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ORIGINAL RESEARCH

Association Among *MIF*, *IFIH1*, and *IL6* Gene Polymorphisms and Non-Segmental Vitiligo in a Chinese Han Population

Danfeng Wang[1,2], Shuhui Min[1,2], Xiao Lin[1,2], Guan Jiang[1,2]

[1]Department of Dermatology, Affiliated Hospital of Xuzhou Medical University, Xuzhou, People's Republic of China; [2]First Clinical Medical College, Xuzhou Medical University, Xuzhou, People's Republic of China

Correspondence: Guan Jiang, Department of Dermatology, Affiliated Hospital of Xuzhou Medical University; First Clinical Medical College, Xuzhou Medical University, Xuzhou, People's Republic of China, Email dr.guanjiang@xzhmu.edu.cn

Objective: The aim of the present study was to investigate the association of single-nucleotide polymorphisms (SNPs) in the macrophage migration inhibiting factor (*MIF*), interferon-induced Helicase C domain 1 (*IFIH1*), interleukin-6 (*IL6*) genes, circulating levels with non-segmental vitiligo (NSV) susceptibility in the Chinese population, and to analyze the relationships between gene polymorphisms and clinical characteristics of vitiligo.

Methods: In this study, genotyping was conducted in 155 patients with NSV and 117 unaffected controls using polymerase chain reaction and snapshot technique. Serum concentrations were determined by ELISA kit.

Results: There were strong associations between *IFIH1* H843R and *IL6*-572G/C polymorphisms and NSV susceptibility ($p = 0.013$; $p = 0.009$). In contrast to previous studies, we found no significant difference in the *MIF*-173G/C polymorphism between the two groups. In addition, the frequency of allelic distribution for *MIF*-173G/C in patients with active NSV was significantly higher than stable NSV ($p = 0.011$), and *IFIH1* H843R with early-onset (≤ 20), active or family history of NSV was significantly higher than late-onset (> 20), stable or no family history of NSV ($p = 0.033$; $p = 0.045$; $p = 0.039$). Serum concentrations of MIF were higher in patients with active NSV, serum IFIH1 and IL6 concentrations were related to the presence of polymorphisms in patients with NSV ($p = 0.009$; $p = 0.011$).

Conclusion: Our results suggested that *IFIH1* H843R and *IL6*-572G/C gene polymorphisms and expression levels are obviously correlated with the onset of NSV. *MIF*-173G/C allele and serum concentrations may be associated with active NSV, and *IFIH1* H843R allele may be associated with youth, active or family history of NSV.

Keywords: vitiligo, *MIF*, *IFIH1*, *IL6*, single nucleotide polymorphism

Introduction

Vitiligo is the most common acquired chronic depigmentation disease that is characterized by selective destruction of functional melanocytes in the skin or hair follicles. Vitiligo affects 0.5% to 2% of the population worldwide,[1] and the incidence of vitiligo in China is 0.5%.[2] Although the disease is not life-threatening, it can lead to disfigurement and seriously affect the patient's appearance. In addition, vitiligo is associated with a variety of autoimmune diseases,[3] which can have a serious impact on mental health and quality of life. Vitiligo is a complex disease that combines genetic and environmental factors as well as metabolic and altered inflammatory and immune responses.[4] Currently, although many vitiligo-related pathogenic genes have been identified and preliminarily confirmed to be related to vitiligo, the exact genetic mechanism of vitiligo cannot be fully explained.

Single-nucleotide polymorphisms (SNPs) of genes refer to the substitution of a single nucleotide at a specific position in the genome and account for most human heritable variations. There is convincing evidence that proinflammatory cytokines play a vital role in the initiation of vitiligo lesions.[5] Several SNPs of inflammatory cytokines have been demonstrated to be associated with vitiligo, but contradictory results exist among different ethnic groups.

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MIF was initially described as a chemotactic lymphocytokine and a negative regulator of the immunosuppressive actions of glucocorticoids. Subsequently, *MIF* has been described as a proinflammatory factor with upstream regulatory roles in innate and adaptive immunity.[6] It has been shown to play a crucial role in several types of immune and autoimmune diseases.[7,8] *MIF* is mainly derived from macrophages and T cells and is secreted in response to several stimuli, including lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α, hypoxia, and oxidative stress.[9–11] In the presence of inflammation, *MIF* allows for prolonged inflammation through the release of other pro-inflammatory cytokines.[12] *MIF*-173G/C(rs755622 [RefSeq NG_012099.1]) is located in the promoter region of chromosome 22. One study on the western Mexico population demonstrated that *MIF*-173G/C will become an extremely attractive target for vitiligo treatment.[13] However, a similar conclusion has not been confirmed in vitiligo patients of different populations.

