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RESEARCH

Identification of variations in genes encoding ATP-dependent potassium channel proteins in patients with primary Raynaud's phenomenon

Primer Raynaud fenomeni olgularında ATP-bağımlı potasyum kanal proteinlerini kodlayan genlerde rastlanan varyasyonların belirlenmesi

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Abstract

Purpose: Primary Raynaud's phenomenon (PRP) is a vascular disorder characterized by recurrent vasospastic response of the fingers and toes to cold or stress. ATP-sensitive potassium (K_{ATP}) channels are widely distributed in vasculatures, and play an important role in the vascular tone regulation. The major vascular isoform of K_{ATP} channels is composed of Kir6.1/SUR2 (KCNJ8/ABCC9). It would be important to determine whether variations of K_{ATP} genes related to PRP is thought to be associated with vasospasm. It is believed that the studies describing mechanisms involved in the pathogenesis of inherited vascular disorders offers the best opportunity for investigation of the early stages of pathogenicity and diagnosis of PRP and associated other diseases. In this study we aim to investigate possible association between genetic variations observed in K_{ATP} channel coding genes and vasospasm associated with PRP.

Materials and Methods: In our study; the cases with PRP, the relation between the variation in the KCNJ8/ABCC9 genes (S422L/V734I or rs72554071/rs61688134) was examined. 50 subjects who were diagnosed with PRP (patient group) and 50 healthy subjects (control group) were included in the study. Variations were determined using the Tetra-Primer Amplification Refractory Mutation System-Polymerase Chain Reaction (T-ARMS PCR) method.

Results: Of the individuals in the patient and control group included in the study, 21 were male and 29 were female. The mean age of the patients was 25.7±3.36 years, and the mean age of the control group was 25.9±3.44

Öz

Amaç: Primer Raynaud fenomeni (PRF), el ve ayak parmaklarının soğuk veya stresle tekrarlayan vazospastik tepkisi ile karakterize vasküler bir hastalıktır. ATP-bağımlı potasyum (K_{ATP}) kanalları vaskülatürlerde yaygın olarak dağılır ve vasküler tonusun düzenlenmesinde önemli bir rol oynarlar. K_{ATP} kanallarının ana vasküler izoformu Kir6.1/SUR2'den (KCNJ8/ABCC9) oluşmaktadır. PRF ile ilgili K_{ATP} gen varyasyonlarının vazospazm ile ilişkili olup olmadığının belirlenmesi önemli olacaktır. Kalıtsal vasküler bozuklukların patogenezinde yer alan mekanizmaları açıklayan çalışmaların, patojenitenin erken evrelerinin araştırılması ve PRF ile ilişkili diğer hastalıkların teşhisi için en iyi fırsatı sunduğuna inanılmaktadır. Bu çalışmada, K_{ATP} kanalı kodlayan genlerde gözlenen genetik varyasyonlar ile PRP ile ilişkili vazospazm arasındaki olası ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmamızda; PRF'li olgularda KCNJ8/ABCC9 genlerindeki (S422L/V734I veya rs72554071/rs61688134) varyasyon arasındaki ilişki incelenmiştir. Çalışmaya PRF tanısı konan 50 kişi (hasta grubu) ve 50 sağlıklı kişi (kontrol grubu) dahil edilmiştir. Varyasyonlar Tetra-Primer Amplifikasyona Dirençli Mutasyon Sistemi-Polimeraz Zincir Reaksiyon (T-ARMS PCR) yöntemi kullanılarak belirlenmiştir.

