

Association of interleukin-6 gene polymorphisms and circulating levels with keloid scars in a Chinese Han population

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ABSTRACT. The aim of the present study is to explore the effect of *IL-6* gene polymorphisms on the development of keloid scar (KS) in the Chinese Han population. Genotyping of *IL-6* was performed by the polymerase chain reaction (PCR), followed by restriction fragment length polymorphism assays (PCR-RFLP). Serum level of *IL-6* was measured using enzyme-linked immunosorbent assay (ELISA). Results indicated that when the *IL-6* -572 CC homozygote genotype was used as the reference group, the GG genotype was found to be associated with a significantly increased risk of KS (GG vs CC: OR = 2.097, 95%CI = 1.100-3.995, P = 0.025). When the *IL-6* -572 C allele was used as the reference group, the G allele was found to be associated with significantly increased risk of KS (G vs C: OR = 1.317, 95%CI = 1.002-1.730, P = 0.048). Furthermore, we observed a marked

increase in serum *IL-6* levels in KS patients with GG genotypes when compared to KS patients harboring the CC genotype. In conclusion, our results suggest that *IL-6* gene polymorphism was associated with keloid scars in the southeastern Chinese Han population.

Key words: Keloid scar, IL-6, Polymorphism

INTRODUCTION

The formation of keloid scars (KS) after skin trauma remains a challenging clinical problem. KS is a benign, proliferative dermal collagen growth characterized by excessive accumulation of extracellular matrix components, mainly type I collagen, fibronectin, and proteoglycans (Wang et al., 2014). Prevalence of KS varies among different ethnic groups, and its incidence has been estimated to be approximately 4-6% in the general population (Emami et al., 2012). In the past decades, studies were performed to identify the exact factors responsible for the incidence and development of KS, yet the mechanism of KS formation remains largely unknown (Rossiello et al., 2009). Keloid formation is a complex and multifactorial process that involves both environmental and genetic risk factors. An increasing body of evidence indicated that genetic factors play an important role in the occurrence of KS (Nakashima et al., 2010; Shih and Bayat, 2010; Zhu et al., 2013). Several genetic markers have been identified to be associated with the diagnosis, prognosis, and development of KS (Wu et al., 2012; Yu et al., 2013; Ogawa et al., 2014).

IL-6, a multifunctional cytokine that regulates the immune response, has been intensively studied in the recent years (Fernandes et al., 2015). IL-6 is secreted by T cells and macrophages that are capable of inducing immune responses, including responses to trauma (Singh et al., 2015). The *IL-6* gene, located on chromosome 7p21-24, has three single nucleotide polymorphisms (SNPs) within its promoter region (Terry et al., 2000). Several studies have demonstrated that SNPs in the -174 promoter region is associated with *IL-6* gene transcription, which subsequently influences the expression and secretion of IL-6 (Jerrard-Dunne et al., 2004). For example, a *IL-6* SNP in the -572 promoter region is the substitution of cytosine (C) by guanine (G) (Wang et al., 2016). This substitution may influence *IL-6* gene transcription and expression level of IL-6, resulting in changes to disease susceptibility (Qi et al., 2012).

Recently, a Japanese study reported keloids are associated with a significant increase in the frequency of -572 G/C polymorphisms as compared with that of healthy controls (0.406 vs 0.230, OR = 2.34, 95%CI = 1.337-4.142; P < 0.001) (Tosa et al., 2016). However, no studies have examined *IL-6* gene polymorphism in patients with KS in the Chinese Han population. Therefore, in the present study, we have explored for the first time the effect of *IL-6* gene polymorphisms on the development of KS in a Chinese Han population.

MATERIAL AND METHODS

Subjects

KS patients (224) between the ages of 18-55 years were recruited from outpatients in the Department of Dermatology, at Zhangjiagang First People's Hospital and Taixing People's Hospital. Patients who had KS due to skin disorders, hypertrophic scars, syndrome,

or genetic or chromosomal disorders were excluded from this case-control study. Clinical diagnoses of KS cases were confirmed by at least two experienced dermatologists. The control subjects were comprised of 246 normal healthy individuals without KS, systemic disorders, autoimmune disorders, and any family history of KS. This study was approved by the ethics committees of our hospital, and written informed consent was obtained from each participant prior to their enrollment in the study.

