• Basic Research •

Association of eleven single nucleotide polymorphisms with refractive disorders from Eskisehir, Turkey

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Abstract

● **AIM:** To investigate relationship between refractive errors and eleven single nucleotide polymorphisms (SNPs) in *HGF, GC, MFN1, GNB4*, and *VDR* genes in Turkish population.

● **METHODS:** A group of 212 participants with myopia (*n*=91), hyperopia (*n*=45), and emmetropia (*n*=76) were investigated in this study. SNPs in *HGF, GC, MFN1, GNB4* and *VDR* genes were studied by SnapShot technique.

● **RESULTS:** The patients in this study consists of 47 female/44 male (age: 23.47±4.30) patients with myopia, 20 female/25 male (age: 31.20±8.02) with hyperopia and 33 female/43 male (age: 25.22±6.60) with emmetropia. The genotype distribution of the rs7618348 polymorphism, which was the only statistically significant one between myopia and emmetropia group. The genotype distribution of the rs3819545, rs3735520, rs7041, and rs2239182 polymorphisms, which were statistically significant between hyperopia and emmetropia groups.

● **CONCLUSION:** The importance of genetic predisposition to refractive errors with respect to etiology of the disease is revealed. It is known that polymorphism studies may differ because of genetic diversity among populations so larger cohort studies are required in different populations to enlighten the etiology of the refractive errors.

● **KEYWORDS:** refractive disorders; myopia; hyperopia; genetics; single nucleotide polymorphisms; Turkey

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INTRODUCTION

Refractive errors, especially myopia is currently a global epidemic and uncorrected refractive errors are the second leading cause of blindness. Refractive errors, namely myopia, hyperopia, and astigmatism, are multifactorial ocular disorders. Their etiology is not completely understood[1]. There are many environmental factors, which are responsible for myopia increase in the world. Intensive near work activity is one of the major factors defined to be related with myopia. Limited outdoor activity is the other important factor associated with myopia. Sports activities, socioeconomic status, education levels, premature birth are other risk factors claimed to be related with myopia.

There are many studies on the genetic contributions to myopic refractive error. There are more than 25 genes associated with myopia[2]. *MYP1, MYP2, MYP3, MYP4, MYP5, PAX6, TGIF, LUM, ARR3* (arrestin 3), *LRPAP1* (LDL receptor related protein associated protein 1), *P4HA2* (prolyl 4-hydroxylase subunit alpha 2), *SLC39A5* (solute carrier family 39 member 5) are the most important genes defined as candidate genes for myopia[3-4]. Studies showed that myopia does not have a single genetic cause, but many susceptibility genes exist[5]. Results of genome-wide association studies (GWAS) meta-analysis support that refractive errors are caused by a light-dependent retina-to-sclera signaling cascade[6]. It has also been shown that genetic factors associated with circadian rhythm and pigmentation play a role in the development of myopia and refractive error[7].

On the other hand, there is a paucity of literature on the genetic basis of hyperopia. There are a few studies which demonstrated the relation between *MFRP* (membrane frizzled-related protein) and *PRSS56* (serine protease 56) genes and hyperopia[4]. The association of hepatocyte growth factor

Table 1 SNPs investigated in this study

No.	Gene name	SNP/rs number	cDNA
1	*HGF*	rs3735520	c.-1652C>T
2	*HGF*	rs2286194	c.1041-116 A>T
3	*HGF*	rs17501108	c.-4607C>A
4	*HGF*	rs12536657	c.1154-45T>C
5	*MFN1*	rs13098637	c.976-194T>C
6	*GNB4*	rs7618348	c.-42-709 G>A
7	*VDR*	rs1544410	c.1024+283G>A
8	*GC*	rs7041	c.1296 T>G
9	*VDR*	rs3819545	c.147-6046T>C
10	*VDR*	rs2239182	c.277+3419 A>G
11	*VDR*	rs2853559	c.68 -6248 T>C

(*HGF*), *MFN1* (mitofusin 1) and *VDR* (vitamin D receptor) genes with myopia has been investigated in a few studies. *HGF* gene has been reported to be associated with low, moderate and high myopia, while *VDR* and *MFN1* genes have been reported to be associated with low and moderate myopia[8-10].

