Original Article

Major Histocompatibility Complex Class I-Related Chain A and Macrophage Migration Inhibitory Factor Gene Polymorphisms in a Turkish Patient Population with Vitiligo

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Abstract

Background: Autoimmunity has been implicated in the etiopathogenesis of vitiligo. **Aim:** We sought to determine whether polymorphisms in the major histocompatibility complex class I-related chain A (MICA) and macrophage migration inhibitory factor (MIF) genes may have a role in the pathogenesis of vitiligo. **Materials and Methods:** We conducted a study including 100 patients with vitiligo and age- and sex-matched 172 control subjects to examine the role of single-nucleotide polymorphisms of MICA gene rs1051792 and MIF genes rs755622 and rs2096525 as risk factors for vitiligo. Real-time PCR combined with the melting curve analysis using fluorescence-labeled hybridization probes was used for genotyping analyses. Mann–Whitney, Kruskal–Wallis, and chi-square (χ^2) tests as well as multivariate logistic regression adjusted for age and gender were used for statistical evaluation. Linkage disequilibrium (LD) and haplotype frequencies were also performed. **Results:** No significant association was observed between the variant alleles of studied genes and vitiligo. Haplotype analysis demonstrated that there was a strong LD between rs755622 and rs2096525 loci of MIF gene ($D' = 0.92$, $r^2 = 0.827$). However, haplotype frequencies in patients were similar to those in controls. **Conclusion:** These preliminary results suggest that the polymorphic variants of MIF rs755622, MIF rs2096525, and MICA rs1051792 genes do not play a critical role in the etiopathogenesis of vitiligo.

Keywords: Gene polymorphism, MHC class I-related chain A, MIF protein, vitiligo

INTRODUCTION

Vitiligo is an acquired cutaneous disorder with a 0.5%–2% incidence worldwide, characterized by the presence of depigmented skin macules due to the loss of functional melanocytes from the epidermis.[1] Although the exact etiopathogenesis has not been clearly elucidated yet, autoimmune response triggered by genetic and environmental factors, directed to melanocytes, is the strongest theory. A hereditary immune defect influencing T and B lymphocyte stimulation and causing either antibody-dependent cytotoxicity or direct T-cell destruction is the main alleged mechanism. However, the precise defects in immune tolerance, which mediate this uncontrolled self-reactivity, are still lacking.

The major histocompatibility complex class I-related chain A (MICA), an oxidative stress-inducible antigen, has been found to contribute to the susceptibility and severity of alopecia areata (AA) and vitiligo.[2,3] On the other hand, macrophage migration inhibitory factor (MIF) is reported to have a role[4,5] in vitiligo and AA and a possible biomarker of vitiligo activity and severity.[6,7] Actually, the literature on MIF is mainly on increased levels of it in blood or serum. However, MIF gene polymorphisms have been found to be associated with disease susceptibility in an active nonsegmental vitiligo

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in Mexico, as well.[8] There are many studies on genetic susceptibility genes, which in certain environments may trigger the immune system to attack "self-antigens" of the pigmentary system, resulting in the development of vitiligo. Because melanocytes are specific targets in both AA and vitiligo, in this study, we aimed to analyze if there is an association between specific MICA or MIF polymorphic alleles and vitiligo in a Turkish patient population.

MATERIALS AND METHODS

The study was approved by the local ethics committee. All the patients and controls were recruited from a single center and all provided written informed consent. A total of 100 patients with vitiligo (53 women, 47 men) were enrolled in the study. Vitiligo patients older than 18 years, showing any clinical picture except segmentary type, and who were not on systemic or topical therapy during previous 2 months were included in the study. Vitiligo was diagnosed on clinical grounds at the Department of Dermatology. The control group consisted of 172 age- and sex-matched dermatology outpatients (88 women, 84 men), with no past history of any systemic, infectious, autoimmune, genetic, or atopic disease. A negative family history for vitiligo was also provided. Patients taking any medication including vitamins were excluded. The main diagnoses in the control group were melanocytic nevus and fibroepithelial polyps.

Peripheral venous blood samples were taken in the morning subsequent to an overnight (12h) fast in EDTA-K$_3$ tubes for genotype analysis. Genomic DNA was isolated from peripheral blood leukocytes by using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). For the detection of the MIF rs755622, MIF rs2096525, and MICA rs1051792 polymorphisms, light SNP assays were used. Light SNP assays are based on simple probe melting curve analysis. They consist of premixed primers and probes. They were developed and optimized according to NCBI "rs" numbers of mentioned polymorphisms by TIB MolBiol (Berlin, Germany). The detection of polymorphisms was performed in a LightCycler (Roche Diagnostics, Mannheim, Germany).

