RESEARCH ARTICLE

Genetic Association of TNF- Alpha Polymorphisms with Generalized Vitiligo in Jordanian Population

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Purpose: This study aims to find the association of Tumor necrosis factor alpha; TNF-alpha promoter polymorphisms and vitiligo in Jordanian population.

Methods: A case-control design was used to compare the genotypes distribution of TNF-alpha (-308 and -238) promoter polymorphisms in 40 vitiligo patients and 40 age- and gender-matched healthy control participants. Polymerase chain reaction (PCR) followed by DNA sequencing was used for genotyping both single nucleotide polymorphisms.

Results: The study revealed the association of TNF-alpha gene polymorphism at rs#361525(p=0.002), but not at rs#1800629(p=1.00) with vitiligo.

Conclusion: Our data suggest that TNF-alpha gene is involved in autoimmune pathogenesis of vitiligo among Jordanians though a large cohort of patients might be needed to confirm such a conclusion.

Key Words: *TNF alpha polymorphism, Vitiligo, Polymerase chain reaction, DNA sequencing*

Vitiligo is an acquired dermatologic pigmentary disorder characterized by white patches on the skin that result from destruction of epidermal melanocytes [1]. It is considered the most common acquired, non-contagious dermatologic disorder with an estimated prevalence of 0.5%-1% in most populations and can occur at any age in both genders, approximately with equal frequencies in all races worldwide [2].

Over the past decades, many researches were conducted trying to investigate the etiology of vitiligo, but the mystery surrounds the exact cause of this complex, polygenic and multifactorial disease. Opinions and hypotheses in this area are numerous, but the most popular and accepted ones are: the autoimmune, the neural and the biochemical hypotheses. All hypotheses contribute to some extent to melanocyte disappearance or destruction, and all are pieces of the convergence theory puzzle of vitiligo etiopathogenesis [3].

Findings of the genetic epidemiology studies of vitiligo, and its association with other autoimmune diseases, as well as discovery of susceptibility genes comprise the base of the framework that generalized vitiligo is a disease with genetic background further enforced by immunotherapy studies of patients with melanoma [4,5].

Vitiligo clusters in families but appears to be segregated in non-simple Mendelian pattern characterized by incomplete penetrance and limited concordance [6]. These results led to exploration of the nongenetic factors that play important role in vitiligo [6,7].

On the other hand, vitiligo was found to be associated with other autoimmune diseases, most commonly autoimmune thyroid disease, suggesting a heritable predisposition as a cause of vitiligo and shared susceptibility genes [8].

The super locus HLA alleles are considered as genetic markers to the actual disease alleles because they are strongly linked to other loci in the major histocompatibility complex region that are associated to wide spectrum of human diseases. It is the most gene-dense and polymorphic region of human genome and is responsible for coding a network of cytokines [9-11].

TNF-alpha is a proinflammatory cytokine. It has been found to be associated with different autoimmune diseases including vitiligo. Various mechanisms have been postulated to explain its role in vitiligo including melanocyte apoptosis, decreased melanogenesis or increased melanocyte cytotoxicity [12].

TNF-alpha gene locus is located within the class III region of the human major histocompatibility complex (MHC) on chromosome 6, and within the promoter region of the TNF-alpha gene, several single nucleotide polymorphisms (SNPs) have been identified that could affect its production. The disturbances that happened have been found to be associated with several autoimmune and infectious diseases, in addition to vitiligo [13,14].

It is anticipated that the discovery of potential genetic cause will provide a clue to new approaches to treatment and, perhaps, even prevention. This is particularly important because vitiligo can impair patients' life and have psychological impact. The only studies conducted in Jordanian population to assess the risk of vitiligo according to genetic variants included SMOC2 [15], PTPN22 [16] and NLAP1 variants [8]. The purpose of this study is to associate TNF-alpha gene polymorphisms with susceptibility to vitiligo in Jordanian population.

MATERIALS AND METHODS

Study subjects

Study cases (vitiligo patients) were recruited from a dermatology clinic at a large public hospital (Al-Basher Hospital) in Amman, Jordan. Vitiligo diagnosis was established by a dermatologist using standard diagnostic criteria. All patients were consented and completed a detailed questionnaire, and the information gathered from the patients included age, gender, medical history including family history, age at disease onset, duration of disease, presence of other autoimmune diseases (as reported by the patient) and other relevant information. The controls (healthy individuals) were matched to cases (of same geographical area) according to age, ethnicity and gender. Human subjects' confidentiality and rights were maintained throughout the study. The study was approved by the Ethical Committee at Jordanian Ministry of Health.