Bulgular: Çalışmaya dahil edilen hasta ve kontrol grubundaki bireylerin 21'i erkek ve 29'u kadındı. Hastaların yaş ortalaması 25.7±3.36, kontrol grubunun yaş ortalaması 25.9±3.44 yıldı. KCNJ8/ABCC9 genlerinin genotip ve alel dağılımı ile PRF hastalığı arasında anlamlı bir ilişki

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years. No significant relationship was found between PRP disease and genotype and allele distribution of KCNJ8/ABCC9 genes.
Conclusion: This study presented the first findings about KCNJ8/ABCC9 gene variations in the Turkish population and may lead to future studies. Studies involving a higher number of cases and more mutations will be able to show whether there is a relationship between K_{ATP} channels and PRP and contribute to the elucidation of PRP pathogenesis in terms of genetic factors.
Keywords:.Primary Raynaud's phenomenon, K_{ATP} channel proteins, KCNJ8/ABCC9 genes, S422L/V734I (rs72554071/rs61688134) variants.

bulunamamıştır.
Sonuç: Bu çalışma Türk popülasyonunda KCNJ8/ABCC9 gen varyasyonları ile ilgili ilk bulguları sunmuş olup, ileride yapılacak çalışmalara öncülük edebilecektir. Daha fazla vaka ve daha fazla mutasyon içeren çalışmaların K_{ATP} kanalları ile PRF arasında bir ilişki olup olmadığını gösterebilecek ve genetik faktörler açısından PRF'nin patogenezinin aydınlatılmasına katkı sağlayacaktır.

Anahtar kelimeler: Primer Raynaud fenomeni, K_{ATP} kanal proteinleri, KCNJ8/ABCC9 genleri, S422L/V734I (rs72554071/rs61688134) varyantları

INTRODUCTION

Raynaud's phenomenon (RP) is a disorder that often involves the upper extremities, less frequently the lower extremities, and is characterized by cyanosis and redness as a result of vasospasm of digital arteries. RP is classified as primary and secondary form. It is named as the Primary Raynaud's Phenomenon (PRP) when there is no underlying disease, and as the secondary form (Raynaud's Syndrome) when associated with an underlying disease[1]. The distinction between these two forms is important, as the prognosis, severity and treatment of both forms differ[2]. The prognosis of the primary form is generally benign and gangrene or tissue loss is very rare[3]. In the secondary form, the presence of causative or concomitant diseases can affect many organs and systems simultaneously[4]. Although pain and discoloration occur as a result of cold and emotional stress, the etiological factors in RP have not been fully elucidated[1]. Although important investigations have been made on the pathophysiological mechanisms of RP, there is limited data describing the underlying abnormal vasospasm. Abnormalities in the vessel wall, vascular tone, neural control and mediators of the circulatory system have been predicted and studied as part of the pathophysiology of this phenomenon. The tendency to vasospasm may occur as a result of acquired changes in hemostasis or the interaction between acquired and genetic factors, in addition to genetic factors[5].

K_{ATP} channels were firstly identified in cardiac myocytes in the heart by Noma. In studies by Noma, K^+ channels, which are normally inhibited by ATP, have been opened by metabolic inhibition by cyanide. Therefore, this channel is called K_{ATP}[6]. K_{ATP} channels are octameric protein complexes containing 4 pore-forming Kir6 subunits and 4 accessory sulfonylurea receptor (SUR) subunits. Two Kir6.x genes (KCNJ8 for Kir6.1, and KCNJ11 for Kir6.2) and two SUR genes (ABCC8 for SUR1 and ABCC9 for SUR2A and SUR2B) have been identified[7]. K_{ATP}, including Kir6.1 and SUR2 proteins, are critical in regulating vascular tone, particularly in coronary arteries. Although K_{ATP} channels have been studied extensively in all aspects of diseases such as Type II diabetes, hyperinsulinism and neonatal diabetes, studies on hereditary vascular disorders have not reached the same level. Studies on Kir6.1/SUR2 genes have attracted attention on vascular functions. Mouse models in which these genes are knockout draw attention to the critical roles these subunits play in the cardiovascular system, particularly in coronary circulation[8, 9]. Studies on possible mutations in the vascular K_{ATP} channel protein (Kir6.1/SUR2) genes (KCNJ8/ABCC9) and the mechanisms of action of these mutations are limited. In existing ones, the identified mutations are insufficient. The hypothesis of this study is to investigate there is a relationship between variations on the genes expressed for K_{ATP} channel proteins and PRP. Genetic association studies on disorders whose cause or mechanism is not clearly explained contribute to shedding light on the genetic basis of such disorders. In this study, we have tried to characterize variations in genes encoding K_{ATP} channel in patients with PRP associated with vasospasm.

