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Research Article

Impact of *ABCG5/G8* Gene Polymorphisms on Sitosterolemia Phenotypes in a Group of Turkish Patients

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Keywords

- Sitosterolemia; ABCG5/G8 Gene; Polymorphisms; Hypercholesterolemia

Abstract

Background; Sitosterolemia is a rare, autosomal-recessive lipid storage disease characterized by xanthoma, premature atherosclerosis, and hematologic abnormalities. The purpose of this study was to investigate the effect of adenosine triphosphate-binding cassette genes (*ABCG) 5 and 8* polymorphisms on sitosterolemia phenotypes in a group of Turkish patients.

Methods: This cross-sectional study included 90 individuals with premature atherosclerosis (n=29), xanthoma (n=32), or hematologic abnormalities (n=29). All underwent sterol level measurement by gas chromatography. All 90 individuals and 157 age-matched healthy controls were tested for two polymorphic variants (c.1895T>C and c.161A>G) of the *ABCG8* gene and one polymorphic variant (c.1810C>G) of the *ABCG5* gene.

Results: There were no significant differences in age or gender between patients and healthy controls (p>0.05 for both). The frequencies of polymorphisms in the *ABCG5/G8* gene were not significantly different between patients and controls (p>0.05). The rate of CC genotype in the *ABCG5* gene in patients with xanthoma and premature atherosclerosis was 1.7 to 2.1 times higher than in patients with hematologic abnormalities. The rate of AA genotype in the *ABCG8* gene in patients with hematologic abnormalities was 3.2 times higher than in patients with xanthoma. In patients with hematologic abnormalities, the mean hemoglobin and platelet values were significantly lower in patients with CC genotype than other genotypes of the *ABCG8* gene.

Conclusion: In Turkish patients with sitosterolemia phenotypes, the frequency of common *ABCG5/G8* gene polymorphisms is similar to that of healthy controls. Our findings suggest that some polymorphisms in the *ABCG5/G8* gene may be linked to sitosterolemia phenotypes (premature atherosclerosis, xanthoma, and hematologic abnormalities (specifically, hemolytic anemia and macrothrombocytopenia)).

BACKGROUND

Sitosterolemia is a rare, autosomal-recessive lipid storage disease caused by defects in adenosine triphosphate-binding cassette genes (*ABCG*) 5 and 8 that affect intestine and liver sterol functions leading to elevated sterol levels. It is characterized by xanthoma, premature atherosclerosis, and hematologic abnormalities [1]. Sitosterolemia patients also tend to have tendon xanthomas, premature atherosclerosis, and coronary artery disease, these clinical features suggest also familial hypercholesterolemia [2]. On the other hand, hematologic abnormalities are the distinctive features that most clearly distinguish sitosterolemia from familial hypercholesterolemia [3]. To date, two polymorphic variants in the *ABCG5* gene and 12 polymorphic variants in the *ABCG8* gene at the sitosterolemia locus have been identified in a study using the HapMap dataset [4]. In a recent study, the most common single nucleotide polymorphisms (SNPs) in the *ABCG5* (rs6720173) and *ABCG8* (rs11887534, rs4148211, rs4148217, and rs6544718) genes have been studied in hypercholesterolemic patients [5]. However, no study has been found to evaluate the most common SNPs of the *ABCG5/G8* genes on sitosterolemia phenotypes yet.

The aim of this study was to analyze the effect of *ABCG5/G8* gene polymorphisms on sitosterolemia phenotypes in a group of Turkish patients.

