Genetic Variations of Vitiligo among Saudi Patients

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Abstract: Vitiligo is a skin disease in which melanocytes (MCs) are eradicated from lesional epidermis, resulting in disfiguring loss of pigment. Our study contained 141 unrelated Saudi young-aged vitiligo patients and 42 healthy volunteers of matched age range. Genotyping the *TAP*1 (C>T) and *LMP*7 (G>T intron 6), was performed with genomic template using PCR/RFLP assays. Our results showed no significant difference between *TAP1* C>T (intron 7) or *LMP7* G>T alleles and healthy controls (p= 0.49 and 0.82, respectively). The OR for the genotypes of the *TAP*1 (C>T) were 1.6 (95% CI= 0.62 to 4.21). The OR of *LMP*7 (intron G>T) genotypes were 1.1 (95% CI= 0.65 to 1.73). However, a major contribution of both *TAP1* and *LMP*7 polymorphisms to vitiligo susceptibility cannot be excluded. Further studies of other alleles within the *TAP* and *LMP* gene regions in Saudi patients is recommended to demonstrate a possible role for MHC class I antigen processing and/or presentation pathway in the antimelanocyte autoimmune response in vitiligo pathogenesis.

Key words: Vitiligo · Polymorphism · *TAP1* · *LMP7* · Genetics

INTRODUCTION

Vitiligo is an acquired cutaneous disease in which melanocytes of the skin are destroyed resulting in melanotic lesions of variable size. It affects both genders and about 1% of the population in all ethnic groups worldwide [1]. The onset of vitiligo usually occurs from age 15 to 25, however it can present as early as infancy and as late as the sixth decade of life. It has a variable clinical spectrum both in severity and in localization [2].

Vitiligo is a multifactorial disease with an etiology that is poorly understood. Two principal hypotheses concerning the etiology of vitiligo include: 1) the self-destruct model, which suggests that biochemical and/or structural defects inherent to patient melanocytes contribute to the initiation and/or progression of melanocyte cytolysis; and 2) the autoimmune model, which suggests that melanocyte death occurs through inappropriate immune system destruction of pigment cells. There is considerable evidence that disease progression in some vitiligo patients involves autoimmune attack of the melanocytes, as evidenced by the presence of both cellular and humoral antimelanocyte autoimmune responses [3]. Another mechanism is the generation of specific quinine and indoles intermediates that can

themselves be cytotoxic to melanocytes. Therefore, elevated oxidative stress resulting from the increased generation of these intermediates is above the threshold that can be combated by genetically susceptible vitiligo melanocytes and consequently cytotoxicity/cell death is induced [4]. In the majority of cases, the triggering factor is not known and cases are classed as idiopathic [5].

Familial clustering of vitiligo has been largely observed. The current dogma is that there is a genetic component(s) that renders the melanocyte fragile and susceptible to apoptosis, that in turn predisposes individuals to developing the disease. About 20% of vitiligo patients have at least one first-degree relative also affected and the relative risk of vitiligo for first-degree relatives of vitiligo patients is increased by at least seven to 10-folds [6].

Vitiligo is not inherited by a simple Mendelian mechanism; rather inheritance patterns demonstrate a complex expression. A number of genes have recently been implicated in vitiligo, including *VIT1*, *catalase*, *tenascin* and *FOXD3*. Furthermore, linkage disequilibrium studies have consistently found a significant association between the histocompatibility antigen (HLA) system and a predisposition to Vitiligo [7].

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Human leucocyte antigen (HLA) molecules are peptide-binding proteins on the surfaces of antigen-presenting cells. The complex of an HLA molecule and bound antigenic peptide form a specific target for T cell recognition [8]. Major histocompatibility complex (MHC) class I molecules are cell surface glycoprotein, which bind intracellularly processed peptides and present them on the cell surface to cytotoxic T lymphocytes. Class I molecules, therefore, play a key role in immune recognition of virally infected and transformed cells. Two groups of proteins that participate in the antigen processing are low molecular weight polypeptides (*LMP*s) and transporters with antigen processing (*TAP*) [9]. Previous study reported a case-control and family-based genetic association for the *TAP*1, *TAP*2, *LMP*7, *LMP*2 and *MECL1* genes in human vitiligo patients [6]. The *LMP/TAP* gene region was found to be significantly associated with vitiligo in Caucasian patients, suggesting a possible role for MHC class I antigen processing and/or presentation in the antimelanocyte autoimmune response involved in vitiligo pathogenesis.