MATERIALS AND METHODS

Sample

The patient group of our study consists of 50 patients who applied to Mersin University Faculty of Medicine, Department of Cardiovascular Surgery and Mersin State Hospital Cardiovascular Surgery, and

were diagnosed with PRP according to the inclusion and exclusion criteria as a result of routine examinations. Inclusion criteria for the study group: Patient between the ages of 16 and 45 who had been admitted to the cardiovascular surgery outpatient clinic in 2015 and 2016 and diagnosed with PRP. The International Consensus Criteria for the Diagnosis of Raynaud's Phenomenon was used to diagnose PRP. The control group was formed according to the exclusion and inclusion criteria. The control group consists of 50 healthy individuals who applied to Mersin State Hospital Cardiovascular Surgery outpatient clinic with vague complaints (extremity pain, headache, feeling unwell, etc.) and with a normal physical examination. Inclusion criteria for the control group: Individuals between the ages of 16 and 45 who had been admitted to the cardiovascular surgery outpatient clinic in 2015 and 2016 with normal physical examination. Exclusion criteria (for both groups): Patients with one or more of the following diseases were excluded from the study: Connective tissue diseases, Buerger's disease (thromboangiitis obliterans), Digital injury sequelae, Chronic drug use (including antiaggregants and anticoagulants), Thoracic outlet syndrome, Carpal tunnel syndrome, Anaemia, Atherosclerosis, Malignancies, Chronic renal failure, Hepatitis associated vasculitis.

The patients were followed up and treated by physicians working in the Department of Cardiovascular Surgery. Genetic analyzes were performed by faculty members and assistants in the laboratories of Pharmaceutical Biochemistry Department. In the study, power analysis was used to determine the number of samples. According to analysis results, it was planned to include at least 90 individuals in the present study by taking the opinion of the Department of Biostatistics and Medical Informatics. 65 people were reached in the patient group. While 10 people were excluded from the study due to exclusion criteria, 5 people refused to participate in the study. 70 people were reached in the control group, but 20 people refused to participate in the study. As a result, the study completed with a total of 100 individuals, 50 individuals in both patient and control groups.

Our study was approved by Mersin University Clinical Research Ethics Committee with the Board Decision dated 22/10/2015 and numbered 2015/324. The study was conducted following the principles of the Declaration of Helsinki and written informed consent was obtained from all included participants.

Genetic analysis

Bibliographic research was conducted using NCBI (https://www.ncbi.nlm.nih.gov/) sub-databases. PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), NCBI's articles, books and so on, is the sub-database in which all relevant information has been reached with this database. The OMIM (http://www.ncbi.nlm.nih.gov/omim) database provided access to information about the genes to be studied and the disease phenotype associated with these genes. In the human genome and other genomes, the most common diversity is single nucleotide polymorphisms (SNP), and detailed information on gene-related SNPs was obtained using the dbSNP (https://www.ncbi.nlm.nih.gov/snp/) sub-database[10].

SNP information

Variations rs72554071 (S422L, NG_041794.1: g.14089C>T) and rs61688134 (V734I, NG_012819.1: g.77219G> A) in the KCNJ8 and ABCC9 genes respectively were studied.

S422L (rs72554071): The amino acid change on the protein sequence causes the polymorphic variation of Serine (S) and Leucine (L) at position 422 (Figure 1).

