

## -119C/G in MYG1 Gene and 49A/G in CTLA4 Gene Polymorphisms in Turkish Patients with Vitiligo

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### ABSTRACT

Vitiligo is a disease characterized by sharply limited white macular depigmentation as a result of melanocyte loss. Disease is divided into three types according to prevalence and localization as localized, general and universal. Etiology of vitiligo is unknown, but clearly shows a complex structure. Genetic predisposition, stress, many influencing factors such as systemic diseases and physical trauma causes vitiligo. In our study, MYG1 gene rs1465073 (-119C/G) and CTLA4 gene rs231775 (49A/G) polymorphisms were evaluated in 106 patients diagnosed with vitiligo and 97 controls. Genotyping was performed using real time PCR method. There is no statistically significant differences between patients and controls in terms of allele frequencies of MYG1 gene rs1465073 and ve CTLA4 gene rs231775 polymorphisms. Beside, there is no statistically significant differences between patients and controls in terms of genotype frequencies of CTLA4 gene rs231775 polymorphism. On contrary to this there is statistically significant differences between patients and controls in terms of genotype frequencies of MYG1 gene rs1465073 polymorphism.

In conclusion, consistent with the literature data, there is a significant association between MYG1 gene rs1465073 polymorphism and vitiligo. But there is no direct association between CTLA4 gene rs231775 polymorphism and vitiligo.

### INTRODUCTION

Vitiligo is a common acquired depigmentation disorder of the skin manifested by the presence of white macules.<sup>1</sup> Vitiligo is divided clinically into two main forms, segmental vitiligo (SV) and non-segmental vitiligo respectively. The latter also includes three major subsets, namely generalized vitiligo, acrofacial vitiligo, and universal vitiligo.<sup>2</sup> The prevalence of this disease

varies from 0.2% to 1% in various global populations without sex predilection.<sup>3</sup> Njoo and Westerhof<sup>4</sup> reported that the etiology of vitiligo is still unknown, but genetic factors, oxidative

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stress, autoimmunity, neurological factors, toxic metabolites and lack of melanocyte growth factors might contribute for precipitating the disease in susceptible people. The genetics of vitiligo can not be explained by simple Mendelian genetics, and it is characterized by incomplete penetrance, multiple susceptibility loci, and genetic heterogeneity factors.<sup>5</sup>

The first study reported genetic association of vitiligo with single nucleotide polymorphisms (SNP) within *CTLA4*.<sup>6</sup> *CTLA4* is a negative regulator of T-cell function, which is suggested to be involved in susceptibility to autoimmune diseases including vitiligo.<sup>7</sup> Many polymorphisms have been identified in the *CTLA4* gene, and one of these polymorphisms +49A/G (rs231775) have been studied in vitiligo.<sup>6-13</sup> Also this polymorphism have been studied in several diseases such as anti-glutamic acid decarboxylase antibody-associated encephalopathy,<sup>14</sup> systemic lupus erythematosus,<sup>15</sup> type 1 diabetes,<sup>16</sup> renal cell carcinoma,<sup>17</sup> Addison's disease,<sup>18</sup> rheumatoid arthritis.<sup>19</sup>

Smicun<sup>20</sup> reported that *MYG1* is a highly expressed gene in freely proliferating melanocytes and down regulated in malignant melanoma cells suggesting that *MYG1* is a melanocyte specific gene. Saaf et al<sup>21</sup> reported that *MYG1* has been consistently up-regulated in skin biopsies from patients with atopic eczema that is a common inflammatory skin disorder. Kingo et al<sup>22</sup> have shown that elevated expression of *MYG1* mRNA in both uninvolved and involved skin in case of vitiligo. Philips et al<sup>23</sup> reported that there are fragmentary expression data from cell cultures, animal and human studies indicating that *MYG1* could be involved in early cellular processes starting from two to four cell embryos but also in adult pathological or stress conditions. Philips et al<sup>24</sup> have proposed that *MYG1* gene may be involved in vitiligo pathogenesis.

To determine whether *CTLA4* and *MYG1* genes are the genetic risk factor for vitiligo in Turkish study group, we analyzed *CTLA4* rs231775 and *MYG1* rs1465073 polymorphisms in patients with vitiligo and healthy controls.

## MATERIALS AND METHODS

### Sample Collection and Genotyping

For the case-control cohort, subjects were recruited at Afyon Kocatepe University Department of Dermatology and Akşehir State Hospital between February 2014 and Jun 2015. Patients were carefully screened, Wood's lamp used to detect vitiligo and those with a history of autoimmune disease were excluded from this study. Patients with vitiligo (n=106) were asked to participate in this case-control study. Patients

attending the related departments for other reasons were included as control subjects (n=97). All participants provided signed informed consent for study before blood collection and studied under a protocol approved by the Afyon Kocatepe University Medical Ethic Committee. The study adhered to the tenets of the Declaration of Helsinki.