TAP is an IFN-γ inducible heterodimer made up of two subunits, *TAP*1 and *TAP*2, which both must be present for peptide binding and translocation. *TAP*1 and *TAP*2 genes encode a heterodimer molecule that forms a heterodimeric complex for delivering antigenic peptides to the endoplasmic reticulum prior to the assembly of class I molecules. The *TAP* complex preferentially binds peptides of certain lengths and of certain amino acid composition [10]. These *TAP*/MHC class I-preferred peptides are produced at a higher rate with the IFN-γ. *TAP*1 and *TAP*2 were also chosen as candidate genes due to their reported associations with other autoimmune diseases such as, celiac disease, Sjogren's syndrome [11] and multiple sclerosis [12], as well as due to their location within the MHC class II genomic region. Downregulation of *TAP*1, *TAP*2, *LMP*2 and *LMP*7 was found to suppress MHC class I molecule surface expression [9].

The nature of the genetic association may vary according to different ethnic backgrounds. In this unreeled study, we investigated the role of *LMP*7/*TAP*1 genes of MHC class I in the pathogenesis of vitiligo Saudi patients.

MATERIAL AND METHODS

Our study samples were selected from outpatient-clinics of different areas among Saudi Arabia. The study included 141 unrelated patients with vitiligo (age range 2-18 y). Informed consents were obtained from the vitiligo families. Patients who had taken systemic or used topical treatment within 2 months prior to study entry were excluded. The healthy control group included 42 healthy volunteers of matched age range without any systemic or dermatological disease. The patients were subjected to précised clinical examination regarding distribution of vitiligo lesions, signs of hypothyroidism, alopecia areata, mucocutaneous candidiasis, or other autoimmune disorders.

Genotyping: Genomic DNA samples were prepared from peripheral leucocytes using Spin Column Genomic DNA kit (Biobasic Inc, Ontario, Canada). To genotype the *TAP1* (C/T intron 7) and *LMP7* (G/T intron 6), we amplified 20 ng of genomic DNA in a 25-µl reaction volume containing 50 mM KCl, 10 mM Tris-Cl, pH 8.3, 1.5 mM $MgCl_2$ and 60 mM of each dNTP and 0.25 U of *Taq* polymerase (Bioron, GmbH). PCR primers were *TAP*1-15 and *TAP*1-16 for *TAP*1 (intron 7) and *LMP*7-7 and *LMP*7-4: for *LMP*7 (intron 6) [6]. PCR samples were subjected to annealing temperatures at 55°C (*TAP1*) and 58°C (*LMP7*) followed by a 5 min final extension, using a thermal cycler Dyad (USA). The underlined nucleotide *TAP*1-16 was changed from the germline sequence (G to C) to create a *Msp*I restriction site at the SNP.

Genotypes were determined by restriction fragment length polymorphism (RFLP) analysis of the PCR products. Following restriction digestion of PCR products overnight under conditions specified by the enzyme supplier (Fermantas, GmbH), restriction fragments were separated on 3% NuSieve gel (FMC Bioproducts, USA) in TBE buffer.

The expected product sizes for the *TAP1* (intron 6, C>T) and *LMP7* (intron 6, G>T) polymorphisms were 183- and 763-bp, respectively. The *TAP1* C>T alleles were differentiated by *Msp*I restriction digestion (C-allele, 161- and 22-bp; T-allele, 183-bp) and the *LMP7* (G>T) alleles were differentiated by *Hha*I restriction digestion (G-allele, 428, 180-and 125-bp; T-allele, 583-and 180-bp).

Data Analysis: The distribution of *TAP1 and LMP7* genotypes in the cases and controls, were compared with that expected from the Hardy-Weinberg equation and with each other using the Chi-squared test. Conditional logistic regression methods were used, in STATA (StataCorp, 1999), to compute odds ratios (OR) for vitiligo risk and 95% confidence intervals (CI), associated with each genotype. For case/control association studies, the significance of observed differences in allelic or genotypic frequencies between vitiligo patient and control populations was determined by using χ^2 tests (SPSS 10.1, SPSS Inc, USA).