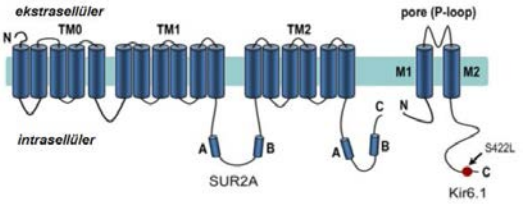

Figure 1. The location of the S422L variation on the K_{ATP} channel[11]

V734I (rs61688134): The amino acid change on the protein sequence is at point 734. Amino acid exchange is the replacement of Valine (V) at position 734 and Isoleucine (I) (Figure 2).

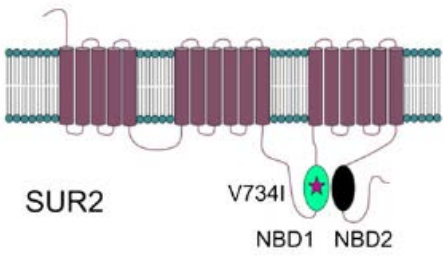

Figure 2. SUR2's topology model (NBD1 and NBD2 are shown in green and black, respectively. V734I in NBD1 is represented as stars.)[12]

Primer design

In the study, primers of the T-ARMS methodology to be used to determine the presence of alleles of both SNPs (S422L and V734I or rs72554071 and rs61688134) were designed using the online primer design tool PRIMER1 (http://primer1.soton.ac.uk/primer1.html) (Table 1)[13]. PCR successes, chemical and kinetic properties and specificity of the designed primers were checked using the Vector NTI 11.0 package program, online IDT and NCBI-BLAST tools, respectively.

Table 1. Designed primers

SNP	System	Primer sequence (5' –3')	Allele	Tm (°C)	Amplicon Length (bp)
S422L (rs72554071)	Forward inner primer	TCCAGAAGGAAATCAAAACACCTT	T	60.1	352
	Reverse inner primer	GGGTTATCTTGCTGTCATGATTACG	C	64.2	388
	Forward outer primer	AAACTACTGGCATCACCACACAAG		63.5	692
	Reverse outer primer	TTTTGTGCTCAAGGCCTGTTACTA		61.8	
V734I (rs61688134)	Forward inner primer	GAACATTGTTTGCTATACTTGCATTA	A	60.0	193
	Reverse inner primer	AAAGAAGGCTCAGATTCATTGAC	G	59.3	224
	Forward outer primer	AGCAATTATTTCCTAGCTGATGAA		58.4	369
	Reverse outer primer	GTTGGAAATATGCTAGCACACTTT		60.1	

Tm: Temperature of melting
SNP: Single nucleotide polymorphisms

Genomic DNA extraction

DNA was isolated from Ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood using the High Pure PCR Template Prepation Kit (Roche, Mannheim, Germany, Catalog#11-976-828-001) according to the manufacturer's instructions.

Tetra primer ARMS PCR

T-ARMS PCR method was used to detect the variations of S422L (rs72554071) and V734I (rs61688134) of KCNJ8 and ABCC9 genes, respectively. In the T-ARMS methodology, four different primers are used to identify alleles. Two of these primers called "outer" Frwouter" and "Revouter" control amplicon, "Frwouter" and "Revinner" primers wildtype amplicon, "Revouter" and "Frwinner" primers amplify mutant amplicon. By placing outer primers at different distances (asymmetric) from the polymorphic nucleotide, the amplicons representing mutant and wild alleles can be distinguished by different sizes in agarose gel electrophoresis since they will be of different lengths[14].

The quantities of PCR components used in the T-ARMS methodology are shown in Table 2.

SimpliAmp™ Thermal Cycler device was used for PCR (Thermo Fischer Scientific, Massachusetts, ABD Catalog#A24811).

Termocycler program of S422L (rs72554071): The

samples were incubated for 4 min at 95˚C to ensure the melting of genomic DNA, followed by 32 cycles of 30 sec denaturation (94˚C), 45 sec annealing (61˚C), and 45 sec extension (72˚C), and an additional 8 min extension at 72˚C at the end of the 32 cycles.