MATERIAL AND METHODS

Setting, study design, and participants

This cross-sectional study was performed by a group of physicians in three departments of Gazi University Hospital in Turkey between March 2014 and April 2018. The study included 90 patients with suspected sitosterolemia who were selected by an expert cardiologist, dermatologist, and hematologist based on clinically important parameters such as premature atherosclerosis, xanthoma, or hematologic abnormalities. Other causes of atherosclerosis, xanthoma, macrothrombocytopenia, and stomatocytes were ruled out. Patients were divided into three subgroups based on clinical and laboratory parameters that indicate sitosterolemia: those with hematologic abnormalities [Group 1], those with premature atherosclerosis [Group 2], and those with xanthoma [Group 3]. All 90 patients and 157 age-matched healthy controls were also tested for three common SNPs (see details below) in the *ABCG*8 gene (c.1895T>C and c.161A>G) and one known SNP in the *ABCG*5 gene (c.1810C>G). The Gazi University Institutional Review Board approved the study.

Diagnosis of sitosterolemia

An individual was considered to have sitosterolemia if testing revealed frankly elevated sitosterol level (>15 µg/mL) and/or genetic diagnostic criteria (pathogenic mutations in *ABCG5/G8* gene) that have been proposed in several reports [6-8]. Detailed methods used to reach the diagnosis are provided. As noted, suspected sitosterolemia was identified by a cardiologist, dermatologist, and hematologist. Premature atherosclerosis was defined according to the European Association of Preventive Cardiology [9]: male subjects aged ≤40 years and female subjects ≤50 years who were referred for elective coronary angiography due to typical chest pain or positive non-invasive test results (*i.e.,* electrocardiogram suggestive of ischemia, positive exercise tolerance test, or suspicious myocardial perfusion scan). The diagnosis of xanthoma involved determining the type of xanthoma and the underlying cause through patient history, physical examination, and relevant laboratory studies. All affected patients in this study had plane (also known as planar) xanthoma, which are yellow-orange patches or slightly raised papules or plaques on skin surfaces. Hematologic abnormalities were defined as macrothrombocytopenia, hemolytic anemia, stomatocytes, or splenomegaly, and patients were only enrolled if their hematologic abnormalities were not explained and were unresponsive to standard treatment. Routine hematological tests, lipid profiles and sterol levels were measured in all 90 suspected patients for sitosterolemia.

Measurement of sterol levels

Plasma sterol levels were measured using an AOCS Official Method Ch 6-91. Sterol derivatives (*i.e.*, silyl ethers) were analyzed using a gas chromatograph (GC 2010; Shimadzu Corporation, Tokyo, Japan) equipped with an HP-5 fused silica capillary column (30 m, 0.25 mm i.d., and 0.25 mm film thickness) (Chrom Tech, Apple Valley, MN, USA). Sterols were quantified using cholestanol as an internal standard. The split ratio was 50:1 and the carrier gas was helium at 0.8 mL/min. Injector, column, and detector (FID) temperatures were 280°C, 260°C, and 290°C, respectively. Plasma samples were collected from the peripheral blood for detailed biochemical analyses including sterol levels and stored at -80 C until analysis. All underwent sterol level measurement by gas chromatography in a specialized research laboratory in Aydın, Turkey

Polymorphisms in ABCG5 and ABCG8 genes

Primers were designed primers and TaqMan probes were used for each mutation site, including the rs6720173 [c.1810C>G (p.Gln604Glu)] polymorphism in *ABCG*5 and the rs6544718 [c.1895T>C (p.Val632Ala)] and rs4148211 [c.161A>G (p.Tyr54Cys)] polymorphisms in *ABCG*8 (SNP; Biotech). For the procedure, 20.5 µL master mix and 0.3 µL hot start Taq DNA polymerase were added into the PCR tube and then 4.5 µL of the patient's DNA was added. The real-time PCR program included an initial denaturation step at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 60 s. The glucose-6-phosphate dehydrogenase (GAPDH) gene was used as an internal control. Allelic discrimination was facilitated by software analysis of the fluorescence data. The homozygous or heterozygous presence of the analyzed variants was reported.

Statistical analyses

All statistical analyses were performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). Nonparametric data were compared using the Mann-Whitney *U* test. Univariate and multivariate analyses were carried out using the chi-square test and the logistic regression model to determine odds ratios (ORs) and 95% confidence intervals (CIs) of probabilities regarding disease phenotypes for sitosterolemia. p values <0.05 were considered statistically significant.