To the best of our knowledge, there are no published studies on the genetic basis of refractive errors in Turkey. Our aim was to investigate the association of five different genes (*HGF, GC, MFN1, GNB4*, and *VDR*) with refractive errors including both myopia and hyperopia in Turkey.

SUBJECTS AND METHODS

Ethical Approval This study was conducted according to the Declaration of Helsinki guidelines and approved by the Clinical Practice Ethics Committee of Eskisehir Osmangazi University, Medical Faculty. All subjects were agreed to participate in the study and biological samples were obtained after written informed consent obtained.

Participants The effect size assumption was used to determine the preliminary sample size. Number of cases was calculated as 133. A total of 212 participants diagnosed with 91 myopia, 45 hyperopia, and 76 emmetropia by ophthalmologist at the Ophthalmology Department of Eskisehir Osmangazi University were enrolled in this study.

The autorefractometer measurements were performed under cycloplegia. Spherical equivalent (SE) was calculated by adding the spherical value and half of the cylindrical value. SE<-0.50 diopters (D) in both eyes was considered to be myopic, while SE>0.75 D in both eyes was considered to be hypermetropic. The inclusion criteria for this study were as follows: 1) patients aged between 18 and 40; 2) 3 measurements obtained under cycloplegia. Patients meeting any of the following criteria were excluded: 1) corneal diseases; 2) lenticular diseases; 3) genetic disorders associated with myopia such as Marfan syndrome and retinitis pigmentosa; 4) previous intraocular surgical intervention; 5) nystagmus; 6) ocular trauma; or 7) patients born preterm.

Single-Nucleotide Polymorphisms Genotyping We have selected eleven single-nucleotide polymorphisms (SNPs; Table 1) that are associated with myopia and hyperopia or have conflicting suggestions about these SNPs in the literature. We genotyped all participants for these SNPs. Genomic DNA was isolated from peripheral lymphocytes using Magna Pure Compact LC (Roche Applied Science, Basel, Switzerland), according to the manufacturer's recommendations.

SNPs in *HGF, GC, MFN1, GNB4* and *VDR* genes were studied by SnapShot technique. SnapShot reactions were performed as recommended by the manufacturer. Electrophoresis of amplified PCR samples related to these polymorphism regions was performed on ABI 3130 Genetic Analyzer and the data were analyzed by using GeneMapper 4.0 Software (Applied Biosystems, Life Technologies, CA, USA).

Statistical Analysis Statistical analysis was performed using the Statistical Package of the Social Sciences 21.0 (IBM, NY, USA). In statistical analysis of the study, continuous data are given as mean±standard deviation. Categorical data are given as percentages (%). Pearson Chi-square analysis was used in the analysis of the created cross tables. Binary Logistic regression analysis was used to determine risk factors. Bonferroni correction adjustment was used for multiple comparison analysis statistically significant evidence of association was determined by *P*-values of 0.05 or below.

RESULTS

Demographic Features of the Study Group The patients in this study consists of 47 female/44 male (age: 23.47±4.30)y patients with myopia, 20 female/25 male (age: 31.20±8.02)y with hyperopia and 33 female/43 male (age: 25.22±6.60)y with emmetropia.

Genotype Results

SNP analysis results in myopia and emmetropia groups Genotypes were wild in each of cases and controls for rs2286194 polymorphism. A statistically significant difference was not observed between myopia and emmetropia group

Table 2 Distribution of genotype and risk allele frequency of rs7618348 (*GNB4* gene) polymorphism between myopia and emmetropia groups

Genotype/allele rs7618348	Myopia (*n*=91; %)	Emmetrope (*n*=76; %)	OR (95%CI)	χ^2	*P*
GG	24 (26.37)	24 (31.58)	Reference		
GA	33 (36.26)	40 (52.63)	1.21 (0.59-2.52)	0.27	0.605
AA	34 (37.36)	12 (15.79)	0.35 (0.15-0.84)	5.68	0.019
A allele	101 (55.49)	64 (42.10)	0.64 (0.41-1.00)	3.93	0.048

Table 3 Distribution of genotype and risk allele frequency of rs3819545 (*VDR* gene) polymorphism between hyperopia and emmetropia groups