Genotyping

Genomic DNA was extracted from whole blood using "Promega-Wizard genomic DNA purification kit, Promega Corporation, USA" according to manufacturer's instructions. After extraction, DNA concentration was estimated spectrophotometrically, and the quality of DNA was also determined using agarose gel electrophoresis. Genotyping of the TNF-alpha -308 (rs#1800629, G/A) and TNF-alpha -238 (rs#361525, G/A) SNPs was performed by PCR followed by DNA sequencing that was carried out by NICEM (National Instrumentation Center for Environmental Management, South Korea).

Statistical analysis

Statistical tests were performed using the SPSS software version 20. For

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each SNP, allele and genotype, frequencies and percentages were calculated, using the suitable assumptions and equations. The SNPs frequencies were tested for Hardy-Weinberg equilibrium (HWE) fulfillment by comparing the observed genotype as counted in the sample with expected genotypes under HWE, before testing for association with phenotypes. Goodness–of-fit X2 test (Fisher exact test) was used to measure departure from HWE calculation for the results. The strength of association of genotypes and alleles with vitiligo was measured by calculating odds ratio for both cases and control groups.

RESULTS

Study population

In total, 80 participants were enrolled distributed equally between both groups including 39 patients with generalized vitiligo and one patient had localized vitiligo. The rest 40 participants were apparently healthy matched controls. There were no significant differences between patients and controls with regards to gender and age (P>0.05). Table 1 shows clinical and demographic characteristics of vitiligo patients.

Notably, about quarter of patients reported family history of vitiligo and another quarter reported family history of autoimmune diseases. Seven vitiligo patients (17.5%) reported presence of other autoimmune disease (five cases of autoimmune thyroid disease, one of alopecia and one of rheumatoid arthritis).

Few patients reported two risk factors for vitiligo: self-history of autoimmune disease and family history of autoimmune disease [3(7.5%)], family history of vitiligo and autoimmune disease [2(5%)] and family history of vitiligo and self-history of autoimmune disease [1(2.5%)]. Most patients believed that vitiligo is caused by emotional stress, especially fears, and 2 patients (5%) reported exposure to chemicals due to their occupation.

Sequencing results

Samples were sequenced in NICEM; National Instrumentation Center for Environmental Management, South Korea. Figure 1 shows an example of sequencing result.

Association analysis

The observed genotype and allele frequencies for both SNPs are shown in Table 2. The distribution of genotypes at SNP-308 was similar between patients and controls (P=1.00), majority being of wild type. In the contrary, there was a significant difference in the distribution of genotypes at SNP-238 (P=0.002) with all controls and most of the patients being of the wild type, while the rest of vitiligo patients being heterozygous and neither individuals in both groups being homozygous for the mutant type.

Genotype analysis by gender both within cases and between cases and controls was performed. Table 3 shows that no significant gender differences were yielded for both SNPs -308 and -238 among patients.

Table 4 shows gender differences in genotype among patient and control groups. There was significantly higher proportion of female vitiligo patients with GA genotype compared to female controls (P<0.05) for SNP-238, but not for SNP-308 with no significant difference among males for both SNPs.

DISCUSSION

Although there are many hypotheses that try to explain possible ways for vitiligo development, the exact triggers for disease development are not known. Recent success in the Human Genome Project, the understanding

TABLE 1

Clinical and demographic characteristics of vitiligo patients (N=40)

Parameter	N (%) or mean (± SD)
Gender, N (%)	
Female	29 (72.5)
Male	11 (27.5)
Age (y)	33.2 ± 14.0
Age of onset (y)	23.6 ± 13.8
Exposure to chemicals, N (%)	2 (5)
Presence of other autoimmune disease, N (%)	7 (17.5)
Reported family history of vitiligo, N (%)	11 (27.5)
Reported family history of other autoimmune disease, N (%)	9 (22.5)

TABLE 2

Allele and genotype distribution of the TNF-α SNPs in Jordanian vitiligo patients (n=40) and controls (n=40)