Termocycler program of V734I (rs61688134): The samples were incubated for 4 min at 95˚C, followed by 32 cycles of 30 sec denaturation (94˚C), 45 sec annealing (50˚C), and 45 sec extension (72˚C), and an additional 8 min extension at 72˚C at the end of the 32 cycles.

The PCR based Tetra Primer (T-ARMS PCR) methodology was used to detect possible variations of S422L (rs72554071) and V734I (rs61688134) of the KCNJ8 and ABCC9 genes, respectively. In the T-ARMS methodology, four different primers are used to identify alleles. In the presence of all four primers in the reaction tube, outer primers, called "Frwouter" and "Revouter," used to amplify the control amplicon, while "Frwouter" and "Revinner" primers amplify the wildtype amplicon, and "Revouter" and "Frwinner" primers amplify the mutant amplicon. By placing the outer primers at different distances (asymmetrically) from the polymorphic nucleotide, the amplicons representing the mutant and wild alleles can be distinguished by different sizes on agarose gel electrophoresis, as they will be of different lengths14. The quantities of PCR components used in the T-ARMS methodology are shown in Table 2. The SimpliAmp™ Thermal Cycler device was used to run PCR reactions (Thermo Fischer Scientific, Massachusetts, USA Catalog#A24811).

Termocycler program for S422L (rs72554071): The samples were incubated for 4 minutes at 95˚C to ensure the melting of entire genomic DNA, followed by 32 cycles of 30 seconds denaturation (94˚C), 45 seconds annealing (61˚C), and 45 seconds extension (72˚C), and an additional 8 minutes extension at 72˚C at the end of the 32 cycles.

Termocycler program for V734I (rs61688134): The samples were incubated for 4 minutes at 95˚C, followed by 32 cycles of 30 seconds denaturation (94˚C), 45 seconds annealing (50˚C), and 45 seconds extension (72˚C), and an additional 8 minutes extension at 72˚C at the end of the 32 cycles.

Gel Electrophoresis

PCR products were analyzed by 2% agarose gel electrophoresis, in 1X Tris-Borate-EDTA (TBE) buffer, containing ethidium bromide. The lengths of the amplicons were determined using DNA ladder (100 bp DNA Ladder, Includes Gel Loading Dye, Purple-Blue-Orange (6X), NEW ENGLAND Biolabs Inc., Massachusetts, USA Catalog # N3231S, Lot # 1091506). The images were visualized and photographed under UV transillumination (DNR Bio-Imaging System, Jerusalem, Israel).

Table 2. Component quantities to be used in T-ARMS PCR method

Reagent	Stock Concentration	Final Concentration	Volume (µL)
Taq Polymerase	5 U/µL	1.25 U	0.25
DNA Sample	100 ng/µL	200 ng	2

Mastermix

Reagent	Stock Concentration	Final Concentration	Volume (µL)
PCR Buffer	10 X	1 X	2.5
Mg^{2+}	25 mM	1.5 mM	1.5
dNTP	2 mM	0.2 mM	2.5
Primer 1	10 µM	0.3 µM	0.8
Primer 2	10 µM	0.3 µM	0.8
Primer 3	10 µM	0.3 µM	0.8
Primer 4	10 µM	0.3 µM	0.8
Total			9.7
ddH_2O			13.05
Mastermix Total			22.75
Total			25.0

PCR: Polymerase Chain Reaction
Mg2+: Magnesium ion
dNTP: Deoxyribonucleotide triphosphate
ddH2O: Double-distilled water

Statistical analysis

Statistical calculations were performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA) on the MS Windows operating system. The t-test was used to compare demographic data, which is shown as mean ± standard deviation. No statistical analysis was performed because there was no allelic variation in the genetic analysis.