IFIH1, also known as melanoma differentiation-associated protein 5 (MDA5), is a cytosolic innate immune receptor that recognizes viral RNA and activates IFN regulatory factor 3 (IRF3) and the proinflammatory transcription factor nuclear factor-kB (NF-kB), to induce inflammation and other antiviral genes.[14] During inflammatory conditions and infections, viral RNA or RNA mimics and lipopolysaccharide (LPS) can activate *IFIH1* production by stimulating distinct signaling pathways.[15] *IFIH1* is an early type I interferon (IFN) β response gene, which plays a critical role in initiating antiviral innate immunity and modulating subsequent adaptive immunity.[16] Several genome-wide association studies (GWAS) have confirmed that *IFIH1* is a vitiligo susceptibility gene.[17,18] A recent GWAS showed that a single nucleotide polymorphism *IFIH1* H843R(rs3747517 [RefSeq NG_011495.1]) in the exon of chromosome 2 was associated with vitiligo in a Chinese population.[19] In order to further study the etiology of vitiligo, we conducted the genotype of phenotype of this locus in depth.

IL6 is a pleiotropic cytokine, produced by macrophages, T cells and B cells, was initially designated as a B cell stimulator that promoted maturity of B-cells and the expression of immunoglobulins.[20] During inflammatory conditions and infections, certain bacterial LPSs, interleukin-1b (IL-1b) and tumor necrosis factor (TNF), are important stimuli for *IL6* production.[21] Through different signaling pathways, *IL6* induces acute phase protein generation, contributing to the development of multiple inflammatory and autoimmune diseases.[22] As a common clinical inflammatory factor, *IL6* has been shown to be significantly elevated in the body fluids of patients with vitiligo.[23] However, SNPs in the *IL6* gene have been identified in various diseases, but few studies concerning *IL6* gene promoter polymorphism at position −572G/C (rs1800796 [RefSeq NG_011640.1]) were conducted on patients with vitiligo.

Therefore, it is of significance to explore the pathogenesis of vitiligo, especially the immunological and genetic backgrounds, for the development of new therapeutic targets for vitiligo. The current study aimed to measure the serum MIF, IFIH1 and IL6 level and the role of the SNP (rs755622, rs3747517 and rs1800796) as a risk for the susceptibility to vitiligo, and to determine its correlation with disease activity and severity of vitiligo.

Materials and Methods

Patients

A total of 155 patients with NSV and 117 sex- and age-matched unaffected controls from the same geographical area were enrolled in the present study. According to the revised classification of vitiligo by the Vitiligo Global Issues Consensus Conference (VGICC) published in 2012,[24] there are four recognized clinical forms of vitiligo: non-segmental, segmental, mixed, and unclassified. We selected the most common type of NSV for the study, and NSV including acrofacial, generalized, mucosal, and universal vitiligo. Participants were excluded if they reported any history of systemic diseases, such as autoimmune diseases, malignancy, hypertension, diabetes mellitus, and family history of autoimmune diseases. All patients were recruited from the dermatology clinic and had no systemic treatment four weeks before enrolment. Control subjects were healthy volunteers who were selected from physical examination center of the hospital during the same period, and volunteers with past or family history of vitiligo were excluded. Both the Ethics Committee of Affiliated Hospital of Xuzhou Medical University approval for the study (approval No. XYFY2021-KL252) and a written informed consent from all participants were obtained. This study complies with Declaration of Helsinki.

Stable vitiligo is defined as static lesions of vitiligo without any new lesions or extension of previously existing lesions occurring over the past six months. By contrast, active vitiligo is defined as the recent appearance of new lesions or enlargement of existing lesions.[25] A halo nevus is a benign melanocytic nevus that is surrounded by a hypopigmented zone.[26]

DNA Extraction

A total of 5 mL peripheral venous blood samples were drawn from each participant into EDTA-K2 anticoagulant tubes and stored at −80°C until DNA extraction. Genomic DNA was extracted from the whole blood by using a commercial Blood Genomic DNA preparation kit (Jizhen Biotechnology, Shanghai, China) following the manufacturer's instructions.

Genotyping Analysis

Genotyping was carried out by the Snapshot sequencing technique. Polymerase chain reaction (PCR) was used for the amplification of target DNA sequences. The specific primer sequences are shown in Table 1.

A total of 15 μL of PCR reaction mixture was prepared in 10.1 μL of PCR water containing 25 mM MgCl 2 (1.5μL), 10 × PCR buffer (1.5 μL), 10 mM dNTPs (0.3 μL), l0 μM forward and reverse primer (0.15 μL each), Taq polymerase (0.3 μL), and sample DNA (1 μL). The PCR program was set under the following conditions: an initial denaturation temperature of 94°C for 3 minutes, followed by 30 cycles of 94°C for 15 seconds, 55°C for 15 seconds, 72°C for 30 seconds, and step of 72°C for 3 minutes. A total of 3 μL of PCR amplification product was then digested with 0.2 μL of Exonuclease I (ExoI) and 0.8 μL of Fast Alkaline Phosphatase (FastAP) and incubated at 37°C for 15 minutes and at 80°C for 15 minutes to remove the remaining primers and dNTPs. After purification of PCR products, the Snapshot multiplex single-base extension reaction was carried out in 6 μL solutions with 2 μL of the purified PCR products, 1 μL of Snapshot MIX (Applied Biosystems), 0.2 μL of extension reaction primer (10 μM), and 2.8 μL of PCR water. The extension primers were as follows: rs755622 (TAAGCCCGGCGCACCGCTCCAA); rs3747517 (TTTTTTTTTTTCGGAAATCATTAACTGTCTCA) ;rs1800796 (TTTTTTTTTTTTTTTTTTTTTTGCCAGGCAGTTCTACAACAGCC). The cycling conditions were 96°C for 1 minute, followed by 30 cycles of 96°C for 10 seconds, 52°C for 5 seconds, and 60°C for 30 seconds. Finally, extension products were further purified by ExoI and FastAP. The results of SNP typing were analyzed using an ABI 3730xl DNA Analyzer (Applied Biosystems) (Figure 1A–C).