Sample collection and genotyping

Venous blood (5 mL) was collected from each participant into 0.1 mM EDTA tubes, and genomic DNA was isolated with the QIAamp DNA Blood Mini Kit, according to manufacturer's directions. Analysis of *IL-6* gene polymorphisms at -174 G/C and -572 G/C were performed via polymerase chain reaction (PCR) followed by restriction fragment length polymorphism assays (PCR-RFLP). The primers and PCR cycling conditions for *IL-6* gene polymorphisms are listed in Table 1. According to the manufacturer's instructions, PCR products were digested overnight with restriction enzymes, and amplified products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Table 1. PCR-RFLP primers and conditions for *IL-6* gene polymorphisms.

SNP		Sequence	Annealing temperature
-174	Forward	5'-CAG AAG AAC TCA GAT GAC TGG-3'	60°C
	Reverse	5'-GCT GGG CTC CTG GAG GGG-3'	
-572	Forward	5'-GGAGACGCCTTGAAGTAACTGC-3'	58°C
	Reverse	5'-GAGTTTCCTCTGACTCCATCGCAG-3'	

Serum IL-6 quantification

Serum samples were taken from enrolled patients and healthy volunteers, and were stored at -70°C until use. The serum level of IL-6 was measured using enzyme-linked immunosorbent assay (ELISA). The ELISA kit was purchased from Beijing KeMei Bio-Tech, Ltd. (Beijing, China). In this study, we use IL-1 to induce expression of IL-6.

Luciferase reporter gene assay

Construction of luciferase reporter gene plasmids was performed as previously described (Chen et al., 2013). A 751-bp sequence of *IL-6* gene was amplified from the genomic DNA of healthy volunteers homozygous for the -572 C allele. The PCR products were digested with Kpn I and Hind III (Takara Biotech., Dalian, China), and were cloned into the pGL3-basic luciferase vector (Promega, Madison, WI, USA). The C allele at nucleotide position -572 was site-specifically mutated to G alleles using a site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Luciferase activity was measured using a dual luciferase reporter assay kit (Promega, Madison, WI, USA) 48 h following transfection, according to manufacturer's specifications.

Statistical analysis

Statistical analysis was performed using the SPSS version 12.0 software for Windows

(SPSS Inc., Chicago, IL, USA). Analysis of Hardy-Weinberg was performed using the chi-square test. Difference in genotype and allele frequencies between patients and controls were evaluated using the chi-square test or the Fisher exact test. Logistic regression analysis was performed to determine the association between *IL-6* gene polymorphisms and KS. All statistical tests were two-sided, and statistical significance was set at a 2-tailed P value of < 0.05.

RESULTS

Patient characteristics

The clinical characteristics of the KS cases and control groups are shown in Table 2. There were no significant differences between case and control groups in terms of mean age, gender, tobacco and alcohol use, and BMI ($P > 0.05$). However, KS patients had significantly higher serum IL-6 levels as compared to those of controls ($P < 0.05$).

Table 2. Clinical characteristics of all participants.

	KS group (N = 224)	Control group (N = 246)	P value
Gender (F:M)	102:122	108:138	0.781
Age (means \pm SD)	25.3 \pm 8.7	24.8 \pm 9.1	0.543
BMI	24.3 \pm 2.5	23.9 \pm 2.7	0.095
Tobacco use	84	92	0.781
Alcohol use	62	70	0.918
Serum IL-6 levels (pg/mL)	84.9 \pm 9.3	71.5 \pm 8.8	0.000

Association between *IL-6* polymorphisms and risk of KS

The genotype distributions of *IL-6* polymorphisms in the KS cases and the controls are shown in Table 3. All observed genotype frequencies in both controls and cases conform to Hardy-Weinberg equilibrium ($P > 0.05$). In this study, we found that *IL-6* -174 polymorphism was not associated with risk of KS (Table 3). However, when the *IL-6* -572 CC homozygote genotype was used as the reference group, the GG genotype was found to be associated with significantly increased risk of KS (GG vs CC: OR = 2.097, 95%CI = 1.100-3.995, $P = 0.025$). When the *IL-6* -572 C allele was used as the reference group, the G allele was found to be associated with significantly increased risk of KS (G vs C: OR = 1.317, 95%CI = 1.002-1.730, $P = 0.048$).