RESULTS

50 subjects who were diagnosed with PRP (patient group) and 50 healthy subjects (control group) were included in the study. The patient group consisted of 21 male and 29 female subjects. Table 3 summarizes demographic data showing the average age and percentages of male (M) and female (F):

Table 3. Demographic data of individuals participating in the study

Variable		Patient	Control
Gender (%)	M	42	42
	F	58	58
Average Age±SD		25.7±3.36	25.9±3.44

M: Male; F: Female; SD: Standard Deviation

According to the T-ARMS methodology, the KCNJ8 gene was determined to be homozygous for C allele in both control and patient groups. Agarose gel electrophoresis image of PCR products for the KCNJ8 gene shows that the electrophoretic band with a length of 692 bp represents the positive control amplicon used to check the success of PCR, and the 388 bp length represents the wildtype amplicon (Figure 4). The genetic analysis found the CC genotype in all patients and controls, but the CT and TT genotypes were not present. These results did not reveal any significant relationship between PRP disease and the distribution of the S422L genotype. When the distribution of alleles was considered, only the C allele was present in the control and patient groups, while the T allele was not detected. Therefore, no significant relationship was found between PRP disease and the distribution of S422L alleles.

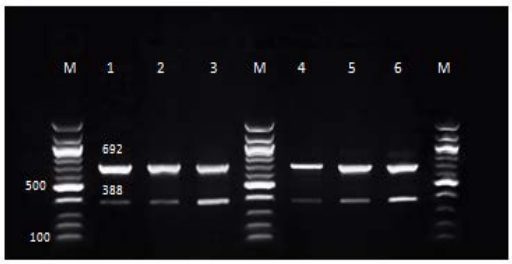

Figure 4. Image of amplified PCR products on agarose gel electrophoresis to identify alleles of the variation S422L (rs72554071) (M: Marker; 1, 2, 3, 4, 5, 6: Cases)

According to T-ARMS methodology, it was determined that the wild-type G allele was homozygous (GG) in both the control and patient groups. The agarose gel image showing the rs61688134 variation in the ABCC9 gene reveals that the 369 bp band represents the control amplicon used to confirm the success of PCR, and the 224 bp band represents the wildtype amplicon (Figure 5). The genetic analysis found that the GG genotype was present in all patients and controls, but the GA and AA genotypes were not detected. These results did not indicate any significant relationship between PRP disease and the distribution of the V734I genotype. When the distribution of alleles was considered, only the G allele was observed in both the control and patient groups, while the A allele was not detected. Therefore, no significant relationship was found between PRP disease and the distribution of V734I alleles.

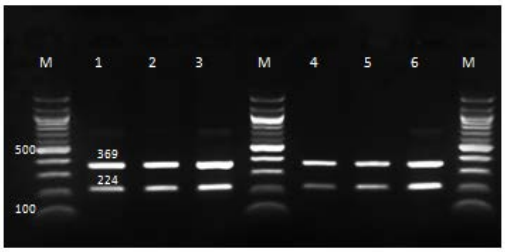

Figure 5. Image of amplified PCR products on agarose gel electrophoresis to identify alleles of variation V734I (rs61688134) (M: Marker; 1, 2, 3, 4, 5, 6: Cases).

Table 4 shows genotype and allele distributions of KCNJ8 S422L (rs72554071), ABCC9 V734I (rs61688134) gene variations.

Table 4. Genotype and allele distributions of KCNJ8 S422L (rs72554071), ABCC9 V734I (rs61688134) Gene Variations

	Genotype and allele	Patient (n=50)	Control (n=50)
KCNJ8 S422L (rs72554071)	CC	50	50
	CT	0	0
	TT	0	0
	C	50	50
	T	0	0
ABCC9 V734I (rs61688134)	GG	50	50
	GA	0	0
	AA	0	0
	G	50	50
	A	0	0