Quantification of Serum Concentrations

Serum was obtained from all individuals at the time of inclusion; cytokine levels were quantified in a subset of 155 NSV patients and 117 control subjects. The determination of serum MIF, IFIH1 and IL6 concentrations was performed by ELISA kit (Jizhen Biotechnology, Shanghai, China), according to manufacturer's protocol.

Statistical Analysis

Data management and analysis were performed using the Statistical Package for Social Sciences software (SPSS), version 23.0. For quantitative data, the mean, median, standard deviation (SD), and interquartile range (IQR) were calculated. Chi-square analysis was used to evaluate the Hardy–Weinberg equilibrium (HWE) and compare genotype and allele frequencies for each SNP between patients and controls. The differences in gender and age distribution between the two groups were analyzed using Chi-square test and Student's *t* test, respectively. To compare nonparametric quantitative determinations, the Mann–Whitney *U*-test, Odds ratios (ORs) and

Table 1 Primer Sequences

Internal Reference	Upstream Primer	Downstream Primer
MIF	5'-GGGACTGGAGCCCTTGA-3'	5'-TCCGCCCGTTCCTCCA-3'
IFIH1	5'-TATCAATGGCAACCACATGC-3'	5'-GAGCCAGAGCTGATGAGAGC-3'
IL6	5'-GAGACTCAGTGGCAATGGGG-3'	5'-CAAGCCTGGGATTATGAAGAAG-3'

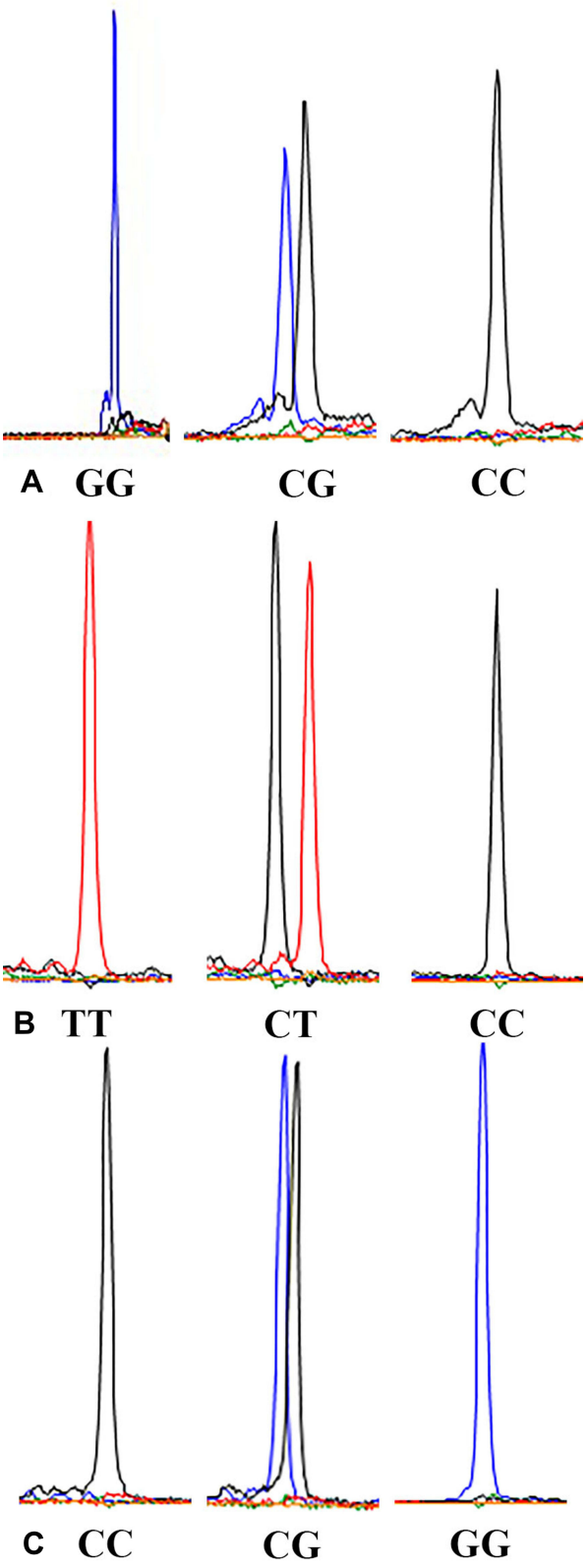

Figure 1 (A) Genotype sequencing of *MIF*-173G/C polymorphism; **(B)** Genotype sequencing of *IFIH1* H843R polymorphism; **(C)** Genotype sequencing of *IL6*-572G/C polymorphism.