Genotype/allele rs3819545	Hyperopia (*n*=45; %)	Emmetrope (*n*=76; %)	OR (95%CI)	χ^2	*P*
TT	9 (20.00)	32 (42.11)	Reference		
TC	21 (46,67)	34 (44.74)	0.46 (0.18-1.14)	2.88	0.093
CC	15 (33.33)	10 (13.15)	0.19 (0.06-0.56)	9.72	0.003
C allele	51 (56.66)	54 (35.52)	0.42 (0.25-0.72)	10.29	0.001

Table 4 Distribution of genotype and risk allele frequency of rs3735520 (*HGF* gene) polymorphism between hyperopia and emmetropia groups

Genotype/allele rs3735520	Hyperopia (*n*=45; %)	Emmetrope (*n*=76; %)	OR (95%CI)	χ^2	*P*
CC	24 (53.34)	25 (32.89)	Reference		
CT	15 (33.33)	33 (43.42)	2.11 (0.92-4.84)	3.17	0.077
TT	6 (13.33)	18 (23.69)	2.88 (0.98-8.49)	3.83	0.055
T allele	27 (30.00)	69 (45.39)	1.83 (1.06-3.16)	4.69	0.030

Table 5 Distribution of genotype and risk allele frequency of rs7041 (*GC* gene) polymorphism between hyperopia and emmetropia groups

Genotype/allele rs7041	Hyperopia (*n*=45; %)	Emmetrope (*n*=76; %)	OR (95%CI)	χ^2	*P*
TT	5 (11.11)	18 (23.68)	Reference		
TG	14 (31.11)	30 (39.48)	0.60 (0.18-1.93)	0.76	0.595
GG	26 (57.78)	28 (36.84)	0.30 (0.10-0.92)	4.68	0.299
G allele	66 (73.33)	86 (56.57)	0.48 (0.27-0.83)	6.79	0.009

Table 6 Distribution of genotype and risk allele frequency of rs2239182 (*VDR* gene) polymorphism between hyperopia and emmetropia groups

Genotype/allele rs2239182	Hyperopia (*n*=45; %)	Emmetrope (*n*=76; %)	OR (95%CI)	χ^2	*P*
AA	5 (11.11)	20 (26.32)	Reference		
AG	30 (66.67)	46 (60.52)	0.38 (0.13-1.13)	3.15	0.083
GG	10 (22.22)	10 (13.15)	0.25 (0.07-0.93)	4.50	0.039
G allele	50 (55.55)	66 (43.42)	0.61 (0.36-1.04)	3.36	0.068

for rs17501108, rs2853559, rs1544410, rs13098637, rs7041, rs3735520, rs2239182, rs12536657 SNPs in terms of genotype and distribution of the allele frequencies. The genotype distribution of the rs7618348 polymorphism, which was the only statistically significant one between myopia and emmetropia group, was shown in Table 2.

SNP analysis results in hyperopia and emmetropia groups

There was no statistically significant difference between hyperopia and emmetropia groups for rs17501108, rs2853559, rs1544410, rs13098637, rs7041, rs3735520, rs2239182, rs12536657, rs3819545 in terms of genotype and distribution of the allele frequencies (P>0.05). The genotype distribution of the rs3819545, rs3735520, rs7041, and rs2239182 polymorphisms, which were statistically significant between hyperopia and emmetropia groups, was shown in Tables 3-6, respectively.

DISCUSSION

rs7618348 in *GNB4* gene might be a risk factor for myopia. rs2239182 and rs3819545 in *VDR*, rs7041 in GC polymorphisms were statistically significant between the hyperopic cases and

controls. Furthermore, T allele in rs3735520 *HGF* might be a slightly protective for the development of axial hyperopia.

Recently, more than 100 genes and 20 loci appear to be involved in refraction errors were identified with the development of next-generation sequencing technologies, GWAS, candidate gene studies, and linkage analyses[3].

HGF gene encodes a protein binding to *HGF* receptor to be involved in regulating cell growth, mobility, and morphogenesis. This protein actually has many distinct roles in like regulation of an inflammation, angiogenesis, tumorigenesis, renewing of tissues, and inhibition of apoptosis. It was first identified as a strong candidate gene in a locus related to eye weight[11]. *HGF* and its receptor have been reported to be extensively expressed in the eye, therefore some SNPs in *HGF* gene play a critical role together in many ocular physiological and pathological processes. In addition, a family-based linkage analysis studied in Chinese population found a potential locus at where *HGF* is located associated with high/severe myopia[12].

