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MBL2 Gene Polymorphism and Risk of Vitiligo in Turkish Patients

Mutlu Karkucak[1*], Berna Solak[2], Hakan Turan[3], Esma Uslu[3], Tahsin Yakut[4], Cihangir Aliagaoglu[3] and Teoman Erdem[2]

[1]Department of Medical Genetics, Sakarya University Training and Research Hospital, Sakarya, Turkey
[2]Department of Dermatology, Sakarya University Faculty of Medicine, Sakarya, Turkey
[3]Department of Dermatology, Düzce University Faculty of Medicine, Düzce, Turkey
[4]Department of Medical Genetics, Uludag University Faculty of Medicine, Bursa, Turkey

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ABSTRACT Mannose-Binding Lectin (MBL) plays an important role in innate immunity. *MBL2* gene polymorphisms affect MBL serum levels. Therefore, this increases the risk of infection and may result in predisposition to autoimmune diseases. The aim of this study was to investigate whether there is an association between the *MBL2* gene codon 54 (allele B: rs1800450, c.161G>A; p.54Gly>Asp) polymorphism and vitiligo in Turkish patients. One hundred and one patients who were diagnosed with vitiligo and 101 control subjects were included in the study. The DNA was analyzed using the Kbioscience Competitive Allele Specific PCR (KASP) technique. *MBL2* gene codon 54 polymorphism frequencies were compared between the two groups. In statistical analysis, the level of significance was set at $p<0.05$. No significant differences in frequencies of the A allele were observed between the patient and control groups. It was observed at similar frequencies in both groups ($p=0.890$). The results suggest that the *MBL2* gene Codon 54 polymorphism is not associated with an increased risk for the development of vitiligo in Turkish patients.

INTRODUCTION

Vitiligo is an acquired and progressive depigmentary disorder characterized by a chronic and progressive loss of functional melanocytes in skin and hair follicles. It affects 0.5-1 percent of the world's population. Although various hypotheses have been put forward regarding the pathogenesis of vitiligo, none of them can explain the mechanism of development of the disease (Dwivedi et al. 2000; Passeron and Ortonne 2005; Taieb et al. 2007; Lv et al. 2013). The aetiology of vitiligo is still unknown, but many factors have been suggested to play a role in the pathogenesis of the disease including oxidative stress, genetic factors, neural factors and autoimmune responses.

Mannose Binding Lectin (MBL), which is one of the innate immune system pattern recognizing molecules, is a C-type serum Lectin. This molecule recognizes major soluble patterns and plays an important role in the innate immunity by activating the complement pathway synthesized by the hepatocytes and phagocytosis. Lectin domain of MBL contributes to the elimination of many microorganisms through the complement pathway and opsonophagocytosis (Dumestre-Perard et al. 2002; Eisen and Minchinton 2003; Terai et al. 2003). The functional *MBL2* gene is mapped on chromosomes (q11.2–q21) and comprises four exons**.** The three functional single-nucleotide polymorphisms (SNPs) in exon 1 of the *MBL2* gene are codon 54 (allele B: rs1800450, c.161G>A; p.54Gly>Asp), 57 (allele C: rs1800451, c.170G>A; p.57Gly>Glu), and 52 (allele D: rs5030737, c.154C>T; p.52Arg>Cys). These variant alleles cause structural changes in MBL, resulting in low plasma levels. Genetic variations or low serum concentrations of MBL have been reported to be associated with increased risks of infectious diseases and autoimmune diseases such as rheumatoid arthritis, Behçet's disease, and systemic lupus erythematosus (Dwivedi et al. 2000; Eisen and Minchinton 2003; Kim et al. 2009; Xu et al. 2013; Turan et al. 2014; Pradhan et al. 2015). A limited number of studies investigating the relationship between MBL-2 codon polymorphism and vitiligo have found different results (Dwivedi et al. 2000; Onay et al. 2007).