SNP	Genotype or allele		Vitiligo patients (N=40)	Control (N=40)	p-value*	Odds ratio
rs#361525-238	Genotype	GG	31	40	0.002	8.22 (2.15-31.4)
		GA	9	0		
		AA	0	0		
	Allele	G	71	80	0.001	
		A	9	0		
rs#1800629-308	Genotype	GG	28	29	1	1.09 (0.48-2.5)
		GA	10	9		
		AA	2	2		
	Allele	G	66	67	0.644	
		A	14	13		

*Fisher exact test

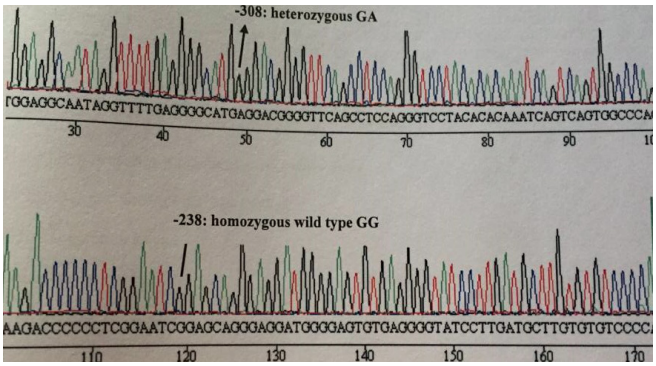

Figure 1) *Heterozygous: shows two peaks over each other, homozygous shows only one peak, Black: guanine, Blue: cytosine, Red: thymine, Green: adenine*

of the effects of mutations on phenotypes and of the effect of genetic control over humoral and cellular immunity have led to the discovery of different candidate genes for vitiligo susceptibility and attributing vitiligo as a complex genetic trait, with different gene-gene and gene-environment interaction [17].

Several TNF-alpha polymorphisms have been reported to be associated with different autoimmune diseases. The association of vitiligo with other autoimmune diseases, the presence of lymphocytes around melanin cells, and the presence of auto-antibodies against different components of melanin cells strengthen the most supported hypothesis for vitiligo, the autoimmune hypothesis [18].

TNF-alpha polymorphisms were not previously assessed in Jordanian population as a risk for vitiligo. The current study explored whether any of the selected TNF-alpha promoter polymorphisms (SNPs) located at positions -308 and -238 is associated with vitiligo in Jordanian population.

The mean age of vitiligo onset was almost 24 years similar to that found in a larger cohort of Caucasian patients in UK and US. We also found that majority of vitiligo patients were women, possibly due to the facts that females have increased susceptibility to develop autoimmune disease and that they more commonly seek medical attention [17]. On the other hand, the age of onset in females was earlier than that in males.

Notably, 27.5% of patients reported family history of vitiligo, 17.5% reported self-history of autoimmune disease, and 25% reported family history of autoimmune diseases. These findings may suggest heritable predisposition to vitiligo and, possibly, shared susceptibility genes as mentioned in previous reports [19].

The analysis of minor allele frequency in healthy control established that both SNPs were in HWE. Minor allele frequency in healthy control for the SNP-

TABLE 3
Genotype differences in genotypes within patients (N=40)

Gender of participants	Genotype at -308			Genotype at -238	
	GG	GA	AA	GG	GA
Male	8	2	1	9	2
Female	20	8	1	22	7
P*	0.585			1	

*Fisher exact test, P<0.05

TABLE 4
Gender differences in genotypes between patients (N=40) and controls (N=40)

SNP Genotype	SNP-308				SNP-238			
	Female		Male		Female		Male	
Genotype	Patient	Control	Patient	Control	Patient	Control	Patient	Control
GG	20	15	8	14	22	24	9	16
GA	8	8	2	1	7	0	2	0
AA	1	1	1	1	0	0	0	0
P*	0.878		0.627		0.012		0.157	

*Fisher exact test, P<0.05

308 was concordant with HAP-Map frequency and close to CEU (European) population. The distribution of AA genotype was 5% in our study, 1.8% for CEU population (European) and 0% for JPT (Asian) population (www.hapmap.org).

However, the frequency of the genotypes distribution at the SNP-238 was nearly matching that for JPT (Asian) population according to the GENO-PANEL, (100% of the GG genotype, and 0% of AA genotype), in contrast to CEU (European) population (according to the same panel, 1.8% and 67% for AA and GG genotypes, respectively) (www.ncbi.nlm.nih.gov). Our findings suggest that SNP-238 G>A in TNF-alpha gene is associated with the occurrence of vitiligo in the study population in contrast to 308 G>A polymorphism.