Yanovitch *et al*[10] found a statistically significant difference in the mild-moderate myopia group in 649 Caucasian patients compared to emmetropes, which also assert a close association between the mild-moderate myopia and rs3735520, SNP in *HGF*. They also reported that the T allele in *HGF* rs3735520 SNP region is a risk allele for myopia in the same study. Results from this study indicate tag SNPs that are rs2286194, rs373552, and rs17501108, also called as haplotypes and rs12536657, rs2286194 in the *HGF* gene are associated with myopia. Vitreous chamber depth is measured longer for rs3735520 SNP in Chinese population[13]. Burdon *et al*[14] have found that *HGF* rs3735520 SNP were associated with patients with keratoconus that this SNP was also associated with *HGF* serum level in genome association study. However, there is no relationship found between rs3735520 polymorphism and high myopia in a study conducted in Chinese population consisting of 1052 high myopia and 1070 controls[15]. To our knowledge, there are not any population-based study for rs3735520 polymorphism in refractive errors except the Chinese population. On the other hand, in a study conducted in the Australian population, an association of rs2286194 with keratoconus was reported[16]. In our study data, no statistically significant difference was observed between myopia and emmetrope groups for rs3735520, rs2286194, and rs17501108 SNPs in *HGF*, but there was a relationship between rs3735520 and hyperopia. Although many studies reveal that rs3735520 polymorphism is associated with myopia, our data failed to support this relationship in myopia group. This risk allele was significantly higher in emmetrope group compared to hyperopia. We presumed that this allele might be a slightly protective allele for the development of axial hyperopia. These data should be supported by studies within larger cohorts. Moreover, there is a study conducted in Australian population in order to determine the effect of *HGF* polymorphisms on fracture defects, which shows that rs12536657 is associated with both mild-moderate myopia and hyperopia[17]. In another study of 403 Spanish-Caucasian hyperopic cases, there is no relationship found between rs12536657 and hyperopia[18]. In our study, no significant difference was found between the rs12536657 SNP and both myopia and hyperopia cases compared to emmetropes. Our study supports the results obtained from the data of Barrio-Barrio *et al*'s research[18], but it contradicts the findings of Veerappan *et al*'s[17] study, which is most probably due to population difference.

The *MFN1* (mitofusin 1) gene encodes the mitochondrial membrane protein and is involved in mitochondrial mobility along with other intramembrane proteins. Considering that the eye is an organ with high energy demand, any defect in mitochondrial function is thought to lead to refractive errors[9]. Only one study, which is conducted in the Chinese population, investigating the effect of rs13098637 polymorphism on refractive errors was found. In this study, an association of rs13098637 polymorphism with mild-to-moderate myopia was revealed. However, no significant relationship was observed in the hyperopia and myopia groups in our study.

SNPs of the *GNB4* gene may be associated with myopia in retinal ON-bipolar cells since the retinal bipolar cells are involved in taking hyperopic images[19]. As a result of genome-wide linkage studies related to myopia in dizygotic twins, Hammond *et al*[20] proposed that the 3q26 region could be a myopia locus for the first time in addition to the other loci they identified. In two mapping studies, including a replication study of *GNB4* rs7618348 SNP, this SNP was found to be highly related to myopia, therefore they suggested that the *GNB4* gene may have a role in the molecular genetic etiology of myopia[19]. On the other hand, no statistically significant relationship was found between myopia and rs7618348 in *GNB4* gene in a controlled, myopia-related SNP study[9]. Statistical outcomes of our study give *P* value for the homozygous rs7618348 in the *GNB4* as 0.019, while the *P* value for the single allele change is 0.048. In particular, homozygous AA genotype has been shown to be associated with myopia in our study (P=0.019). Considering the frequency distribution of the A allele, a significant difference was found between myopia and emmetrope groups and was concluded as a risk allele. However, in order to determine its exact role and examine its relationship with myopia, it necessitates big population studies besides functional ones.