RESULTS

All our patients experienced active progressive vitiligo lesions. None of the patients had segmental vitiligo, autoimmune diseases, concomitant dermatological diseases or thyroid dysfunction. Skin phototypes according to Fitzpatrick classification [13] was as follows: type II (n= 6), type III (n= 36), type IV (n= 90), type V (n= 9) in the vitiligo group and type III (n= 18), type IV (n= 24) in the control group. Types I to VI represented patients as having very white or freckled, white, white-to-olive, brown, dark brown and black skin, respectively. Family history of similarly affected persons was present in 38% of the cases, the affected members may be daughters, sons or cousins. Vitiligo patients were stratified according to Acros-Burgos *et al.* [7] as early age of onset group <20 y. The mean age of the vitiligo cases ± standard deviation (SD) was 5.9 ± 3.6 y (range 2-18 y) and the maxima onset at 8 year-old age.

Allele and Genotype Frequencies: The single-nucleotide polymorphisms (SNPs) used in this study as genetic markers focused on the *TAP1* C>T (intron 7) and *LMP7* G>T (intron 6) loci. Preliminary case/control analyses of a patient population consisting 141 unrelated Saudi vitiligo patients and 42 healthy controls revealed insignificant differences in allele and/or genotype frequencies

(Table 1). Our results showed no significant difference between the *TAP*1 genotype (intron 7) or *LMP*7 genotype (intron 6) and the healthy controls (p= 0.23 or 0.42, respectively). The OR for risk of developing vitiligo was 2 (95% CI: 0.77-5.09) in individual having *TAP*1-CC genotype. In addition, the OR of *LMP*7-GG genotypes was 1.6 (0.62 to 4.21). The frequencies of the *TAP*1 C-allele and *LMP*7 G-allele were the commonest in the vitiligo patients than control, however the results were not significant (54.3%, p= 0.49 and 47.9%, p= 0.82 respectively). The OR for risk of developing vitiligo was 1.2 (95% CI: 0.73-1.93) in individual having *TAP*1-C allele and 1.1 (95% CI: 0.65-1.73) in individual having *LMP*7 G-allele.

*TAP*1/ *LMP*7 haplotypes were identified from *TAP*1 and *LMP*7 combined genotypes for all subjects (Table 2 and 3), except for double heterozygotes (CT/GT). This group was not evaluated further, as it is not possible to determine the specific haplotype frequencies directly from single digest results and other direct methods are difficult to perform. The frequency of the CG haplotype (sum of combined genotypes: CC/GG, CC/GT and CT/GG) was not significantly higher in the case population compared to controls (p= 0.47) and the OR for risk of developing vitiligo disease was 1.4 (95% CI: 0.54-3.71) in those with an CG haplotype compared to those with the other haplotype.

Table 1: Allele and genotype frequencies of *TAP1* (C/T intron 7) and *LMP7* (G/T intron 6) candidate genes in Saudi vitiligo patients (age of onset 2-16 y) compared to controls

			Allele frequency:				
			Vitiligo patients		Controls		
Gene	Polymorphism	Allele	Count	%	Count	%	*P*-value
TAP1	C/T intron 7	C	153	54.3	42	50.0	0.49
		T	129	45.7	42	50.0	
LMP7	G/T intron 6	G	135	47.9	39	46.4	0.82
		T	147	52.1	45	53.6	
			Genotype frequency:				
			Vitiligo patients		Controls		
Gene	Polymorphism	Allele	Count	%	Count	%	*P*-value
TAP1	C/T intron 7	CC	36	25.5	6	14.3	0.23
		CT	81	57.4	30	71.4	
		TT	24	17.0	6	14.3	
LMP7	G/T intron 6	GG	30	21.3	6	14.3	0.42
		GT	75	53.2	27	64.3	
		TT	36	25.5	9	21.4	

Table 2: Distribution of combined genotype for *TAP1* (C/T intron 7) and *LMP7* (G/T intron 6) SNPs in case and control populations. n= number of subjects. See text for additional details

Population	n	combined genotypes								
		CC/GG	CC/GT	CC/TT	CT/GG	CT/GT	CT/TT	TT/GG	TT/GT	TT/TT
Case	141	27	9	0	3	63	15	0	3	21
Control	42	6	0	0	0	24	6	0	3	3

