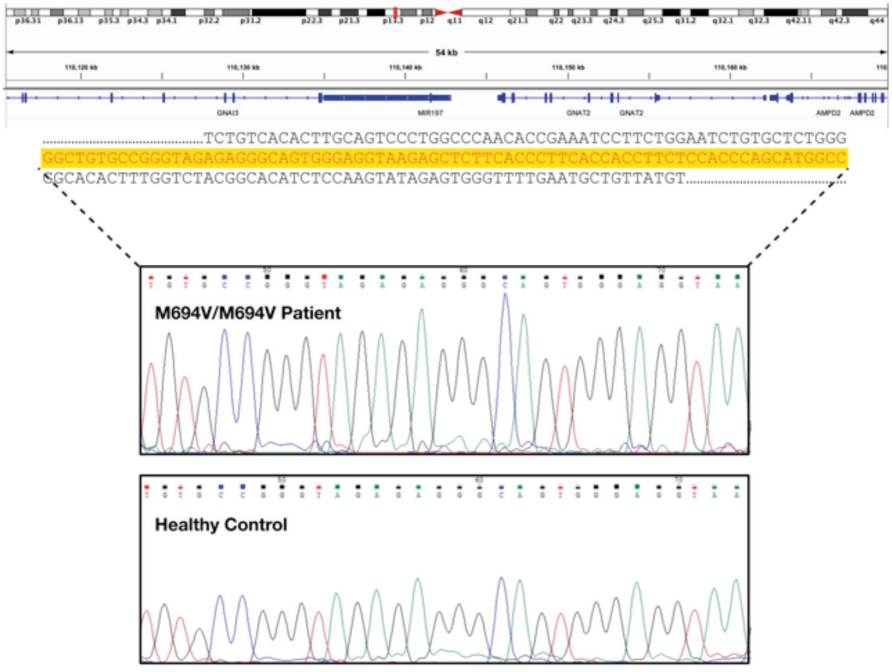

Figure 1. Sequencing results of the *MIR197* locus. In the chromosomal region, *MIR197* gene was showed in blue and the gene sequence was highlighted with yellow[30]

individuals with sequence analysis showed that the miRNA expression differences we identified in our patients were not due to genetic variations in the *MIR197* coding region.

The dysregulation of miRNAs can lead to the disruption of biological pathways and the development of various diseases. Studies have shown that miRNAs are closely related to the pathogenesis of many autoinflammatory and autoimmune diseases.[31]

Genetic variations in miRNA sequences are very important as they can affect both the expression levels and the functionality of miRNA. SNPs may influence miRNA expression at every step of the miRNA biogenesis. It is known that differences in the sequence may cause damage to the hairpin stem structure and subsequent disruption of its processing, resulting in the degradation of miRNA.

Due to their variety of functions, the regulation of miRNA activity is a complex process involving various mechanisms. These mechanisms include transcriptional regulation, epigenetic modifications, RNA editing, and regulation by other proteins or non-coding RNAs.[32] DNA methylation and histone modifications are transcriptional regulation mechanisms that can affect miRNA expression levels by controlling the transcription machinery's access to the miRNA gene locus. Post-transcriptional regulatory mechanisms include alternative splicing and enzymatic modifications of RNA. Additionally, alternative Drosha cutting generates different types of pre-miRNA products, increasing their diversity and, in some situations, leading to the degradation of specific miRNAs.[33] These modifications can also modulate the half-life of miRNAs for the regulation of their activity in a time-wise manner. Non-coding RNAs like lncRNA may capture newly synthesized pri-miRNAs or regulate transcription to alter miRNA expression. Every cellular molecule involved in miRNA biogenesis and function can be responsible for the altered miRNA profile in disease situations.[34] Genetic code differences of these epigenetic molecules and target mRNA 3'UTR sites may lead to increased or decreased synthesis, altered functions, splicing variants, and disrupted protein-protein interactions.

Conclusion

In this study, we determined that the previously demonstrated dysregulation in miR-197-3p expression seen in FMF patients is not explained by variations in the *miR-197* gene locus. We also did not find any SNPs upon analyzing open-access GWAS data on the Turkish population.[29] To our knowledge, ours is the first study to investigate *MIR197* variations in autoinflammatory diseases. This study is limited by the small sample size, and only the coding region was analyzed. Increasing the sample size and sequencing the promoter region may provide better information about the altered expression of miR-197-3p in FMF patients. Our results suggest that further investigation of other epigenetic factors such as promotor methylation, histone modifications, other non-coding RNAs, and alterations in post-transcriptional processing may be implicated in the differential expression of miR-197-3p.

Ethics

Ethics Committee Approval: Approved by national ethics committee (23.01.2013 date, GO13/54-07 number - Hacettepe University Non-invasive Clinical Research Ethics Committee).

Informed Consent: Written consent was acquired from the patients and controls involved in the study.

Authorship Contributions

Concept: L.K., Design: L.K., Y.Z.A.U., Data Collection or Processing: E.N., L.K., Analysis or Interpretation: E.N., B.Ş., Literature Search: E.N., Writing: E.N., B.Ş., Y.Z.A.U.

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