Table 3. Distribution of *IL-6* polymorphism in KS and control cases.

Position	Genotypes	Case (%) N = 224	Control (%) N = 246	OR (95%CI)	P value
-174	GG	99 (44.2)	103 (41.9)		
	GC	101 (45.1)	113 (45.9)	0.930 (0.633-1.366)	0.768
	CC	24 (10.7)	30 (12.2)	0.832 (0.455-1.522)	0.646
	G allele	299 (66.7)	319 (64.8)		
	C allele	149 (33.3)	173 (35.2)	0.919 (0.701-1.204)	0.582
-572	CC	93 (41.5)	117 (47.6)		
	CG	101 (45.1)	111 (45.1)	1.133 (0.773-1.660)	0.559
	GG	30 (13.4)	18 (7.3)	2.097 (1.100-3.995)	0.025
	C allele	287 (64.1)	345 (70.1)		
	G allele	161 (35.9)	147 (29.9)	1.317 (1.002-1.730)	0.048

Associations between *IL-6* -572 polymorphism and expression level of *IL-6*

Lastly, we explored the association between *IL-6* -572 polymorphism and expression level of *IL-6*. We observed a marked increase in *IL-6* serum levels in KS patients with the GG genotype when compared to KS patients harboring the CC genotype (Figure 1A). As illustrated in Figure 1B, significant luciferase activity was induced in HEK293 cell lines in the presence of *IL-1*. The reporter gene expression driven by the -572G allelic *IL-6* promoter was approximately 1.55-fold higher as compared with that driven by its -572C allelic counterpart (1.85 ± 0.19 vs 1.20 ± 0.13 , $P < 0.05$).