Genotype distributions in the SNP-308G>A did not differ between the two study groups, being in agreement with the findings of study by Yazici (2006) that failed to confirm the association between the polymorphism and vitiligo in Turkish population, as well as of a Mexican study by Salinas, et al. (2012) who also found no association of SNP-308G>A with vitiligo in general, but only with vitiligo vulgaris. The allele frequencies and the load of the allele A did not differ significantly between patients and controls (P> 0.05) in both above studies.

On the contrary, a study by Laddha, et al conducted on Indian population, detected a significant association between SNP-308 and vitiligo (P<0.0001), with -308 A allele increasing the risk by 4-folds (OR=4.326, CI=3.623-5.165).

Although the Iranian study (2009) showed that -308 G>A polymorphism was more common in patients than in controls and the difference -in genotypes- was significant in female gender.

The genotype distribution in the female patients was not in HWE, and this may confer the need to explain these results with caution. Our study failed to confirm association depending on gender differences, most probably due to uneven distribution of genders. These contradictory results may be due to differences in genetic background of different populations and the complex nature of the disease that permit the environmental and ethnic factors to have an impact on vitiligo prevalence. Regarding SNP (-238G>A), the shortage of reports was a challenge. There was only one study that reported its association with vitiligo in Indian population [20].

The presence of -238 A allele was found to increase the risk of vitiligo by 6-folds (OR=6.35, 95% CI=5.320-7.590) in the above study and by 8-folds in our study (OR=8.22, 95% CI=2.15-31.4), the wide confidence interval can be attributed to small sample size. The difference in genotypes between patients and controls remained significant after stratification of the studied groups by gender. Notably, only female vitiligo patients had the GA genotype of -238 A allele polymorphism compared to control female group (P=0.012).

The large sample size in the Indian study detected different genotype distribution percentages in the control group, compared to our study. Genotypes were distributed at 81%, 18% and 1% for GG, GA, AA respectively.

Scanning other studies resulted in finding diversity in genotype distributions in the control and patient groups. Study of Boraska, et al. (2008) of the association of TNF promoter polymorphisms and type 1 diabetes in Croatian population revealed a different distribution in the control group, 93%, 7% and 0% for GG, GA, and AA respectively. On the other hand, in the Turkish study of association of multiple sclerosis with TNF-alpha polymorphisms, 0% of patients had GG genotype, and the majority of both patient and control group participants had the AA genotype (95.3%) [21].

Overall, the above studies lead us to the question, whether TNF-alpha polymorphisms have any real influence on disease susceptibility, or they are just silent and exist due to linkage disequilibrium with HLA alleles. Remarkably, we demonstrated a unique gender differences in genotypes distribution at -238 polymorphism. Only female vitiligo patients had the GA genotype, which obviously leads to more areas of comparisons with other studies.

TNF-alpha polymorphisms were reported to be associated with several diseases, including vitiligo. However, failing to find an association does not imply any causation. Single nucleotide polymorphism may contribute to diseases, but there may be a complex pattern of effect, due to haplotype and linkage disequilibrium with other susceptibility loci that together share the path and cause the disease.

CONCLUSION

In conclusion, this study is the first to investigate two TNF gene promoter polymorphisms (-308 and -238) in Jordan. This study observed the limited association of TNF-alpha gene polymorphism at -238 (rs#361525), but not at at-308 (rs#1800629) with vitiligo in Jordanian population.

TNF-alpha gene lies within MHC region that is highly polymorphic. Therefore, the influence of TNF-alpha gene polymorphisms on vitiligo development may be related to its linkage to a genetic variant in this area that affects its genetic expression, rather to its genetic polymorphisms.

It is important to evaluate these findings in additional investigations with a larger sample that will be sufficiently powered to detect a true association. In addition, a measurement of TNF-alpha level in plasma should parallel this study.

LIMITATIONS OF THE STUDY

Our sample size was relatively small (40 patients and 40 controls) in addition to unequal proportion of male and female participants. TNF-alpha levels were not assessed since all patients included in the study were not treatment-naïve, thus, their TNF-alpha level could have been affected by therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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