95% confidence intervals (CIs) were used to analyze the risk for NSV associated with the SNPs. A two-sided *p* value of less than 0.05 was considered statistically significant and 80% statistical power. The Bonferroni correction was applied to multiple tests, and *p* values were corrected accordingly to 0.0167 (adjust *p* value = alpha/n, alpha = 0.05, n = 3).

Results

Clinical Characteristics of the Study Population

There were no statistically significant differences in gender and age distribution among the vitiligo and control patient groups ($p > 0.05$). The demographic characteristics are summarized in Table 2. Both vitiligo patients and controls were in Hardy-Weinberg equilibrium with *MIF*-173G/C, *IFIH1* H843R, and *IL6*-572G/C genotype distribution ($p > 0.05$) (Table 3).

Association Between *MIF*-173G/C Polymorphism and Vitiligo

No significant differences in the genotype and allele frequencies of the *MIF*-173G/C polymorphism were found between patients with vitiligo and healthy controls ($p > 0.0167$) (Table 4). Notably, compared to the estimated sample size with PASS statistical software, the smaller sample size (155 patients and 117 controls) of this study decreased the statistical power significantly. Therefore, this conclusion we reached should be analyzed conservatively.

Association Between *IFIH1* H843R Polymorphism and Vitiligo

The genotype and allele frequencies of the *IFIH1* H843R polymorphism among vitiligo cases and control subjects are shown in Table 4. There were significant differences in the distribution of the CT genotype between vitiligo patients and healthy controls ($p = 0.030$, OR = 1.766, 95% CI = 1.056–2.953). According to the dominance model,

Table 2 Demographic Data Among the Patient Groups

Parameter	Cases (N=155)	Controls (N=117)	*P*
Gender			0.905
Male	81 (52.26%)	62 (52.99%)	
Female	74 (47.74%)	55 (47.01%)	
Mean age (years)	24.77 ± 18.07	23.38 ± 10.64	0.460
Age of onset			
Early (≤ 20 years old)	96 (61.94%)		
Late (> 20 years old)	59 (38.06%)		
Disease duration (months)	20.15 ± 39.21		
Disease activity			
Active	102 (65.81%)		
Stable	53 (34.19%)		
Family history			
Positive	18 (11.61%)		
Negative	137 (88.39%)		
Halo nevus			
Positive	9 (5.81%)		
Negative	146 (94.19%)		
Types			
Acrofacial	95 (61.29%)		
Generalized	57 (36.77%)		
Mucosal	2 (1.29%)		
Universal	1 (0.65%)		

Table 3 Hardy-Weinberg Balance Test Results of Non-Segmental Vitiligo and Control Group

SNP	Genotype			χ^2	P
	n (%)				
Vitiligo group					
*MIF*rs755622	GG	CG	CC	0.482	0.786
	97 (62.58)	53 (34.19)	5 (3.23)		
*IFIH1*rs3747517	TT	CT	CC	0.058	0.971
	60 (38.71)	74 (47.74)	21 (13.55)		
*IL6*rs1800796	CC	CG	GG	0.001	0.999
	78 (50.32)	64 (41.29)	13 (8.39)		
Control group					
*MIF*rs755622	GG	CG	CC	0.663	0.718
	80 (68.38)	35 (29.91)	2 (1.71)		
*IFIH1*rs3747517	TT	CT	CC	0.352	0.839
	63 (53.85)	44 (37.60)	10 (8.55)		
*IL6*rs1800796	CC	CG	GG	0.032	0.984
	76 (64.96)	37 (31.62)	4 (3.42)		

the carriers of the H843R C allele (CT + CC) showed an 1.847 fold risk ($p = 0.013$, OR = 1.847, CI = 1.136–3.004) to develop vitiligo than carriers of the T allele (TT). A significant difference in allele distribution was observed before and after Bonferroni correction between the two groups, and the C allele was present in 37.42% of patients and 27.35% of controls ($p = 0.013$).

Association Between *IL6*-572G/C Polymorphism and Vitiligo

The genotype and allele distribution of the *IL6*-572G/C polymorphism are summarized in Table 4. There were significant differences in the distribution of the CG and GG genotypes between vitiligo patients and healthy controls ($p = 0.046$, OR = 1.685, 95% CI = 1.009–2.816; $p = 0.043$, OR = 3.167, 95% CI = 0.988–10.145). According to the dominance model, the carriers of the −572 G allele (CG + GG) showed an 1.830-fold risk ($p = 0.016$, OR = 1.830, CI = 1.117–2.997) for the development of vitiligo than carriers of the −572 C allele (CC). A significant difference in allele distribution was observed before and after Bonferroni correction between the two groups, and the G allele was present in 29.03% of patients and 19.23% of controls ($p = 0.009$).