Mann–Whitney U, Kruskal–Wallis, chi-square (χ^2) tests, and multivariate logistic regression analysis estimating age, gender, and smoking status adjusted odds ratio (aOR) were used for statistical evaluation. The statistical significance for deviations from the Hardy–Weinberg equilibrium (HWE) was determined using the Pearson χ^2-test. These statistical analyses were performed with SPSS statistics for Windows (version 21; SPSS Inc., Chicago, IL, USA). Linkage disequilibrium (LD) and haplotype frequencies were estimated using the Haploview software and compared between cases and controls using a contingency χ^2-test.[9] In addition, the NCSS 2000 statistical package (Kaysville, Utah, USA) was used to evaluate the power analysis.

RESULTS

A total of 272 subjects (100 vitiligo and 172 controls) were included in this study. Table 1 depicts the clinical characteristics of the vitiligo patients including the clinical type of the disease, duration, family history, leukotrichia, stability within 1 year, and associated diseases. The mean ages were 38.6 ± 12.0 years (range = 18–76) and 38.5 ± 9.1 years (range = 18–72 years) for patients and controls, respectively. There was no significant difference among the study and control groups in terms of mean age and sex distribution. We had 85% power to detect an effect size (W) of 0.20 using two degrees of freedom (α = 0.05). The genotypic and allelic distributions of MIF rs755622, MIF rs2096525, and MICA rs1051792 polymorphisms for patients and controls are shown in Table 2. All genotype distributions were in accordance with the HWE among the controls and patients. We did not find any associations between vitiligo and variant alleles of MIF rs755622 (aOR = 0.87, 95% CI = 0.53–1.45), MIF rs2096525 (aOR = 0.80, 95% CI = 0.48–1.34), and MICA rs1051792 (aOR = 0.96, 95% CI = 0.66–1.38). In patients with vitiligo, the evaluation of the relationships between the studied polymorphisms and the disease onset, family history, the clinical type of disease, the presence of leukotrichia, the presence of associated disease, and changes in the vitiliginous area within past 1 year showed no significant difference (data not shown).

Table 1: Characteristics of the patients with vitiligo

	Vitiligo (*n* = 100)
Age (years)	
Mean ± SD	38.6±12.0
Range	18–76
Disease onset	
<40 years, *n* (%)	81 (81.0)
>40 years, *n* (%)	19 (19.0)
Duration, years (mean ± SD)	9.95±11.4
Sex	
Male, *n* (%)	47 (47.0)
Female, *n* (%)	53 (53.0)
Family history, *n* (%)	26 (26.0)
Clinical type of disease	
Focal	25 (25.0)
Acrofacial	25 (25.0)
Generalized	50 (50.0)
Leukotrichia	33 (33.0)
Change in the vitiliginous area within past 1 year	
Newly diagnosed cases, <1 year	21 (21)
Enlargement	49 (49)
No enlargement	23 (23)
Repigmentation	7 (7)
Presence of associated disease	66 (66.0)

Enlargement: Increase in the vitiliginous area within past 1 year

**Table 2: Distribution of genotypes and allele frequencies for patients with vitiligo and control group**

	Controls, *n* (%)	Patients with vitiligo, *n* (%)	aOR (95% CI)*	*P* value
MIF rs755622 (-173G/C)				
CC	123 (75.5)	74 (74.0)	1.0[a]	-
CG	46 (26.7)	25 (25.0)	1.08 (0.61–1.92)	0.78
GG	3 (1.8)	1 (1.0)	1.54 (0.16–15.28)	0.71
CG + GG	49 (28.5)	26 (26.0)	1.11 (0.63–1.93)	0.73
C allele frequency	0.85	0.87	1.0[a]	
G allele frequency	0.15	0.13	0.87 (0.53–1.45)	0.61
MIF rs2096525				
TT	122 (70.9)	76 (76.0)	1.0[a]	-
CT	48 (27.9)	23 (23.0)	1.30 (0.73–2.31)	0.38
CC	2 (1.2)	1 (1.0)	1.00 (0.09–11.52)	0.99
CT + CC	50 (29.1)	24 (24.0)	1.29 (0.73–2.28)	0.38
T allele frequency	0.85	0.88	1.0[a]	
C allele frequency	0.15	0.12	0.80 (0.48–1.34)	0.40
MICA rs1051792 (129Met/Val)				
GG	76 (42.2)	41 (41.0)	1.0[a]	-
AG	68 (39.5)	48 (48.0)	0.78 (0.46–1.34)	0.37
AA	28 (16.3)	11 (11.0)	1.38 (0.62–3.06)	0.43
AG + AA	96 (55.8)	59 (59.0)	0.90 (0.54–1.48)	0.67
G allele frequency	0.64	0.65	1.0[a]	-
A allele frequency	0.36	0.35	0.96 (0.66–1.38)	0.81