RESULTS

The demographic and clinical characteristics of the 90 patients with suspected sitosterolemia are summarized in Table 1. The patients were 57 (64%) males and 33 (36%) females of median age 30 years (range, 1-82 years). The 157 controls comprised 102 (65%) males and 55 (35%) females of median age 31 years (range, 2-77 years). There were no significant differences between patients and controls with respect to age or sex (p>0.05 for both).

Demographic data

Among 90 suspected patients, the 29 patients with premature atherosclerosis were 12 females (42%) and 17 males (58%); the 32 patients with xanthoma were 10 females (32%) and 22 males (68%); the 29 patients with hematologic abnormalities were 11 females (38%) and 18 males (62%). There was no statistically significant difference in gender between the three subgroups (p>0.05). The median ages were as follows: premature

Table 1: Demographic characteristics of the suspected sitosterolemia patients

		Patients (*n*=90)
Median age in yrs (range)		30 (1-82)
Sex (Males/Females)		57(64%)/33(36%)
CLINICAL FEATURES		
Group 1 Hematological abnormalities *n* (%)		29 (32.0%)
Group 2 Premature atherosclerosis *n* (%)		29 (32.0%)
Group 3 Xanthomas *n* (%)		32 (36.0%)
LABORATORY FINDINGS		
Hemoglobin (g/dL)		13(7-16)
Platelets (10^3/µL)		234.000(8000-482.000)
Total cholesterol (mg/dL)		166(101-331)
Triglyceride (mg/dL)		109(34-581)
Plasma HDL (mg/dL)		46(23-127)
Plasma LDL (mg/dL)		105(47-243)
Plasma sitosterol **(**µg/mL**)**		16.4(1-234)
Plasma campesterol **(**µg/mL**)**		4.4(1-62)
Plasma stigmasterol **(**µg/mL**)**		2.3(0-36)
POLYMORPHIC GENES		
***ABCG5* gene**	c.1810C>G allele (p.Gln604Glu)	126(0.70)/54(0.30)
***ABCG8* gene**	c.1895T>C allele (p.Val632Ala)	149(0.82)/31(0.18)
	c.161A>G allele (p.Tyr54Cys)	106(0.58)/74(0.42)

Table 2: The frequency of *ABCG5/ABCG8* gene polymorphisms in the patients and healthy controls

		Patients (*n*=90)	Controls *(n=157)*	*p*
rs6720173polymorphism/ ABCG5				
Genotypes	CC	45 (50%)	98 (62%)	0.81
	GC	36 (40%)	51 (32%)	
	GG	9 (10%)	8 (6%)	
Allele	C/G	126(0.70)/54(0.30)	247(0.78)/67(0.22)	
rs6544718 polymorphism/*ABCG*8				
Genotypes	CC	63 (70%)	121 (77%)	0.77
	CT	23 (26%)	33 (21%)	
	TT	4 (4%)	3 (2%)	
Allele	C/T	149(0.82)/31(0.18)	275(0.87)/39(0.13)	
rs4148211 polymorphism/*ABCG*8				
Genotypes	AA	34 (38%)	68 (44%)	0.71
	AG	38 (42%)	60 (38%)	
	GG	18 (20%)	29 (18%)	
Allele	A/G	106(0.58)/74(0.42)	196(0.62)/118(0.38)	

atherosclerosis subgroup 41 years (range, 18-50 years); xanthoma subgroup 40 years (range, 10-82 years); hematologic symptomatişc abnormality subgroup 12 years (range, 1-40 years). There was no significant difference between the mean ages of premature atherosclerosis and xanthoma subgroups (p>0.05). The mean age of the subgroup with hematologic abnormalities was significantly lower than that of the other subgroups (p<0.05 for both).