Associations Between the *MIF*-173G/C, *IFIH1*H843R, and *IL6*-572G/C Polymorphisms and Clinical Characteristics of Vitiligo

Stratified analysis of genotype, allele frequency, and clinical phenotype of vitiligo for the *MIF*-173G/C, *IFIH1* H843R and *IL6*-572G/C polymorphisms are shown in Tables 5 and 6. The *MIF*-173G/C polymorphism in the active vitiligo group or with halo nevus vitiligo group was significantly higher than the stable vitiligo group or without halo nevus vitiligo group ($p < 0.05$), and the −173 C allele frequency in the active vitiligo group was significantly higher than the stable vitiligo group, which was statistically significant ($p < 0.05$). The *IFIH1* H843R polymorphism in the early onset (≤ 20 years old), active or family history groups was significantly higher compared with the late onset (> 20 years old) vitiligo group, stable vitiligo group or no family history group ($p < 0.05$). The H843R C allele frequency in the early-onset vitiligo group, active vitiligo group or family history group was significantly higher than in the late-onset vitiligo group, stable vitiligo group or no family history group ($p < 0.05$). However, there was no correlation between the *IL6*-572G/C gene polymorphism and the clinical characteristics of patients with vitiligo ($p > 0.05$).

Table 4 *MIF-173G/C, IFIH1H843R, IL6-572G/C* Genotypes and Allele Frequencies in Patient Groups

	Cases (N=155)		Controls (N=117)		χ^2	*P* value	OR(CI 95%)
	N.	%	N.	%			
MIF-173G/C							
Genotype:							
GG	97	62.58	80	68.38	1.249[a]	0.529[a]	
CG	53	34.19	35	29.91	0.704	0.401	1.249 (0.743–2.100)
CC	5	3.23	2	1.71	NA	0.464	2.062 (0.390–10.913)
Allele:							
G	247	79.68	195	83.33			
C	63	20.32	39	16.67	1.170	0.279	1.275 (0.820–1.983)
Dominant model analysis:							
GG	97	62.58	80	68.38			
CC+CG	58	37.42	37	31.62	0.985	0.321	1.293 (0.778–2.148)
IFIH1H843R							
Genotype:							
TT	60	38.71	63	53.85	6.420[a]	0.040[a]	
CT	74	47.74	44	37.60	4.735	0.030	1.766 (1.056–2.953)
CC	21	13.55	10	8.55	3.570	0.059	2.205 (0.960–5.066)
Allele:							
T	194	62.58	170	72.65			
C	116	37.42	64	27.35	6.106	0.013	1.588 (1.099–2.295)
Dominant model analysis:							
TT	60	38.71	63	53.85			
CC+CT	95	61.29	54	46.15	6.167	0.013	1.847 (1.136–3.004)
IL6-572G/C							
Genotype:							
CC	78	50.32	76	64.96	6.833[a]	0.033[a]	
CG	64	41.29	37	31.62	3.997	0.046	1.685 (1.009–2.816)
GG	13	8.39	4	3.42	4.100	0.043	3.167 (0.988–10.145)
Allele:							
C	220	70.97	189	80.77			
G	90	29.03	45	19.23	6.866	0.009	1.718 (1.143–2.582)
Dominant model analysis:							
CC	78	50.32	76	64.96			
CG+GG	77	49.68	41	35.04	5.814	0.016	1.830 (1.117–2.997)

Note: [a]Chi-square test and P value were compared between vitiligo group and control group.
Abbreviation: NA, not available.

MIF, IFIH1, IL6 Concentrations in Vitiligo Patients, Association with the *MIF, IFIH1, IL6*

We found differences in serum MIF, IFIH1, and IL6 concentrations between patients and controls. When we stratified the serum MIF, IFIH1 and IL6 concentrations according to the activity index of the patients, a higher MIF, IFIH1 and IL6 concentration were found in active patients in comparison with the stable ones [39.23 ng/mL (32.48–52.49) vs 37.89 ng/mL (32.57–40.71), $p = 0.029$; 38.61 ng/mL (34.66–45.68) vs 30.55 ng/mL (27.86–35.59), $p < 0.001$; 34.67 pg/mL (27.29–40.58) vs 27.67 pg/mL (21.18–30.68), $p < 0.001$]; and with the control groups [39.23 ng/mL (32.48–45.68) vs 24.78 ng/mL (13.89–31.27), $p < 0.001$; 38.61 ng/mL (34.66–45.68) vs 23.57 ng/mL (17.49–29.10), $p < 0.001$; 34.67 pg/mL (27.29–40.58) vs 19.78 pg/mL (12.57–27.48), $p < 0.001$] (Figure 2A–C).

Regarding *MIF* genotype analysis, active NSV patients with CC+CG genotypes exhibited significantly higher mean serum levels of MIF [37.58 ng/mL (31.01–52.13) vs 28.67 ng/mL (25.42–34.23), $p = 0.002$]. Regarding *IFIH1* genotype analysis, active NSV patients with TT and CC+CT genotypes exhibited significantly higher mean serum

Table 5 Distribution of *MIF-173G/C, IFIH1 H843R*, and *IL6-572G/C* Polymorphisms in Clinical Features of Vitiligo