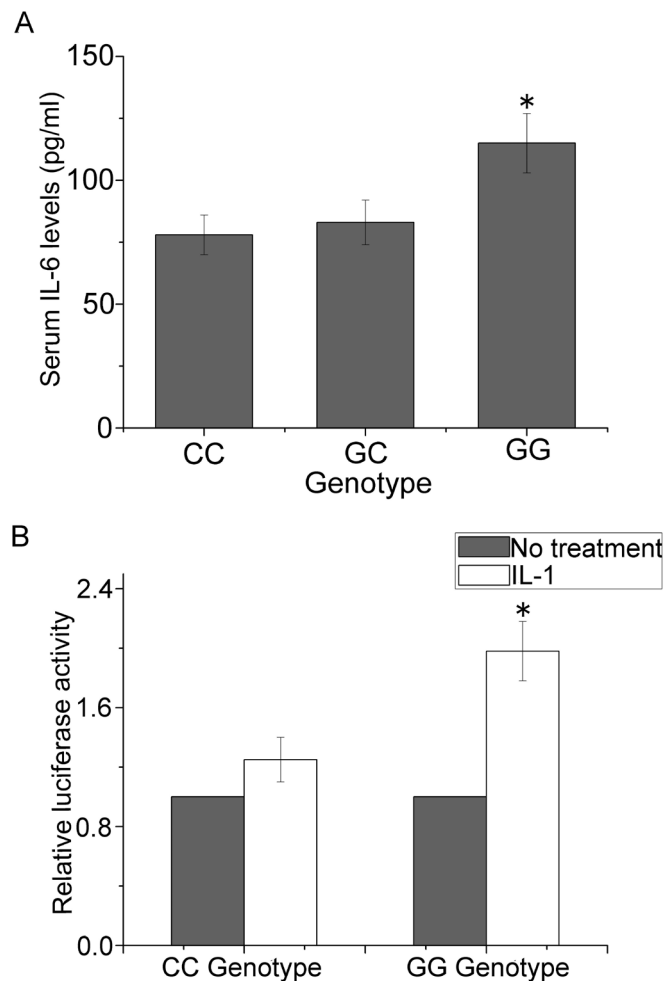


Figure 1. Associations between *IL-6* -572 polymorphism and expression of *IL-6*. **A.** serum levels of *IL-6* were measured by ELISA in KS patients with various genotypes; * $P < 0.05$ as compared to the CC genotype. **B.** cells were incubated for 48 h in the absence or presence of *IL-1*. Firefly luciferase activity was normalized for transfection efficiency by co-transfection with a *Renilla* pRL-TK plasmid; * $P < 0.05$ as compared to control.

DISCUSSION

The present study is the first demonstration of an association between *IL-6* polymorphisms and KS in a Chinese Han population. Using a case-control design, we investigated the relationship between two common *IL-6* polymorphisms and KS in southeastern China. We found that the *IL-6* -572 C > G polymorphism was closely associated with incidence of KS. Carriers with the GG genotype in the *IL-6* gene were shown to be at higher risk for developing KS. Keloid is defined as an abnormal raised, ill-defined scar that grows beyond the boundary of the original site of a skin injury. It is characterized by a flattened/smooth appearance, and is the size of tumor with varying shapes and sizes (Marneros and Krieg, 2004). Previous studies have demonstrated that KS is familial in nature, and its reported presence in identical twins has long indicated a genetic component to its disease pathology. However, the exact etiopathogenesis of KS remains unclear (Marneros et al., 2001; Yu et al., 2013). Using case-control association approaches, studies were performed to attempt to test candidate gene loci that may be involved in formation of keloid scars. Yu et al. (2013) reported that the vitamin D/VDR pathway plays an important role in the development of KS. Han et al. (2014) found that *ADAM33* polymorphism may be associated with keloid scars in the northeastern Chinese population. Findings from another studies suggested the common genetic factors can predispose individuals to the development of KS (Zhu et al., 2013). In the present study, we found that *IL-6* gene polymorphism was associated with keloid scars in the southeastern Chinese Han population.

IL-6 is a 27 kDa glycoprotein that is composed of 184 amino acids, and is secreted by activated inflammatory cells such as lymphocyte and macrophage cells (Li et al., 2015). It has been confirmed that IL-6 is involved in many metabolic processes, such as tumor growth, differentiation, and regulation of the immune microenvironment. IL-6 plays a role in a variety of biological functions by activating multiple signaling pathways. For example, it activates the Ras/Raf/MEK/ERK1/2 pathway to promote tumor cell proliferation (Naka et al., 2002; Ara and Declerck, 2010). The *IL-6* gene is located on chromosome 7p21, and is composed of 6 exons. There are several polymorphisms in the promoter region of the *IL-6* gene. Of these polymorphisms, the two most commonly involved and researched are -174 G > C and -572 C > G. Both of these polymorphisms are reported to influence the transcription rate of *IL-6* (Chérel et al., 2009). In the present study, we found the *IL-6* -572 GG genotype was associated with significantly increased risk of KS. Furthermore, we observed a marked increase in IL-6 serum levels in KS patients with the GG genotypes when compared with KS patients harboring the CC genotype. Our findings indicated that genetic factors play an important role in the occurrence of KS.

Several limitations in this study need to be addressed. This study was a single-center cohort investigation on a relatively small sample size, and therefore further studies with large independent cohorts are required to confirm the results. Next, we did not detect IL-6 expression in KS tissues. Detection of IL-6 protein in KS tissues will help to clarify the effect of the genetic variants of *IL-6* gene on KS formation.

CONCLUSION

In summary, our results suggest that the *IL-6* gene polymorphism is associated with keloid scars in the southeastern Chinese Han population.

Conflicts of interest

The authors report no conflict of interest.

ACKNOWLEDGMENTS

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