IGF2BP2 gene polymorphism in the pathogenesis of vitiligo

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ABSTRACT. Vitiligo is a disorder of pigmentation presenting with loss of melanocytes in the epidermis and hair follicles. The gene for insulin-like growth factor binding protein 2 (IGF2BP2) is located on the third chromosome and it plays role in growth, development, cellular differentiation, and metabolism. In the present study, we aimed to investigate the rs1470579 and rs4402960 gene polymorphisms of IGF2BP2 in the patients with vitiligo, which were confirmed to have a relationship with insulin resistance. The study was conducted with a total of 100 patients with vitiligo between the ages of 18 to 60 years and 100 healthy controls. Gene polymorphism was investigated in deoxyribonucleic acid (DNA) samples isolated from blood cells using the real-time polymerase chain reaction (PCR) method. There was no statistically significant difference in the genotype and allele frequencies of the rs1470579 and rs4402960 polymorphisms on IGF2BP2 between the groups ($p>0.05$). Our study results show that the IGF2BP2 gene has no significant role in the pathogenesis of vitiligo.

Key words: Vitiligo; IGF2BP2; Polymorphism

INTRODUCTION

Vitiligo is an acquired and idiopathic disorder of pigmentation with progressive course and high frequency, presenting with loss of melanocytes in the epidermis and hair follicles, which is characterized with depigmented macules with distinct margins (Bolognia JL, et al. 2008) Its frequency in Turkey has been reported to be 0.15% to 0.32% (Arıcan O, et al. 2003). The frequency of vitiligo is higher between the ages of 10 to 30 and it has generalized localized, universal and segmental clinical types. Although its etiopathogenetic has not been fully understood yet, degradation of melanocytes by the neurochemical mediators released from the nerves in skin, auto degradation-cytotoxicity caused by the toxic effects of some intermediate products and free radicals such as H_2O_2 released during melanin synthesis, and autoimmunity related with humoral and cellular immune systems are several theories which have been suggested (Falco OB, 2000). Current evidence indicates increased insulin resistance in patients with vitiligo Pietrzak A (2012). Karadag et al. [5], reported increased insulin resistance in non-diabetic patients with vitiligo.

Insulin-like growth factor (IGF) system consists of different groups, such as IGF-1, IGF-2, IGF-binding proteins (IGFBP) and IGF receptors (IGFR) (Florini JR, Fu Z, et al.). IGF-2 gene is known to play a role in the growth, development, cellular differentiation, and cellular metabolism Allan GJ, et al.

Insulin-like growth factor binding protein 2 (IGF2BP2) is a member of the mRNA-binding protein family, which involves functions related to the RNA localization, stability, and translation. The IGF2BP2 gene is located on the 3q27.2 chromosome, and it is about 181,3 kb, including 16 exons Christiansen J, et al. IGF2BP2 functions as the regulator of IGF2, by binding to the 5'UTR region of the IGF2 mRNA Zeggini E, et al.

Several studies have demonstrated that carriers with rs1470579 and rs4402960 mutations of the IGF2BP2 gene have the risk of type 2 diabetes mellitus. It has been shown that levels of insulin secretion may vary in patients with type 2 diabetes having different genotypes of IGF2BP2 Zhang LF, et al. Altogether, these literature data made us investigate whether IGF2BP2 gene rs1470579 and rs4402960 polymorphisms plays a role in the development of vitiligo.

METHODS

Participants

This study was approved by the local Ethics Committee, which is the Presidential of Non-Interventional Researches Ethical Committee, T.R. Fırat University (Date: 18.11.2014, No: 02). The study included a total of 200 participants, of whom 100 were patients between the ages of 18 to 60 years, who were diagnosed with vitiligo in the Dermatology Clinic, Hospital of School of Medicine, Fırat University, and 100 were healthy control individuals. An informed consent was obtained from everyone.

PCR analysis

Blood (2-3 cc) was withdrawn into the ethylenediaminetetraacetic acid (EDTA) tubes, and DNA was isolated in the Molecular Biology and Genetics Laboratory, using the DNA isolation kit (Pure Link™ genomic DNA kits). The purities and quantities of isolated DNA samples were evaluated using the micro-volume spectrophotometer. Ready-to-use master mix was used for quantitative polymerase chain reaction (QPCR), while real-time polymerase chain reaction device was used for the analysis (BIONEER brand ExiCycler96 Model Q-PCR). Samples of DNA were stored at -20°C until analysis.

The primers of related regions of mutation were then designed, and polymorphisms were analyzed by real-time PCR, using proper protocols. Single point mutations (SNP) were screened in the patients and control group using proper primer sets and probes, by multiplying the target sequences (Table 1).

Table 1. Primer sequences used for the IGF2BP2 gene regions.