Parameter	rs755622			X^2	*P*	rs3747517			X^2	*P*	rs1800796			X^2	*P*
	GG	CG	CC			TT	CT	CC			CC	CG	GG		
Gender				0.485	0.831				2.432	0.296				1.673	0.433
Male	52	26	3			34	34	13			39	33	9		
Female	45	27	2			26	40	8			39	31	4		
Age of onset				1.204	0.579				7.224	0.027				0.856	0.652
Early	60	34	2			45	39	12			51	37	8		
Late	37	19	3			15	35	9			27	27	5		
Family history				1.234	0.478				6.979	0.031				3.207	0.201
Positive	10	7	1			12	4	2			6	9	3		
Negative	87	46	4			48	70	19			72	55	10		
Types				6.972	0.435				3.410	0.857				4.102	0.779
Acrofacial	63	30	2			40	42	13			45	42	8		
Generalized	33	21	3			19	30	8			30	22	5		
Universal	0	1	0			0	1	0			1	0	0		
Mucosal	1	1	0			1	1	0			2	0	0		
Disease activity				6.527	0.029				7.020	0.030				0.807	0.668
Active	57	40	5			47	42	13			51	41	10		
Stable	40	13	0			13	32	8			27	23	3		
Halo nevus				7.762	0.014				0.145	0.930				0.349	1.000
Positive	6	1	2			4	4	1			5	4	0		
Negative	91	52	3			56	70	20			73	60	13		

Table 6 Allele Frequencies of *MIF-173G/C*, and *IFIH1H843R* Polymorphisms in Clinical Phenotypes of Vitiligo Patients

Parameter	rs755622		X^2	*P*	OR (95% CI)	rs3747517		X^2	*P*	OR (95% CI)
	G	C				T	C			
Gender			0.068	0.794	1.076 (0.619–1.872)			0.021	0.884	1.035 (0.653–1.640)
Male	130	32				102	60			
Female	117	31				92	56			
Age of onset			0.088	0.767	1.089 (0.618–1.920)			4.571	0.033	1.670 (1.042–2.675)
Early	154	38				129	63			
Late	93	25				65	53			
Family history			0.550	0.458	0.736 (0.327–1.657)			4.017	0.045	2.277 (1.001–5.182)
Positive	27	9				28	8			
Negative	220	54				166	108			
Disease activity			6.460	0.011	0.431 (0.222–0.835)			4.254	0.039	1.655 (1.024–2.677)
Active	154	50				136	68			
Stable	93	13				58	48			
Halo nevus			0.656	0.418	0.644 (0.221–1.880)			0.136	0.712	1.209 (0.441–3.313)
Positive	13	5				12	6			
Negative	234	58				182	110			

levels of IFIH1 [38.61 ng/mL (34.66–45.37) vs 32.11 ng/mL (29.54–38.99), $p = 0.017$; 38.61 ng/mL (34.67–46.57) vs 32.11 ng/mL (29.54–38.99), $p < 0.001$]. Regarding *IL6* genotype analysis, active NSV patients with CC and CG +GG genotypes exhibited significantly higher mean serum levels of IL6 [36.32 pg/mL (30.56–47.35) vs 27.68 pg/mL (23.59–34.54), $p < 0.001$; 34.64 pg/mL (23.59–38.45) vs 26.68 pg/mL (20.32–30.37), $p = 0.011$] (Figure 2D–F).