2ml aliquots of peripheral blood samples were collected from the participants and stored in EDTA-coated vacutainers. Genomic DNA was extracted from a 200µl peripheral blood sample by using a EZ1 DNA Blood 200 µl kit (Qiagen, GmbH, Germany). Then, DNA amount and DNA purity were quantified for each DNA sample by Nanodrop ND-1000 spectrophotometer V 3.7 (Thermo Fisher Scientific; DE, USA). DNA samples were stored at -20°C until use. Each genomic DNA sample was analysed for *CTLA4* rs231775 and *MYG1* rs1465073 polymorphisms.

### Genotyping

*MYG1* rs1465073 genotyping was performed by real-time polymerase chain reaction (PCR) on a LightCycler 480 Real-Time PCR System (Roche Diagnostics, Vienna, Austria) using LightCycler\_ FastStart DNA Master HybProbe, (Roche Diagnostics, Germany), LightSNIP rs1465073 *MYG1* Reagent Mix (Tib Molbiol, Germany). Amplicon was determined with fluorescence using specific probes that hybridized at the annealing phase of PCR cycle. After preparation of the master mixture (1.0 µl Reagent Mix, 2.0 µl FastStart DNA Master HybProbe, 1.6 µl 25mM MgCl<sub>2</sub> and 13.4 µl sterile PCR-grade H<sub>2</sub>O), 18 µl of the reaction mixture and 2 µl of the isolated genomic DNA template or the control template were loaded to 96-Well Plate for PCR analysis. System under the following thermocycling conditions: 10 s at 95°C for DNA denaturation followed by 45 cycles of PCR (10 s denaturation at 95°C, 10 s annealing at 60°C, and 15 s extension at 72°C). After the PCR, a melting curve analysis was performed by heating to 95°C for 20 s followed by cooling to 40°C for 20 s to achieve maximum hybridization and then heating slowly at 0.2°C/s to 85°C. After the melting curve analysis, a final cooling was performed at 40°C for 30 s. The fluorescence signals recorded in the respective channels were then converted to melting peaks by plotting the negative derivative of the fluorescence with respect to the temperature ( $-dF/dT$  vs  $T$ ). The results of melting peaks in the different fluorescence channels allowed us to discriminate among the homozygous as well as the heterozygous genotypes.

Genotyping for *CTLA4* rs231775 with the snipsig kit (Qiagen, California) by Rotor Gene-Q (Qiagen, Hilden, Germany), a

15 µl reaction mixture was performed. The reaction mixture contained 10 µl 2X precision master mix, 1 µl genotyping primer/probe mix, 4 µl RNase/DNase free water, 5µl genomic diluted DNA in water according to positive control. System under the following thermocycling conditions for *CTLA4* rs231775: 2 minutes at 50°C and 5 minutes at 95°C for qPCR enzyme activation followed by 45 cycles of PCR (15 seconds denaturation at 94°C, 60 seconds extension at 60°C, Data collection). Allelic Discrimination Analyse was performed for detection the polymorphism.

### Statistical Analysis

Allele and genotype frequencies were estimated by an allele counting method. Odds ratios were computed for estimating the risk of vitiligo with respect to different genotypes. Statistical analysis was performed using the SPSS 18.0 program. In patients and controls allele and genotypic frequencies related to studied polymorphisms were compared using  $\chi^2$  test.

## RESULTS

Genotype and allelic frequencies of rs231775 polymorphism of the *CTLA4* gene were evaluated in 106 vitiligo patients and 97 healthy controls. rs231775 polymorphism has three possible genotypes AA, AG and GG. The distribution of genotypic frequencies of rs231775 polymorphism in the controls was 41,2% for AA, 45,4% AG for, and 13,4% for GG and in the case group the distribution of genotypic frequencies was 49,1% for AA, 42,5% AG for and 8,5% for GG. There were not significant differences between cases and controls in terms of genotype frequencies ( $P>0.05$ ). The distribution of allele frequencies of rs231775 polymorphism in the controls was 63,91% for A and 36,09% for G, and in the case group the distribution of allelic frequencies was 70,29% for A and 29,71% for G. There were no significant differences between cases and controls in terms of allele frequencies ( $P>0.05$ ).