Comparison of ABCG5/G8 gene polymorphisms in sitosterolemia phenotypes

The frequencies of polymorphisms (c.1810C>G in the *ABCG*5 gene, c.1895T>C, and c.161A>G in the *ABCG*8 gene) were not significantly different between patients and controls (p>0.05) (Table 2). The rate of AA genotype (c.161 A>G) in the *ABCG*8 gene was significantly higher in patients with hematological abnormalities than in patients with xanthoma (p<0.05). The rate of AA genotype (c.161 A>G) in the *ABCG*8 gene in patients with hematological abnormalities was 3.2 times higher than in patients with xanthoma (OR: 3.2; 95%CI:1.1-9.4). The rate of CC genotype (c.1810 C>G) in the *ABCG*5 gene was significantly higher in patients with premature atherosclerosis and xanthoma than in patients with hematological abnormalities (p<0.05). The rate of CC genotype (c.1810 C>G) in the *ABCG*5 gene in patients with xanthoma and premature atherosclerosis was 1.7 (OR: 1.7; 95% CI:1.0-2.8) to 2.1 times (OR: 2.1; 95% CI:1.2-3.7) higher than in patients with hematologic abnormalities (Table 3). In patients with hematologic abnormalities, the mean hemoglobin and platelet values were significantly lower in patients with CC

Table 3: Comparison of laboratory parameters between different genotypes in *ABCG5/G8* gene in patients with premature atherosclerosis, xanthoma and hematological abnormalities

	ABCG5 gene rs6720173polymorphism				OR
Disease Groups	CC n=45	CG/GG n=45	*Univariate P*	*Multivariate P*	95%CI)
Patients with premature atherosclerosis (n, %)	19(66%)	10(34%)	0.37	-	-
Patients with xanthoma (*n, %*)	18(58%)	14(42%)			
Patients with hematological abnormalities (n, %)	8(28%)	21(72%)	0.03	0.01	
hematological abnormalities (*n, %*)	8(28%)	21(72%)	0.03	0.01	1.7(1.0-2.8)
Patients with xanthoma (*n, %*)	18(58%)	14(42%)			
Patients with premature atherosclerosis (n, %)	19(66%)	10(34%)	0.004	0.001	2.1 (1.2-3.7)
Patients with hematological abnormalities (n, %)	8(28%)	21(72%)			
	ABCG8 gene rs6544718 polymorphism				OR
	CC n=63	CT/TT n=27	*Univariate P*	*Multivariate P*	95%CI)
Patients with premature atherosclerosis (n, %)	22(76%)	7(24%)	0.13	-	-
Patients with xanthoma (*n, %*)	19(60%)	13(40%)			
Patients with hematological abnormalities (n, %)	22(76%)	7(24%)	0.13	-	-
Patients with xanthoma (*n, %*)	19(60%)	13(40%)			
Patients with premature atherosclerosis (n, %)	22(76%)	7(24%)	1		-
Patients with hematological abnormalities (n, %)	22(76%)	7(24%)			
	ABCG8 gene rs4148211 polymorphism				OR
	AA n=34	AG/GG n=56	*Univariate P*	*Multivariate P*	95%CI)
Patients with premature atherosclerosis (n, %)	11(38%)	18(62%)	0.2	-	-
Patients with xanthoma (*n, %*)	8(25%)	24(75%)			
Patients with hematological abnormalities (n, %)	15(52%)	14(48%)	0.04	0.03	3.2 (1.1-9.4)
Patients with xanthoma (*n, %*)	8(25%)	24(75%)			
Patients with premature atherosclerosis (n, %)	11(38%)	18(62%)	0.29	-	-
Patients with hematological abnormalities (n, %)	15(52%)	14(48%)			

OR:Odds Ratio, 95%CI: Confidence interval

genotype than other genotypes of the *ABCG8* gene (p<0.05). In patients with hematologic abnormalities, the mean HDL value was significantly higher in patients with AA genotype than in other genotypes of the *ABCG8* gene (p<0.05). As well, frequencies of the patient's subgroups and other laboratory values (*i.e.*, hemoglobin, lipid profile) were not significantly different among those genotypes in three polymorphisms (c.1810 C>G in *ABCG*5 and c.161 A>G and c.1895 T>C in *ABCG*8) (all p>0.05) (Table 4).