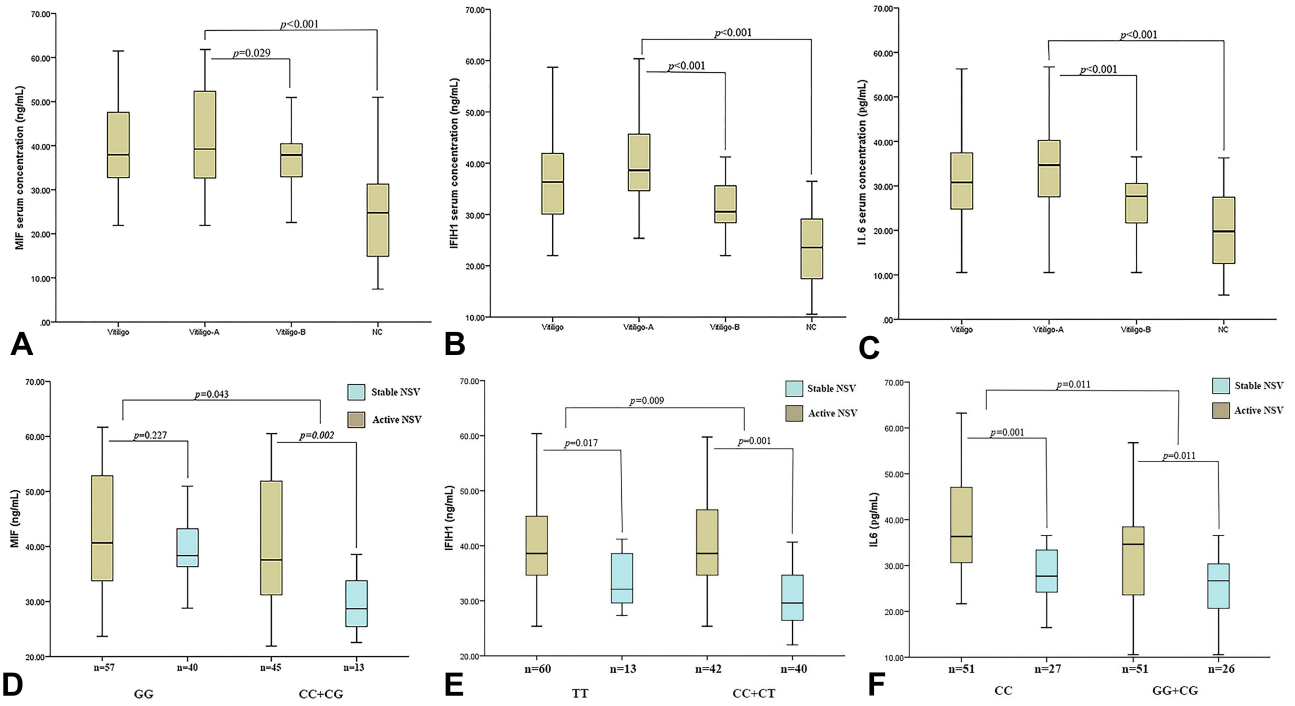

Figure 2 Serum MIF, IFIH1 and IL6 concentrations in study groups, and serum MIF concentrations according to the genetic model of dominant inheritance. (**A**) Serum MIF concentrations in study group; (**B**) serum IFIH1 concentrations in study group; (**C**) serum IL6 concentrations in study group; (**D**) genetic model of dominant inheritance by SNP −173G/C *MIF* genotypes; (**E**) genetic model of dominant inheritance by SNP H843R *IFIH1* genotypes; (**F**) genetic model of dominant inheritance by SNP −572G/C *IL6* genotypes *p* - value: Mann–Whitney *U*-test.

Abbreviations: NSV, non-segmental vitiligo; vitiligo – A, active vitiligo; vitiligo – B, stable vitiligo; NC, numerical control.

Discussion

Vitiligo is a complex, multifactorial, and polygenic disease with a multifaceted pathogenesis. It is well known that the interaction between genetics and environment influences the onset and evolution of vitiligo. Currently, more attention is paid to the factors that influence the autoimmune response.

MIF is a key proinflammatory cytokine that plays a role in inflammation, autoimmune, metabolic diseases, and cancer.[27] In recent years, the *MIF*rs755622(−173G/C) polymorphism has been shown to be associated with the pathogenesis and progression of autoimmune diseases, such as rheumatoid arthritis (RA),[28] psoriasis,[29] systemic sclerosis (SSc),[30] and systemic lupus (SLE).[31] A study in a population of western Mexicans confirmed a significant association between *MIF* gene polymorphisms (−794CATT5-8 and −173G/C) and NSV.[13] Due to resource and technical limitations of the present study, we focused on the *MIF*-173G/C gene polymorphism, and the results showed that there was no statistical difference in genotype and allele frequency of the *MIF*-173G/C polymorphism between vitiligo patients and the healthy control group ($p > 0.05$). This discrepancy between findings may be due to genetic heterogeneity in vitiligo susceptibility between ethnic populations or the small sample size involved in this study. Further analysis of the correlation between the *MIF*-173G/C allele distribution and clinical manifestations in patients with non-segmental vitiligo showed that it was more significant in patients with active vitiligo than in patients with stable vitiligo.

Evidence has shown that the occurrence of the *MIF*-173G/C polymorphism is related to its high transcriptional activity and protein expression. In vitro experiments have confirmed that −794CATT5-8 and −173 C significantly enhanced *MIF* gene transcription.[32] The *MIF*-173 C allele has been associated with increased *MIF* levels in peripheral blood in a population-validated study.[33] Multiple studies have found that the serum *MIF* concentration in patients with vitiligo vulgaris is significantly higher than the control group, and the serum *MIF* concentration in the active phase is higher compared with the stable phase.[6,15,34–36] Ma et al[37] and Garcia-Orozco et al[13] further found that *MIF* mRNA

levels in skin lesions of patients with vitiligo were significantly increased, and serum *MIF* concentration and in situ expression were correlated with active non-segmental vitiligo, further suggesting that *MIF* plays a role in the pathogenesis of non-segmental vitiligo, especially in active patients. In our study, we found significant differences between patients and controls in terms of serum MIF levels. Besides, serum MIF concentrations were not related to the presence of polymorphisms in patients with vitiligo. In conclusion, we found no association between *MIF* gene polymorphisms and the risk of NSV in Han population. However, further studies with a large sample are necessary to verify the association between NSV and the MIF gene polymorphisms.

IFIH1 encodes interferon-induced RNA helicases, which recognize the intracytoplasmic viral dsRNA, modulates interferon (IFN) responses and products of pro-inflammatory cytokines, including IL6, IL8, and TNF-α that have been proposed to play an essential role in the pathogenesis of vitiligo.[36,38] In addition, *IFIH1* plays a role as a virus-associated pattern recognition receptor (PRR) to activate innate immune responses and has a central role in triggering autoimmune responses, such as vitiligo.[18] Some scholars have found that the activation of MDA5 could induce some chemokines of keratinocytes by using Poly (I : C) to simulate viral infection, thus exacerbating melanocyte death in vitiligo.[39] Therefore, the MDA5 signaling pathway can be used as a potential therapeutic target for viral invasion of vitiligo.