Each *P* value was based on chi-square (χ^2) analysis

aOR = adjusted odds ratio

*Adjusted for age and gender

[a]Reference values for aOR
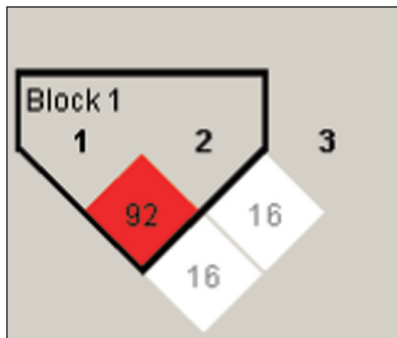
Figure 1: Haplotype analysis and LD patterns (estimated as r² and D′) of MIF rs755622 and rs2096525 (block 1). There was strong LD between rs755622 and rs2096525 (D′ = 0.92, r² = 0.827) of MIF gene (the number of boxes indicates decimal places of D′)

Lewontin's standardized disequilibrium coefficient (D′) was calculated as a measure for LD between the rs755622 and rs2096525 polymorphisms in the MIF gene [Figure 1]. The rs755622 and rs2096525 were found to be in strong LD (D′ = 0.92, r² = 0.827). Haplotype frequencies are shown in Table 3. The most frequent haplotype among the patients and controls was CT (0.860 and 0.837, respectively). There were not any significant differences in the haplotype frequencies between patients and controls.

DISCUSSION

Vitiligo is an autoimmune disease against melanocytes. So far, neither the triggering mechanism of T-cell-mediated destruction of melanocytes nor the gene polymorphisms disrupting regulatory mechanisms are clearly delineated. Research on new molecules is still needed.

MICA is a highly polymorphic cell surface glycoprotein of nonclassical MHC class I gene in humans. MICA gene encodes a ligand for NKG2D (NK group 2, member D) receptor, that is expressed mainly on natural killer (NK) cells, γδ T cells, and CD8⁺ αβ T cells. In this way, MICA attracts cytotoxic or NK cells bearing NKG2D.[10] The role of NK cell activation has been shown in nonsegmental vitiligo,[11] and MICA-interacted NKG2D⁺ CD8⁺ skin effector memory T cells were increased in vitiligo skin.[12] On CD8+ αβ T cells and NK cells, NKG2D signaling elicits the target cell lysis.[13-15] Indeed, compared with healthy controls, increased melanocyte-specific CD8+ T cells were found in the blood of patients with vitiligo, and it was correlated with disease activity.[16-18] The upregulation of MICA contributing to melanocyte destruction has been reported in vitiligo.[3]

It is certain that polymorphisms in various genes may reduce the threshold for lymphocyte activation and thereby increase the susceptibility for autoimmunity. The single-nucleotide polymorphism (SNP) rs1051792 causing

Table 3: Haplotype analysis of the MIF rs755622 and rs2096525 polymorphisms in patients with vitiligo and control subjects

Haplotype	Frequency of haplotype	Frequency in controls	Frequency in vitiligo	*P* value
MIF rs755622/rs2096525				
CT	0.845	0.860	0.837	0.48
GC	0.132	0.120	0.139	0.52
GT	0.013	0.015	0.012	0.74

Haplotype frequencies and haplotype association analyses were estimated using Haploview software

a valine (Val) to methionine (Met) exchange at position 129 of the MICA protein is of specific interest. It separates MICA into isoforms that bind NKG2D with high (Met) and low affinities (Val).[19] Thus, it has recently been shown that the MICA-129 Met variant elicits a stronger NKG2D signaling, resulting in a faster costimulation of CD8+ T cells.[19]

Accordingly, MICA-129 (rs1051792) polymorphism was a reasonable suspect, and it was not investigated in vitiligo before. However, MICA-129 polymorphism did not show a statistically significant difference between vitiligo and control group. Some studies had suggested that NK cells may play a trigger role at the beginning of the autoimmune process, so we reanalyzed our patients putting them in two groups, according to the duration of the illness, whether 1 year or longer. Even so, no significant difference was detected between the two groups. Our results do not suggest a role for MICA 129 SNP in the etiopathogenesis of vitiligo.