Genotype and allelic frequencies of rs1465073 polymorphism of the *MYG1* gene were evaluated in 106 vitiligo patients and 102 healthy controls. rs1465073 polymorphism has three possible genotypes CC, CG or GG. The distribution of genotypic frequencies of rs1465073 polymorphism in the controls was 13,8% for CC, 45,7% for CG and 40,4% for GG, and in the case group the distribution of genotypic frequencies was 36,8% for CC, 17,9% for CG and 45,3% for GG (Figure 3). There were significant differences between cases and controls in terms of genotype frequencies ( $P<0,05$ ). The distribution of allele frequencies of rs1465073 polymorphism in the controls was 36,70% for C and 63,29% for G, and in the case group the distribution of allelic frequencies was 45,75% for C and 54,24%

for G.

We obtained the family information of all the vitiligo patients. In all, 29 of 106 has a history of vitiligo in their family. Comparison of rs231775 and rs1465073 polymorphisms in terms of genotype/allele frequencies between the two patient groups, with or without a family history of vitiligo, shows that, there were no significant differences between two groups.

## DISCUSSION

The researchers are trying to explain the pathogenesis of vitiligo with different hypotheses. The three main prevailing theories of pathogenesis of vitiligo are centered on neurochemical, autoimmune and oxidative stress aspects, but none of these hypotheses explain the entire spectrum of the vitiligo disorder.<sup>25</sup> Also Spritz<sup>26</sup> reported that the most popular theory of the vitiligo development is a multifactorial hypothesis according to which genetic conditions predispose vitiligo macules to occur as a result of specific environmental factors. Possible connections between *CTLA4* and several autoimmune diseases have been demonstrated, which suggests that *CTLA4* may play a role in regulating self-tolerance by immune system and in regulating immune system involvement in the pathogenesis of many disorders.<sup>27</sup> Song et al<sup>13</sup> suggested that it appears that defective *CTLA4* expression, function or both are associated with autoimmune diseases including vitiligo. Blomhoff et al<sup>8</sup> suggested that vitiligo when not associated with an autoimmune disorder is not influenced by *CTLA4* polymorphism. Birlea et al<sup>12</sup> showed that association of generalized vitiligo with *CTLA4* rs231775 seems to be secondary to epidemiological association with other concomitant autoimmune diseases. Also, Kemp et al<sup>6</sup> noted that there is no association between the *CTLA4* microsatellite polymorphisms and vitiligo, at least when the disorder occurs in the absence of an autoimmune disease. LaBerge et al<sup>9</sup> examined *CTLA4* SNPs including rs231775 for association generalized vitiligo and the expanded vitiligo-associated autoimmune phenotype. None of the SNPs tested showed association. In our study none of the vitiligo patients has concomitant autoimmune disease. Beside, we did not find an association of genotype and allele frequencies for rs231775 polymorphism with vitiligo patients. Our results are in line with some studies.<sup>7,10,11,13</sup> Contrary to this, Itrli et al<sup>28</sup> reported that *CTLA4* microsatellite polymorphisms are associated with vitiligo in Turkish patients.

*MYG1* gene contains 10 polymorphisms that are defined as SNPs but two polymorphisms are potentially functional. SNP is located at 119 bp upstream of *MYG1* translation start site (ATG) and *MYG1* promoter polymorphism -119C/G (rs1465073).<sup>24</sup>

When searching the literature, it is shown that there are few study about *MYG1* gene -119C/G (rs1465073) polymorphism related to vitiligo. Philips et al. (24) reported that promoter polymorphism 119C/G in *MYG1* gene is associated to vitiligo susceptibility. Diwivedi et al<sup>29</sup> studied rs1465073 polymorphism in 846 vitiligo patients and 726 controls and they reported that *MYG1* -119C/G promoter polymorphism was may be a genetic risk factor for susceptibility and progression of vitiligo. In our study, there were significant differences between cases and controls in terms of genotype frequencies of -119C/G (rs1465073) polymorphism. Despite the small sample size, finding significant association between studied polymorphism and vitiligo shows that this polymorphism have potential risk for vitiligo.

Vitiligo occurrence among members of patients' families indicates that genetic factors play a role in pathogenesis of the disease.<sup>30</sup> Nath et al<sup>31</sup> reported that the relative risk of vitiligo for the first degree relatives of patients increased by at least 7-10 fold. Contrary to this, comparison of rs231775 and rs1465073 polymorphisms in terms of genotype/allele frequencies between the two patient groups, with or without

a family history of vitiligo, shows that, there were no significant differences between two groups.

In conclusion, our study has demonstrated a significant correlation between *MYG1* rs1465073 polymorphism and vitiligo, but there was no association for *CTLA4* rs231775 polymorphism. However, these polymorphisms must be studied with more number of cases or cell cultures for expression data, may be with another probable candidate genes.

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