Table 3: Distribution of *TAP1* (C/T intron 7) and *LMP7* (G/T intron 6) combined haplotype in the study population. n= number of alleles. Double heterozygotes CT/GT were excluded from the haplotype counting as it is not possible to determine the specific haplotype frequencies directly from single digest results

population	n	Haplotype (%)			
		CG	CT	TG	TT
Case	156	66 (42.3)	24 (15.4)	6 (3.8)	60 (38.5)
Control	36	12 (33.3)	6 (16.7)	3 (8.3)	15 (41.7)

DISCUSSION

We did not find an association between the *TAP*1 (intron 7) and *LMP7* (intron 6) polymorphisms and the development of vitiligo in Saudi patients (young age group).

Failure to find an association might be due to the sample size, although a minor contribution of both *TAP*1 and *LMP7* polymorphisms to vitiligo susceptibility cannot be excluded as *TAP*1 CC genotype and *LMP7* GG genotypes showed higher frequency in patients than in controls though not significant. The remaining contribution to the disease might be due the *TAP/LMP* cluster and *MECL*1 locus [6]. Although insignificance and *TAP* gene polymorphism have been found in various diseases including vitiligo, *TAP* gene polymorphisms were reported to alter the peptide-processing pathway. However, *TAP* gene association has not been found in ankylosing spondylitis [14], celiac disease [14, 15] and insulin dependent diabetes mellitus [14, 16].

Among different ethnic populations, several studies found no differences in *TAP*1 and *TAP*2 allele frequencies between vitiligo and control subjects [17]. Discrepancy in *TAP1* and *LMP7* loci association between our results and other ethnic populations with high significant results might be related to different patient clinical characteristics. *TAP*1 allele distributions in Saudi patients and controls were not similar to those in some populations; Japanese, Tunisian and Caucasian [18] populations and ethnic differences were not prominent.

LMP genes are closely linked to MHC class II genes and are believed to be involved in the antigen-processing pathway. Polymorphisms in the amino acid composition of the *LMP2* and *LMP7* proteins have been reported in humans [19, 20]. Controversial results were reported on the association of the *LMP*2 gene polymorphism with extraspinal disease in HLA-B27 positive subjects with ankylosing spondylitis [21-23]. *LMP* genes were rarely studied in patients with vitiligo although these molecules are involved in the antigen-processing pathway.

Previous study reported that, there is no significant difference in allele counts and frequencies in six out of eight markers examined in vitiligo [6]. In addition, they reported no significant differences for seven out of eight markers genotyped. However, they showed genetic association between vitiligo and *LMP/TAP* in 2 alleles only. Many HLA associations have been reported between specific alleles of complement, class I and class II MHC genes with vitiligo in various ethnic and racial subpopulations [24], but no common HLA association is observed. Also, several studies have reported HLA associations in early onset versus late-onset vitiligo cases [7, 25, 26].

All our patients belonged to the early age of onset group which was proved by Arcos-Burgos *et al.* [7] to have a dominant mode of inheritance, compared to older age of onset group (after 30 years) of vitiligo patients who have a recessive mode of inheritance influenced by environmental effects.

The results of this study showed that vitiligo patients have no significantly different frequency of harbouring *TAP*1 and *LMP7* at risk haplotypes than those in control group. The identification of evidence of a significant interaction (patients with both of two or more risk genotypes) may not necessarily indicate that the two genes are synergistic. They may instead influence risk via independent mechanisms. Gene-gene interactions might be important for the development of vitiligo and this interaction needs to be explored.

To conclude, we did not find any association between *TAP*1 (C/T intron 7) and *LMP*7 (G/T intron 6) and our young aged Saudi group of vitiligo patients. Other studies [6, 27, 28] found evidence for genetic association between *LMP/TAP* gene cluster and vitiligo susceptibility. They suggested that *LMP* and *TAP* expression and/or function might influence the peptide repertoire presented by vitiligo patient antigen-presenting cells, by affecting antigen processing by the immuno-proteasome or by preferential transport to peptides for MHC class I presentation.

Further studies of other alleles of *TAP* and *LMP* gene region in Saudi patients is recommended to demonstrate a possible role for MHC class I antigen processing and/or presentation pathway in the antimelanocyte autoimmune response in vitiligo pathogenesis.

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