*Address for correspondence:
Dr. Mutlu Karkucak
Sakarya University Training and Research Hospital, Department of Medical Genetics,
Korucuk Campus, 54000, Sakarya/Turkey
Telephone: 02644445400
E-mail: mutlukarkucak@hotmail.com

The aim of this study was to investigate the association between codon 54 polymorphism in *MBL2* gene coding MBL and susceptibility to vitiligo.

MATERIAL AND METHODS

Study Subjects

One hundred and one patients diagnosed with vitiligo clinically and/or histopathologically, and 101 systemically and dermatologically healthy individuals with no personal or familial history of vitiligo were included in this study. Patients with a dermatological disease other than vitiligo or who have systemic medical diseases were excluded from the study. The study protocol was approved by the Local Ethical Committee of Duzce University.

DNA Isolation and MBL 2 Genotyping

The venous blood samples obtained from the patients and control subjects were collected in EDTA tubes. Genomic DNA was extracted from whole blood using a DNA isolation kit (RTA Laboratuvarlari Biyolojik Urunler Ilaç ve Makine San. Tic. A.S., Gebze, Kocaeli, Turkey) according to the manufacturer's instructions and samples were stored at -20 °C until the performance of PCR.

Sequences containing the *MBL2* gene codon 54 polymorphism (allele B: rs1800450, c.161G>A; p.54Gly>Asp) were submitted to LGC Genomics for KASP SNP assay design. Primers for the KASP SNP assays were designed using the LGC's primer picker software in the form of two allele-specific forward primers and one common reverse primer. PCR reactions were performed in a total volume of 20 µl including DNA (5 µl), KASP Assay Mix (0,3 µl), KASP Master Mix (10 µl) and distilled water (4,9 µl). Genotyping reactions were performed using LGC Genomics standard cycling conditions in a hydrocycler (LGC Genomics). The following cycling conditions were used: 15 minutes at 94°C; 10 touchdown cycles of 20 seconds at 94°C, 60 seconds at 61-55°C (dropping 0.6°C per cycle), and 26 cycles of 20 seconds at 94°C, 60 seconds at 55°C. Fluorescence detection of the reactions was performed using a BMG FLUOstar Omega plate reader (BMG LABTECH GmbH, Offenburg, Germany), and the data was analyzed using the Klustercaller Version 3.4.1.36 software (LGC Genomics).

Statistical Analysis

Age was expressed as mean ± standard deviation and categorical variables were presented as frequencies (%). Independent samples t-test and Pearson chi-square test were performed for intergroup comparisons. Statistical analyses were performed using SPSS v.13 and the level of significance was set at p=0.05. Genotype data for control subjects and patients were in Hardy-Weinberg equilibrium (P > 0.05).

RESULTS

The mean ages and gender distribution of the patient and control groups are shown in Table 1. The age and gender distribution did not differ between the patient and control groups.

The investigation of the MBL 2 gene codon 54 polymorphism revealed that in the vitiligo

Table 1: Clinical characteristics and genotype distribution between vitiligo patient group and control group

SD=Standard deviation

	Vitiligo patient group n=101 (%)	*Control group n=101 (%)*	*p value*
Gender (Female/Male)	50(49.5)/51(50.5)	50(49.5)/51(50.5)	1
Age (years±SD)	39.9±15.9	40.2±11.7	0.646
MBL2 gene codon 54 polymorphism (rs1800450, c.161G>A)			
G/G genotype	73(72.3)	74(73.3)	0.705
G/A genotype	25(24.8)	22(21.8)	
A/A genotype	3(2.9)	5(4.9)	
G Allele	171(84.7)	170(84.2)	0.890
A Allele	31(15.3)	32(15.8)	

patient group, 73 (72.3%) had the G/Ggenotype, 25 (24.8%) had the G/A genotype and 3 (2.9%) had the A/A genotype. In the control group, 74 (73.3%) had the G/G genotype, 22 (21.8%) had the G/A genotype and 5 (4.9%) had the A/A genotype. With respect to genotype distribution, no significant difference was observed between the vitiligo patients and the controls (p=0.705). The frequency of the A allele was fifteen percent in the patient group and sixteen percent in the control group, hence similar rates in both groups were detected. No significant differences were observed between the patient and control groups with respect to the frequencies of the A allele (p=0.890) (Table 1).