DISCUSSION

Sitosterolemia is a rare, autosomal-recessive lipid storage disorder that has been described in persons of Hutterite, Amish, Northern European, Japanese, and Chinese ancestry [10-14]. The true prevalence is unknown due to underdiagnosis; however, the current estimate is 1 in 2,600.000 for *ABCG*5 and 1 in 360.000 for *ABCG*8 [15]. To date, there have been only eight reported index cases and their relatives of sitosterolemia in patients of Turkish origin, seven index cases who lived in Turkey at the time of diagnosis and another who lived in Germany [16-18]. A systematic review of few data demonstrated sitosterolemia in 10% of the subjects diagnosed with premature atherosclerosis, and in 30% of the involved subjects diagnosed with xanthoma and hematologic abnormalities [12]. Age of onset and the spectrum of clinical manifestations have varied greatly among previously reported patients with hematological abnormalities, premature atherosclerosis, and xanthoma. Premature atherosclerosis and xanthoma can present at any age in patients with sitosterolemia [12]. We found a similar median range of age in both patient groups, including premature atherosclerosis and xanthoma. In contrast to other phenotypes, hematological abnormalities were mostly found in children in our study. These findings suggest that pediatricians should suspect sitosterolemia in children who have unexplained hematological abnormalities (stomatocytes, hemolytic anemia, and/or macrothrombocytopenia).

The frequencies of sitosterolemia-related polymorphisms (c.1810 C>G in the *ABCG*5 gene and c.161 A>G and c.1895 T>C in the *ABCG*8 gene) vary widely across different populations [4,19-21]. The c.1895 T>C variant in *ABCG*8 is the most frequent in Caucasians, but it is typically uncommon or absent in Asian populations, where polymorphic variants of c.1810 C>G in *ABCG*5 and c.161 A>G in *ABCG8* are most frequent [21]. The frequencies and distributions of common SNPs (c.1895 T>C and c.1810 C>G) in the Turkish population are similar to what has been observed in other Caucasian and African American populations [4]. These three SNPs were also investigated in Chinese hypercholesterolemic patients [5]. For c.161 A>G variants in the *ABCG8* gene, the percentages of AA, AG, and GG

Table 4: Comparison of disease groups between different genotypes in the *ABCG5/G8* gene