Since the vitamin-D binds to the vitamin D-binding protein, most of it does not circulate freely in the plasma. Thus, the *GC* gene, which encodes this binding protein, has been the

subject of epidemiological and genetic studies in which vitamin D levels can be associated with myopia. There are few studies aimed to establish the link between SNPs in the *GC* gene and myopia phenotype. In one of these studies, the relationship of rs7041 variant with myopia could not be established[8]. In addition, in another Mendelian randomized study to determine the relation of low vitamin-D level to myopic refractive defects, SNPs known to affect vitamin D level were investigated and the study group reported that *GC* rs7041 polymorphism was not associated with myopia[21]. Our results support these two studies. However, as an interesting thing in our findings, the G allele was found to be significantly different between the hyperopic and emmetropic groups. In the literature, no study has been found to examine the relationship between rs7041 SNP and hyperopia. With the replication of our study data, more enlightened relationship might be established between rs7041 and hyperopia.

Many SNP regions on *VDR* have been associated with many different phenotypes as it encodes a ligand-induced transcription factor that plays a role in the transcriptional regulation of vitamin D[21]. Mutti *et al*[8] suggested that there was no relationship between vitamin D levels in the blood and myopia first, but then although there is no strong experimental evidence, it has been reported that there may be a relationship between *VDR* SNPs and refractive defects. In the Meta-analysis conducted by Tang *et al*'s[22] study group, no association was found with myopia as a result of two-step statistical correction for *VDR* SNPs rs3819545, rs2239182, and rs2853559. In our study, none of the four SNPs examined for *VDR* (rs1544410, rs3819545, rs2239182, rs2853559) were associated with myopia, and our results are consistent with the literature in terms of myopia. However, it was found that homozygous genotypes of rs3819545 and rs2239182 SNPs were statistically significant between hyperopia and emmetropic cases (P<0.05). No other publications investigating the relationship between the examined *VDR* polymorphisms and hyperopia have been found. In this respect, our study data is the first to suggest the relationship of these polymorphisms with hyperopia.

In our study, 212 cases were examined to determine the relationship between refractory errors and related SNPs in *GC, GNB4, HGF, MFN1* and *VDR* genes. rs7618348 in *GNB4* gene might be a risk factor for myopia. rs2239182 and rs3819545 in *VDR*, rs7041 in *GC* polymorphisms were statistically significant between the hyperopic cases and controls. Furthermore, T allel in rs3735520 *HGF* might be a slightly protective for the development of axial hyperopia. Our results reveal the importance of genetic predisposition to refractive errors with respect to etiology of the disease. It is known that polymorphism studies might differ because of genetic diversity among populations. Results of polymorphism studies are therefore varied particularly in where a combination of many different ethnic groups found in countries such as Turkey. In addition, it is important not to ignore the gene-environment interactions and the effect of modifying genes when evaluating genetic risk factors. It is possible to get more exact outcomes with researches in large cohorts and populations with different ethnic backgrounds.

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REFERENCES

1 Harb EN, Wildsoet CF. Origins of refractive errors: environmental and genetic factors. *Annu Rev Vis Sci* 2019;5(1):47-72.

2 Wojciechowski R. Nature and nurture: the complex genetics of myopia and refractive error. *Clin Genet* 2011;79(4):301-320.

3 Cai XB, Shen SR, Chen DF, Zhang Q, Jin ZB. An overview of myopia genetics. *Exp Eye Res* 2019;188:107778.

4 Li J, Zhang Q. Insight into the molecular genetics of myopia. *Mol Vis* 2017;23:1048-1080.

5 Meng WH, Butterworth J, Bradley DT, Hughes AE, Soler V, Calvas P, Malecaze F. A genome-wide association study provides evidence for association of chromosome 8p23 (MYP10) and 10q21.1 (MYP15) with high myopia in the French population. *Invest Ophthalmol Vis Sci* 2012;53(13):7983-7988.

6 Tedja MS, Wojciechowski R, Hysi PG, *et al*. Genome-wide association meta-analysis highlights light-induced signaling as a driver for refractive error. *Nat Genet* 2018;50(6):834-848.

7 Hysi PG, Choquet H, Khawaja AP, *et al*. Meta-analysis of 542, 934 subjects of European ancestry identifies new genes and mechanisms predisposing to refractive error and myopia. *Nat Genet* 2020;52(4): 401-407.