n: The number of participants
A: Adenine, G: Guanine, C: Cytosine, T: Thymine

DISCUSSION

PRP is a disorder characterized by redness and cyanosis as a result of vasospasm of the digital arteries[1]. Vasospastic effect is a defining characteristic of PRP, which is classified as a vascular disease. K_{ATP} channels, which are widely distributed in vasculature and play a significant role in regulating vascular tone, have been the focus of study using knockout mouse models. For example, the Kir6.2 knockout mouse model exhibits hyperinsulinemia in humans and lacks insulin-dependent glucose secretion[15]. Similarly, the SUR1 knockout mouse model exhibits similar characteristics as the Kir6.2$^{-/-}$ model. The Kir6.1/SUR2 knockout mouse model has drawn attention to the important role of these genes in the cardiovascular system, particularly in coronary circulation[8,9]. The study of S422L/V734I variations in the KCNJ8/ABCC9 genes, which encode these proteins, was motivated by the analysis of these knockout mouse models and the prominence of Kir6.1/SUR2 proteins in vascular functions.

Although K_{ATP} channels have been extensively studied in a variety of diseases, such as type II diabetes, hyperinsulinism, and neonatal diabetes, research on hereditary vascular disorders has not reached the same level. Studies on possible mutations in the vascular K_{ATP} channel protein genes (Kir6.1/SUR2) and the mechanisms of action of these mutations (KCNJ8/ABCC9) are limited. PRP has been studied in relation to a number of diseases, including cardiovascular and cardiac involvement, migraine, H syndrome, hereditary vascular retinopathy, and scleroderma, as well as with polymorphism studies on different genes.

Shemirani et al. studied the genetic risk for clotting factors that could predispose to abnormal microvascular thrombosis in patients with RP in its primary form. They studied 200 controls and 200 patients of Hungarian origin. 158 of the patients were female and 42 were male, with an average age of 42.47 ± 13.7 years. They investigated variations in the Factor V G1691A (FVLeiden), prothrombin G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T genes. As a result of the study, they found that prothrombin G20210A variations of FVLeiden had no effect on the etiology of RP in its primary form. However, the frequency of C677T variation in the homozygous MTHFR gene was significantly lower in patients compared to the control group[16]. Takats et al. examined the prevalence and possible association of hereditary prothrombotic risk factors in patients with PRP and migraines. They included 142 patients (101 females, 41 males) with both migraines and PRP and 58 patients (57 females, 1 male) with only PRP in their study. They used Real-time PCR to detect variations in the protombin G20210A, MTHFR C677T, and FXIII-A V34L genes. As a result of their study, they determined that the C677T variation in the MTHFR gene may have a significantly higher allele frequency in patients with PRP and migraines. FVLeiden, G20210A, and V34L variations did not have a significant effect on the development of migraines in PRP[17]. Gaudy-Marqueste et al. observed a heterozygous variation in exon 9 of the Lamin B Receptor (LBR) gene in a 76-year-old Caucasian woman with RP. Studies on the patient's lymphoblastoid cells showed no abnormalities, but the patient's fibroblasts showed decreased LBR and decreased levels of lamin proteins, as well as spotted chromatin and dysmorphic nuclei. As a result of these findings, the authors showed that the Arg372Cys (R372C) variation had a dominant-negative effect on LBR interacting proteins, likely due to decreased stabilization of the mutant protein and increased proteasome-mediated degradation[18]. Fujita et al. demonstrated the presence of a new mutation in the SLC29A3 gene in a 48-year-old male patient with H syndrome and consanguineous parents. This patient also reported massive skin involvement, retroperitoneal fibrosis, and RP. After a long follow-up period, the patient's genomic DNA was obtained, and a homozygous mutation was detected at exon 5, where c.625G > A resulted in a glycine-to-arginine conversion. This mutation was not detected in 50 healthy individuals[19].