Macrophage MIF is another regulatory cytokine functioning in innate and adaptive immunity.[20] MIF activates lymphocytes, granulocytes, monocytes/macrophages, and concentrate macrophages at the inflammation loci.[5,20]

Research indicates that MIF is implicated in the pathogenesis of autoimmune diseases by a couple of mechanisms.[21] Macrophage infiltration has been demonstrated in vitiligo lesions, and increased macrophage numbers are also observed in perilesional vitiliginous skin.[22] MIF can inhibit the random migration of macrophages, leading to the accumulation of them at the inflammation area and contribute to the pathogenesis of vitiligo.[5] MIF itself also functions as an initial proinflammatory mediator, which upregulates many cytokines, including TNF-α and IL-6 that have been shown to be increased in vitiliginous skin[5] and serum in vitiligo vulgaris patients.[23] Third, MIF may take part by inhibiting NK cells and causing prolonged inflammation and macrophage infiltration in the area, which all end up with the tissue destruction and the release of new antigens. This is called epitope spreading and is a particular mechanism facilitating the progression of autoimmune reactions.[24]

In vitiligo, MIF levels detected in the peripheral blood mononuclear cells, serum, and the skin of vitiligo patients were associated with disease severity and activity.[5] Recent studies repeated the same conclusion that serum MIF levels were correlated with the severity and activity of vitiligo.[6,7] Likewise, in another controlled study, serum MIF levels in vitiligo patients were associated with the duration and clinical type of the disease.[4]

Genetic factors are known to influence circulating MIF levels and the disease susceptibility. *In vitro* and *in vivo* studies also suggested that rs755622 found in the promoter region of the gene was associated with an increased gene expression and protein levels of MIF.[25] A very recent study showed that MIF polymorphisms rs5844572 and rs755622 were associated with vitiligo susceptibility in active nonsegmental vitiligo.[8]

In this study, we investigated the role of MIF gene polymorphisms (rs755622, rs2096525), but we did not find a statistically significant difference between the two groups in terms of genotype and allele distributions.

In our study, we also performed extended haplotype analysis for the MIF gene loci. The most frequent haplotype among the patients and controls was CT (0.860 and 0.837, respectively). The rs755622 and rs2096525 were found to be in strong LD (D′ = 0.92, r² = 0.827). However, there were not any significant differences in the haplotype frequencies between patients and controls.

The main limitation of the study is the lack of serum measurements of MICA and MIF. It is difficult to make an interpretation as the relationship between the three studied genetic polymorphisms of MICA/MIF genes and the serum levels of them cannot be clearly revealed. Moreover, the incriminated SNPs may not be relevant in our study population.

Despite the accumulated data pointing to the important role for MICA and MIF proteins in vitiligo, our results do not support any significant relationship in terms of MICA or MIF gene polymorphisms. The decision to either promote or inhibit autoreactive T cells is probably dependent on a wide variety of factors, which we partially know. Furthermore, the reason causing continuous immune response is also not clear. The identification of new receptors, ligands, or even cell subsets and factors affecting the vulnerability of target cells might help clarify the process.

Conclusions

We studied three genetic polymorphisms of MICA and MIF genes related with NK cell activity, but we were

unable to detect any significant association. Because of the complex nature of the autoimmune processes, we prefer to avoid clear-cut conclusions. Yet, we can at least say these MIF rs755622, MIF rs2096525, and MICA rs1051792 SNPs do not seem to play a critical role in the etiopathogenesis of vitiligo. We think these results should be assessed as preliminary, because of the relatively small sample size, and there is a need for further larger-scale studies including other loci of MIF and MICA genes.

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Conflicts of interest

There are no conflicts of interest.

Author contribution

İEA, İB, PV, SD: Concept and design. İEA: Clinical studies, manuscript preparation. İB, PV, SD: Experimental studies and statistical analysis. İEA, İB, PV, SD: Data analysis, manuscript editing and manuscript review.

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