8 Mutti DO, Cooper ME, Dragan E, Jones-Jordan LA, Bailey MD, Marazita ML, Murray JC, Zadnik K, CLEERE Study Group. Vitamin D receptor (VDR) and group-specific component (GC, vitamin D-binding protein) polymorphisms in myopia. *Invest Ophthalmol Vis Sci* 2011;52(6):3818-3824.

9 Zou YC, Lei JH, Wang Y, Xu S. Correlation between polymorphisms in the MFN1 gene and myopia in Chinese population. *Int J Ophthalmol* 2015;8(6):1126-1130.

10 Yanovitch T, Li YJ, Metlapally R, Abbott D, Viet KN, Young TL. Hepatocyte growth factor and myopia: genetic association analyses in a Caucasian population. *Mol Vis* 2009;15:1028-1035.

11 Zhou G, Williams RW. Eye1 and Eye2: gene loci that modulate eye size, lens weight, and retinal area in the mouse. *Invest Ophthalmol Vis Sci* 1999;40(5):817-825.

12 Han W, Yap MK, Wang J, Yip SP. Family-based association analysis of hepatocyte growth factor (HGF) gene polymorphisms in high myopia. *Invest Ophthalmol Vis Sci* 2006;47(6):2291-2299.

13 Chen JH, Chen HY, Huang SL, Lin JW, Zheng YQ, Xie ML, Lin WJ, Pang CP, Zhang MZ. Endophenotyping reveals differential phenotype-genotype correlations between myopia-associated polymorphisms and eye biometric parameters. *Mol Vis* 2012;18:765-778.

14 Burdon KP, MacGregor S, Bykhovskaya Y, *et al*. Association of polymorphisms in the hepatocyte growth factor gene promoter with keratoconus. *Invest Ophthalmol Vis Sci* 2011;52(11):8514-8519.

15 Yang X, Liu XQ, Peng J, Zheng H, Lu F, Gong B, Zhao GQ, Meng Y, Guan HZ, Ning MZ, Yang ZL, Shi Y. Evaluation of MYOC, ACAN, HGF, and MET as candidate genes for high myopia in a Han Chinese population. *Genet Test Mol Biomarkers* 2014;18(6):446-452.

16 Sahebjada S, Schache M, Richardson AJ, Snibson G, Daniell M, Baird PN. Association of the hepatocyte growth factor gene with keratoconus in an Australian population. *PLoS One* 2014;9(1):e84067.

17 Veerappan S, Pertile KK, Islam AF, Schäche M, Chen CY, Mitchell P, Dirani M, Baird PN. Role of the hepatocyte growth factor gene in refractive error. *Ophthalmology* 2010;117(2):239-245.e1-2.

18 Barrio-Barrio J, Bonet-Farriol E, Galdós M, Noval S, Pueyo V, Breeze CE, Santos JL, Alfonso-Bartolozzi B, Recalde S, Patiño-García A. *HGF*-rs12536657 and ocular biometric parameters in hyperopic children, emmetropic adolescents, and young adults: a multicenter quantitative trait study. *J Ophthalmol* 2019;2019:7454250.

19 Andrew T, Maniatis N, Carbonaro F, Liew SH, Lau W, Spector TD, Hammond CJ. Identification and replication of three novel myopia common susceptibility gene loci on chromosome 3q26 using linkage and linkage disequilibrium mapping. *PLoS Genet* 2008;4(10):e1000220.

20 Hammond CJ, Andrew T, Mak YT, Spector TD. A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genomewide scan of dizygotic twins. *Am J Hum Genet* 2004;75(2):294-304.

21 Cuellar-Partida G, Williams KM, Yazar S, *et al*. Genetically low vitamin D concentrations and myopic refractive error: a Mendelian randomization study. *Int J Epidemiol* 2017;46(6):1882-1890.

22 Tang SM, Lau T, Rong SS, Yazar S, Chen LJ, MacKey DA, Lucas RM, Pang CP, Yam JC. Vitamin D and its pathway genes in myopia: systematic review and meta-analysis. *Br J Ophthalmol* 2019;103(1):8-17.