A-T alteration

1	rs1470579(A) P1	CATACGAGTT [A] ATCCTGCCT
2	rs1470579(T) P2	CATACGAGTT [T] ATCCTGCCT

G-T alteration

1	rs4402960(G)-P1	ACAGTAGATT[G]AAGATACTGATT
2	rs4402960(T)-P2	ACAGTAGATT[T]AAGATACTGATT

Statistical analysis

Statistical analysis was performed using the SPSS version 22 (SPSS Inc., Chicago, IL, USA) software. Gene frequencies of each allele were determined and equilibrium control of the study population was assessed by the Hardy-Weinberg equilibrium test and Chi-square test. The frequency of each genotype was expressed in percent (%). A p value of <0.05 was considered statistically significant.

RESULTS

The present study included 100 patients with vitiligo in total composed of 52 women and 48 men. Of 100 healthy controls, 57 were women and 43 were men. The mean ages of the patients with vitiligo and healthy controls were 32.30 ± 12.54 years and 35.05 ± 11.13 years, respectively, indicating a non-statistically significant difference (p>0.05) (Table 2). The rs1470579 and rs4402960 genotype and allele frequencies in patients and control group are shown in Table 3. The biochemistry values of the patient and control groups are listed in Table 4.

Table 2. Clinical characteristics of patient and control groups.

Variables	Vitiligo	Control	P
N	100	100	
Sex (M/F)	52/48	57/43	p>0.05
Age* (year)	32.30□12.54	35.05 ± 11.13	p>0.05
BMI* (kg/m^2)	31.60□42.68	23.85 ± 4.01	p>0.05

BMI: Body Mass Index

Table 3. Distribution of the rs1470579 and rs4402960 genotypes and alleles in the patient and control groups.

Variables	Genotype Allele	Vitiligo (n=100)	Control (n=100)	X2	P
	AA	75(0.750)	74(0.740)		
	AT	14(0.140)	16(0.160)	0.187	0.91
rs1470579	TT	11(0.110)	10(0.100)		
	A	161(0.805)	158(0.790)	0.139	0.708
	T	39(0.195)	42(0.210)		
	GG	58(0.580)	70(0.700)		
	GT	11(0.110)	11(0.110)	4.005	0.135
rs4402960 TT	31(0.310)	19(0.190)			
	G	147(0.735)	159(0.795)	2.002	0.157
	T	53(0.265)	41(0.205)		

Table 4. Biochemistry results of patient and control groups.

Variables	Vitiligo	Control	P
Glucose*	114.18 ± 37.01	93.45 ± 14.218	p<0.001
Triglycerides*	150.17 ± 54.35	94.80 ± 39.46	p<0.001
(mg/dL)			
LDL-cholesterol*	113.11 ± 30.48	72.03 ± 28.71	p<0.001
(mg/dL)			
HDL-cholesterol*	50.65 ± 19.56	47.53 ± 7.60	p>0.05
(mg/dL)			

DISCUSSION

Vitiligo is a genetically and non-genetically associated multi-factorial disease. Degradation of melanocytes leads to depigmentation of the skin. Vitiligo is related with incomplete penetrance, more than one sensitive regions, and genetic heterogeneity. About 30% of the patients with vitiligo have also a family history. In the first genetic study on this subject, the susceptibility gene on the 17p13 chromosome was found to be SLEV1. The following studies have revealed which is related with the 1p31.3-p32.2 chromosome (AIS1 locus), and 7q and 8p chromosomes (AIS2 and AIS3 loci).

Rashad et al., determined that the frequency of ACE allele showed significant differences between the patients with vitiligo and healthy controls. In the study of (Lee et al., 2015) including patients with rheumatoid arthritis and vitiligo, TNF-α was found to play an important role in the pathogenesis of vitiligo (Li et al. 2015) demonstrated that the BSMI bb genotype was associated with the risk for vitiligo in the East Asian populations. In addition, a recent study has shown that the gene for Forkhead box protein 3 (FOXP3), which is important for cellular proliferation and functions of the CD4+ and CD25+ regulatory T cells, play a role in the pathogenesis of vitiligo as an immunoregulator (Jahan P, 2015). In another study, cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphism was not found to contribute to the pathogenesis of vitiligo (Liang J, et al. 2015). In another genetic research, a family has been defined with the heterozygous mutation for the suppressor gene of cytokine signaling-4 (SOCS4), and having autoimmune diseases like vitiligo, alopecia, and hypothyroidism (Arts P, et al. 2015).

CONCLUSION

The review of literature revealed no study investigating the possible relationship between the IGF2BP2 gene and vitiligo. Our study is the first which addressed to us found that the alleles of rs1470579 and rs4402960 gene regions on IGF2BP2 were not significantly different in terms of statistical results between the patient and control groups. However, there was a statistically significant difference in glucose, triglyceride, and low-density lipoprotein levels between the patient and control groups. Therefore, we conclude that the IGF2BP2 gene does not have a significant role in the pathogenesis of vitiligo. However, further studies are required to establish a definite conclusion.

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