In European populations, *IFIH1*rs2111485 was found to be associated with vitiligo by GWAS as a protective gene.[18,19] The second GWAS in non-Hispanic European population confirmed that *IFIH1*rs1990760 and rs2111485 were susceptible loci of vitiligo, especially rs2111485.[40] Similar results have been reported by Onan et al[41] with reference to a Turkish population, further indicating that rs2111485 is a susceptible locus of vitiligo in Western populations. In the present study, rs3747517 (H843R), another SNP found in the *IFIH1* gene, is a missense variant located at exon 13 of *IFIH1*.[42] In humans, the variant of the *IFIH1* gene that encodes the H843R polymorphism has been associated with different susceptibilities to multiple viral infections and autoimmune diseases, such as type I diabetes mellitus (T1DM),[43] chronic viral hepatitis,[44] systemic lupus erythematosus,[45] and psoriatic arthritis.[46] In the present study, significant associations were observed between *IFIH1* H843R alleles and vitiligo susceptibility. Consistent results have been reported by Cheng et al[20] in the Chinese Han population but did not specifically explore the genotype and allele frequency of this locus in relationship to the risk of vitiligo and its clinical impacts. In addition, we found that the *IFIH1* H843R polymorphism genotype and allele frequency were higher in patients with early-onset (≤ 20), active or family history of vitiligo was significantly increased compared with late-onset (> 20), stable or no family history of vitiligo ($p < 0.05$). This may demonstrate differences in the genetic background of early-onset and late-onset vitiligo, active and stable vitiligo, with or without family history of vitiligo. To clarify, as definitively as possible, the contribution of *IFIH1* promoter polymorphisms to vitiligo risk and phenotype in this study, we also determined the relationship between H843R polymorphisms and the serum levels of IFIH1. In the first instance, there were significant differences between patients and controls in terms of serum IFIH1 levels. Besides, serum IFIH1 concentrations were related to the presence of polymorphisms in patients with vitiligo.

IL6 is a well-known pro-inflammatory cytokine that has been implicated in vitiligo pathogenesis. Monocytes and macrophages are the major producers of *IL6*, but keratinocytes and melanocytes can also generate *IL6* after stimulation. *IL6* plays an important role in the human cytokine network, inducing the expression of various proteins responsible for acute inflammation and regulating endocrine and metabolism. *IL6* is a paracrine inhibitor of human melanocyte proliferation and melanin production,[47] and can significantly increase the expression of intercellular adhesion molecule-1 (ICAM-1) on the surface of melanocytes, which contributes to the adhesion between leukocytes and melanocytes, resulting in melanocyte damage and loss in patients with vitiligo.[48] In addition, *IL6* can induce the activation of polyclonal B cells, which may directly inhibit the growth of melanocytes and immune damage, leading to skin discoloration lesions.[49] Besides, elevated *IL6* expression in the skin and serum of vitiligo patients have been repeatedly identified,[23,50,51] and the same results were found in our study.

There were significant racial differences between Asian and Caucasian populations in *IL6* gene promoter region polymorphisms, and the allele C at the *IL6*-572G/C locus has been frequently observed in East Asian populations.[52–54] Generally, the darker the skin color, the higher the probability of incidence of vitiligo. The difference between populations at two loci may be involved. The −572G/C is the most common polymorphic site in the *IL6* promoter

region, and we found that this site can result in an overproduction of *IL6* and promote vitiligo development.[55,56] Singh et al[49] showed that the *IL6*-572G/C polymorphism was associated with vitiligo susceptibility in the Gujarat population and had a reduced risk of vitiligo in individuals with GC+CC genotypes compared with GG genotype ($p < 0.05$). Aydıngöz et al found no correlation between the *IL6*-174G/C polymorphism and vitiligo in Turkish population.[57] In our study, we observed that the CG+GG genotype and G allele appeared more frequently among vitiligo patients, whereas the G allele carriers showed a 1.718-fold higher risk for vitiligo development compared with C allele carriers. In addition, we found no potential genetic association between *IL6*rs1800796 and clinical features in patients with vitiligo.

There were certain limitations in the current study. The smaller sample size in the investigation made the negative results less convincing. We did not further verify the correlation between variant genotypes and protein transcription levels due to the high cost of the experiment. Furthermore, we investigated only one polymorphism from each of the three genes and had a relatively small sample size. Therefore, confirming the genetic effects of multiple polymorphisms in *MIF, IFIH1*, and *IL6* need to be further investigation.

Conclusion

The present study indicated that the *IFIH1* H843R and *IL6*-572G/C polymorphisms play a role in NSV susceptibility in the Chinese population, and the significantly high prevalence of these two polymorphisms was observed in NSV. The H843R C allele may be associated with the incidence of vitiligo in youth (≤ 20 years old), active or family history groups. However, no statistical significance was observed between the *MIF*-173G/C polymorphism and NSV, which contradicts previous finding in other populations. The −173C allele may be associated with vitiligo incidence in the active NSV group in Chinese population. Moreover, the serum concentrations of MIF is associated with active NSV, and the serum IFIH1 and IL6 concentrations were related to the presence of polymorphisms in patients with NSV. Further investigation with a larger sample size and different ethnic groups is required to verify the results.

Disclosure

The authors report no conflicts of interest in this work.

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