DISCUSSION

Although the aetiopathogenesis of vitiligo has not been fully known so far, genetics factors and autoimmunity are thought to have an important role in the pathogenesis of vitiligo. Studies have shown the importance of genetic factors (positive family history) for development vitiligo (Nordlund 1997; Dwivedi et al. 2000; Njoo and Westerhof 2001). Yet, the abnormal immune responses seen in patients with vitiligo in some studies suggest that there might be an autoimmune component in development the disease. Moreover, many genes (such as ACE, CDH1, PTPN22, and HLA) have been linked to the development of vitiligo (LaBerge et al. 2008; Lv et al. 2013; Czajkowski and Mêciñśka-Jundzi[33] 2014; Tarlé et al. 2015). One of these, the PTPN22 gene, with its +1858C/T polymorphism, has been shown in studies to have an association with vitiligo and other autoimmune diseases. In the studies exploring the ACE I/D polymorphism, another gene polymorphism, have demonstrated that there is an increased risk of vitiligo in individuals who have the D/D genotype in particular. Even though it has been argued that the polymorphisms in many genes such as CTLA4, VDR and CDH1 contribute to the development of vitiligo, the aetiopathogenesis of vitiligo has not been fully clarified (Dwivedi et al. 2000; Birlea et al. 2011; Spritz 2012; Lv et al. 2013; Patwardhan et al. 2013; Czajkowski and Mêciñska-Jundzi[33] 2014; Garcia-Melendez et al. 2014; Rashed et al. 2015).

It has been suggested in some epidemiologic studies that the polymorphisms in the *MBL2* gene cause, by affecting MBL levels, proneness to infections and susceptibility to autoimmune diseases. Particularly the three structural variant genotypes of *MBL2* gene in exon 1 have been shown to reduce the MBL levels considerably as compared to a homozygous wild-type genotype (Dwivedi et al. 2000; Wallis 2002; Zhang et al. 2013). In the present study, the researchers investigated the relationship between the codon 54 (allele B: rs1800450) polymorphism of the *MBL2* gene, one of the innate immune system regulating genes, and susceptibility to vitiligo in Turkish patients. Only a few studies have discussed the association between the *MBL2* gene and vitiligo. Onay et al. (2007) have explored in their study the codon 54 and codon 57 polymorphisms in the *MBL2* gene. While they found no difference between the patient and control groups with respect to the codon 57 polymorphism and the disease, there was a statistically significant difference with respect to codon 54 polymorphism and vitiligo development. They argued that there might be a connection between disease development and codon 54 polymorphism. Dwivedi et al. (2009) have investigated in their study the codon 52, codon 54 and codon 57 polymorphisms in the *MBL2* gene. When they compared the patient and control groups, they found no statistical correlation between the three polymorphisms of the *MBL2* gene and vitiligo development. Similar to the study of Dwivedi et al. (2009), the researchers also did not find any relationship between the disease development and codon 54 polymorphism of the *MBL2* gene. When the researchers reviewed these studies in terms of allele frequencies, they observed that in the study by Onay et al. (2007) the B allele frequency was significantly higher in the patient group (20%) than in the control group (3%), whereas in the study of Dwivedi et al. (2009) the B allele frequencies of the groups were close to each (12% in the control group and 16% in the patient group). In this study, the allele frequencies were similar in the patient group (15%) and control group (16%). The differences between the results of these similar studies may have been because of the population variations occurring due to different geographical areas.

Relatively small numbers of patient and control group members and exclusion of other *MBL2* gene polymorphisms (codon 52 and codon 57) were the limitations of this study.

CONCLUSION

In conclusion, no significant difference was found between vitiligo patients and healthy controls in terms of the MBL codon 54 polymorphism. It can be considered that the codon 54 polymorphism in the *MBL2* gene may not confer a role in vitiligo susceptibility of the Turkish patients.

RECOMMENDATIONS

Future studies are needed with Turkish patients involving larger sample sizes and other *MBL2* gene polymorphisms as a disease susceptibility factor in vitiligo.

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