Ermiş et al. investigated the presence of the KCNJ8 gene S422L variation in individuals diagnosed with Early Repolarization Syndrome (ERS). The early repolarization and control groups were studied in two groups. There were 78 males and 22 females in the group diagnosed with early repolarization, with an age range of 33.1 ± 9.7 years. In the control group, there were 70 males and 30 females, with an age range of 35.8 ± 9.9. The variation was absent in the patient group and was found in one sample in the control group. There was no statistically significant relationship between ERS and the KCNJ8 gene S422L variation[20]. Fedele et al. investigated the role of genetic polymorphisms in ion channels in the pathology of ischemic heart disease and coronary microvascular dysfunction. In the study, 155 patients

with coronary artery disease were in group 1, 146 patients with endothelium-dependent and/or non-obstructed microvascular dysfunction were in group 2, and 41 patients with normal coronary arteries were in group 3. Variations in the KCNJ8/KCNJ11 genes from K_{ATP} channel protein genes were studied. No significant correlation was found between the S422L variation in the KCNJ8 gene and the three groups studied[21]. In the study conducted by Veeramah et al., the S422L variation in the KCNJ8 gene was investigated in relation to the high frequency J wave syndrome in Ashkenazi Jews. In this study, 722 people genotyped the S422L variant in the European, Middle Eastern, Ashkenazi Jewish, and Ashkenazi non-Jewish populations. As a result, it was found that the S422L variation was almost nonexistent in the entire population except Ashkenazi Jews, where 23 (7.9%) of 291 Ashkenazi Jews were heterozygous and the overall allele frequency was 4%. The only exception to this result was the heterozygous presence of the S422L allele in 3 of the 48 Romans with non-Ashkenazi Jews. However, the allele frequency in Ashkenazi Jews was significantly higher than in other non-Jewish populations and in previous controls, and the change was recorded as heterozygous[22]. Minoretti et al. investigated whether the V734I variation encoded in the ABCC9 gene is associated with myocardial infarction (MI). The study included 584 patients under 60 years old and 873 control groups. As a result of the genetic studies, they provided the first important evidence that the 734I allele in the ABCC9 gene could affect susceptibility to early-onset MI in the population[23]. Smith et al. examined the pathophysiological mechanism of the V734I variation in exon 17 of the ABCC9 gene, which is estimated to create a 6.4 times higher risk of Acute Myocardial Infarction (AMI) before age 60. They found that the 734I allele in ABCC9 could affect susceptibility to AMI by disrupting the vascular response, and that this allele found in an AMI-related ion channel gene was the first human mutation[24].

Based on the results described above, it can be concluded that the S422L and V734I variations in the KCNJ8 and ABCC9 genes, respectively, may not be significantly associated with the development of PRP in the study population. This conclusion is based on the fact that the CC genotype was found in all patients and controls for the S422L variation in the KCNJ8 gene, and the GG genotype was found in all patients and controls for the V734I variation in the ABCC9 gene. When the distribution of alleles was considered, only the C allele was present in the control and patient groups for the S422L variation, and only the G allele was present in the control and patient groups for the V734I variation. No significant relationship was found between PRP and the distribution of these variations.

One limitation of our study is that it can not represent the profile of the entire country, as data from only two centers were used. Another limitation is that the number of cases in the disease and control groups is relatively low. As a result of the genetic analysis, we did not find these two mutations in any of the cases. However, the number of samples studied is insufficient to conclude that there is no relationship between these mutations and PRP. Therefore, we believe that studies involving a higher number of cases and more mutations will be able to shed more light on whether there is a relationship between K_{ATP} channels and PRP and contribute to a better understanding of the genetic factors involved in PRP.

It is worth noting that these conclusions are based on a relatively small sample size, and further studies with larger sample sizes may be necessary to confirm these findings. Additionally, factors such as environmental influences, lifestyle, and other genetic factors may also be involved in the development of PRP, and these should also be considered in future studies.

Yazar Katkıları: Çalışma konsepti/Tasarımı: AEY, MA, MY; Veri toplama: MA, MY, ÖV, HS, AEY; Veri analizi ve yorumlama: MA, AEY; Yazı taslağı: MA, MY, ÖV, NS, AEY; İçeriğin eleştirel incelenmesi: MA, MY, ÖV, NS, AEY; Son onay ve sorumluluk: MA, MY, ÖV, NS, AEY; Teknik ve malzeme desteği: ÖV, NS; Süpervizyon: AEY; Fon sağlama (mevcut ise): yok.
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