Parameters in	***ABCG5 gene rs6720173polymorphism***			***ABCG8 gene rs6544718 polymorphism***			*ABCG8 gene rs4148211 polymorphism*		
Premature atherosclerosis	CC n=45	CG/GG n=45	*P*	CC n=63	CT/TT n=27	*P*	AA n=34	AG/GG n=56	*P*
Hemoglobin (g/dL)	13.6±1.6	14.1±1.1	0.5	13.8±1.5	13.9±1.4	0.85	14.0±1.0	13.8±1.6	0.66
Platelets (10^3/μL)	275,730±19,860	242,000±8,290	0.22	254,650±11,780	287,240±38,640	0.27	279,500±20,150	250,670±17,150	0.35
Total cholesterol (mg/dL)	183,6±52,6	183,2±45,2	0.98	185,4±50,1	179,9±48,2	0.8	184.8±46,1	182.8±51,5	0.92
Plasma sitosterol level (μg/mL)	37,7±10,6	18,9±6,4	0.26	33,5±9,6	22,5±3,3	0.57	47.9±19.8	37.2±19.1	0.51
Plasma campesterol level (μg/mL)	5,8±1,6	5,1±3,1	0.81	6,8±1,4	3,5±1,1	0.26	5,3±2,1	5,9±1,6	0.85
Plasma stigmasterol level (μg/mL)	-	-	-	-	-	-	1.7±0,7	2.8±0,8	0.57
Plasma LDL **(**mg/dL**)**	112,5±42,9	115,1±37,9	0.86	114,4±39,3	111,4±44,6	0.87	108.6±28,2	116.7±45,2	0.65
Plasma Triglyceride **(**mg/dL**)**	157,4±31,2	112,4±12,7	0.32	148,1±27,1	123,8±28,8	0.61	130,8±27,7	147,5±29,7	0.72
Plasma HDL **(**mg/dL**)**	45,1±11,8	37,6±8,5	0.08	42,6±12,3	43,1±5,5	0.9	42.1±5,1	42.8±1,9	0.87
Hematological abnormalities									
Hemoglobin (g/dL)	11.3±2.6	11.8±1.2	0.52	11.3±1.6	12.9±1.3	**0.04**	11.8±1.3	11.6±2.1	0.83
Platelets (10^3/μL)	135,930±42,960	118,850±27,190	0.74	98,030±39,410	217,820±69,120	**0.02**	129,450±37,890	117,940±25,980	0.8
Total cholesterol (mg/dL)	148,1±28,6	159,4±27,2	0.6	177,4±43,1	176,9±46,2	0.95	150.3±20,9	170.1±14,1	0.22
Plasma sitosterol level (μg/mL)	24,4±11,6	32,6±10,4	0.73	31,4±13,9	26,3±16,3	0.84	52.5±19.1	44.5±14.8	0.68
Plasma campesterol level (μg/mL)	2,6±0,6	11,5±4,7	0.46	11,3±5,2	6,3±2,7	0.64	4,8±1,0	14,3±7,1	0.27
Plasma stigmasterol level (μg/mL)	2,9±0,4	13,1±7,1	0.69	-	-	-	2.4±0,7	13.1±9,7	0.69
Plasma LDL **(**mg/dL**)**	58,5±14,9	91,1±30,9	0.33	86,2±31,7	93,5±33,2	0.78	75.3±25,3	106.3±30,2	0.11
Plasma Triglyceride **(**mg/dL**)**	92,3±24,2	89,4±11,7	0.9	96,2±37,3	69,8±16,2	0.26	94,2±16,3	85,1±10,3	0.66
Plasma HDL **(**mg/dL**)**	50,3±10,2	51,1±10,7	0.92	52,6±15,3	45,5±15,1	0.51	57.6±10,8	44.1±4,8	**0.04**
Xanthomas									
Hemoglobin (g/dL)	13.5±1.2	13.1±1.3	0.48	13.6±1.3	12.8±1.1	0.07	13.3±1.0	13.4±1.3	0.96
Platelets (10^3/μL)	252,120±18,190	288,000±14,780	0.15	260,180±17,180	280,840±16,240	0.42	233,660±24,850	277,370±13,350	0.14
Total cholesterol (mg/dL)	191,2±49,5	181,6±48,2	0.64	177,4±42,1	193,9±55,2	0.4	206.6±55,1	179.4±46,1	0.27

Plasma sitosterol level (µg/mL)	23,9±5,2	35,2±12,4	0.32	29,3±6,8	26,1±7,3	0.77	59.6±34.8	44.1±14.8	0.2
Plasma campesterol level (µg/mL)	5,4±1,4	21,5±14,1	0.12	5,4±1,4	18,5±12,1	0.14	6,6±2,9	11,3±6,1	0.63
Plasma stigmasterol level (µg/mL)	4,2±1,4	2,7±0,1	0.68	-	-	-	4.7±2,1	2.1±0,7	0.48
Plasma LDL (mg/dL)	121,3±50,3	111,2±44,1	0.59	101,7±32,3	131,4±56,6	0.1	130.2±43,6	111.7±47,2	0.44
Plasma Triglyceride (mg/dL)	112,3±14,2	121,4±12,7	0.63	112,1±9,1	122,8±17,8	0.6	119,1±24,7	116,7±51,7	0.92
Plasma HDL (mg/dL)	54,5±23,1	50,2±18,5	0.6	54,8±24,3	48,1±15,5	0.41	54.6±19,8	51.3±3,8	0.74

OR:Odds Ratio, 95%CI: Confidence interval

genotypes were 27%, 50%, and 23%, respectively. For c.1895 T>C variants in the *ABCG8* gene, the percentages of CC, CT, and TT genotypes were 65%, 32%, and 3%, respectively. For c.1810 C>G variants in the *ABCG5* gene, the rates of CC, CG, and GG genotypes were 13%, 57%, and 30%, respectively. A similar rates of SNPs in *ABCG8* gene were found in our study whereas the rate of SNP in *ABCG5* gene was different from our study. This difference may be related to disease phenotypes. In contrast to our study population, only hypercholesterolemia patients were included in the Chinese study [5]. Kaya et al., previously reported the CC genotype (c.1895 T>C) and AA genotype (c.161 A>G) in the *ABCG8* gene in the first index child in Turkey and some family members with sitosterolemia, as well as three additional index cases, all of which were later reported [16,18]. Although all of the index cases had hematological abnormalities, only four of their relatives had xanthoma. Another study suggests that two polymorphic variants (c.1895 T>C and c.161 A>G) in *ABCG8* gene are linked to hematologic abnormalities in patients with sitosterolemia [22]. Similarly, patients with the CC genotype (c.1895 T>C) and AA genotype (c.161 A>G) of the *ABCG8* gene had more hematological abnormalities (specifically, hemolytic anemia and macrothrombocytopenia), whereas patients with the CC genotype (c.1810 C>G) of the *ABCG5* gene had significantly more premature atherosclerosis and xanthoma. Our findings suggest that some SNPs in the *ABCG5/G8* genes may influence disease phenotypes in patients with sitosterolemia.

Different phenotypic features may be related to environmental or genetic factors that can modify gene expression or sterol absorption. The Turkish diet, which is rich in vegetable or olive oil, is a contributing factor for elevated blood levels of plant sterol in patients with sitosterolemia, whereas it is well known that a sterol-rich diet can help reduce cholesterol levels and prevent atherosclerosis in patients with hypercholesterolemia. [23]. Sitosterolemia is now being investigated in patients with hypercholesterolemia [3,6,7,12,24]. Recent studies suggested that the use of a plant sterol diet and ezetimibe to lower cholesterol is a controversial strategy because of inter-individual variations due to genetic polymorphisms [5,25]. In a Chinese study, the mean value of HDL before and after ezetimibe treatment was significantly higher in patients with AA genotype than in patients with other genotypes of the *ABCG8* gene [5]. Similarly, we found a higher HDL mean value in the AA genotype in patients with hematological abnormalities than in other genotypes of the *ABCG8* gene. Our findings suggest that some SNPs in the *ABCG8* gene could be useful for monitoring lipid profiles after ezetimibe treatment in patients with sitosterolemia.

No previous studies have examined the utility of *ABCG5/G8* gene polymorphisms in a group of Turkish cohorts with suspected sitosterolemia. Due to a lack of resources, the majority of the genetic variants in the *ABCG5/G8* gene in our cohort were not studied. Another limitation of this study was that no sterol measurements were performed in healthy controls. Because plasma sterol levels are not routinely measured in many countries, including Turkey. A more detailed scientific study with a greater number of participants is required to confirm our findings.

CONCLUSIONS

In Turkish patients with sitosterolemia phenotypes, the frequency of common *ABCG5/G8* gene polymorphisms is similar to that of healthy controls. Our findings suggest that some polymorphisms in the *ABCG5/G8* gene may be linked to sitosterolemia phenotypes (premature atherosclerosis, xanthoma, and hematologic abnormalities (specifically, hemolytic anemia and macrothrombocytopenia)).

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AUTHORS' CONTRIBUTIONS

Z.K., G.A., and E.A. recruited patients. A.Y., M.A.E., and A.T. analyzed and interpreted the data. Z.K. wrote the manuscript, which was reviewed, edited and finally approved by all authors.

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