

Full Length Research Paper

Comparison of the G-174C polymorphism of interleukin (IL)-6 in different countries

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Polymorphism of G-174C in the interleukin-6 (IL-6) promoter could affect both the transcription and secretion of IL-6 and may be involved in inflammation related to and the pathogenesis of infectious diseases and chronic diseases. However, IL-6 G-174C polymorphism may differ in various ethnic groups. We recruited 300 Chinese healthy subjects among two ethnic populations in order to assess the nature of the IL-6 G-174C polymorphism in different ethnic populations of China. Common polymorphic loci in the IL-6 G-174C were determined by TaqMan SNP genotyping assays. We found that the C allele frequency at the -174 promoter region of IL-6 was extremely low in both Han and Uyghur ethnic groups. Therefore, it was concluded that the G to C variant at -174 of IL-6 is extremely rare in Chinese Han and Uyghur healthy populations, which are different from other American and African countries.

Key words: Interleukin-6 (IL-6), G-174C, polymorphism, Infectious diseases healthy population.

INTRODUCTION

Interleukin-6 (IL-6), one of the confirmed multifunctional pro-inflammatory cytokines which is demonstrated to be associated with infectious diseases (Jang et al., 2004; Nagabhushanam et al., 2003; Boulware et al., 2011; Sobti et al., 2010; Ruff et al., 2007; Newsom-Davis et al., 2004; Poudrier et al., 2001) and cardiovascular diseases (Pola et al., 2003; Lalouschek et al., 2006; Hojo et al., 2002; Plutzky, 2001; Tentolouris et al., 2004; Jenny et al., 2002), tuberculosis, AIDS and stroke, have been given more and more attentions by researchers. IL-6 demonstrates a crucial role in anti-viral immunity and in modulating T and B cell responses. Therefore, it can be conceived that the development of neutralizing antibodies against any of these cytokines as a consequence of autoimmunity affects the cellular functions and clearance of pathogens and predisposes the host to infectious

diseases. In addition, IL-6- deficient mice have been shown to be susceptible to various bacterial infections, including *Mycobacterium*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (van der Poll et al., 1997; van Enkevort et al., 2001; Diao and Kohanawa, 2005)

It is well established that IL-6 is also a key mediator in the inflammatory response associated with atherosclerosis and thus involved in many cardiovascular diseases, with many studies suggesting IL-6 may play a central role in the inflammatory response to *cerebral ischemia*, *carotid artery atherosclerosis* and *coronary heart disease* (Lalouschek et al., 2006; Hojo et al., 2002; Plutzky, 2001). The change from glycine (G) to cytosine (C) at position 174 of the IL-6 gene creates a potential binding site for the transcription factor NF-1, resulting in repressed gene expression. The G allele, on the other hand, is associated with higher circulating IL-6 levels (Fishman et al., 1998). IL-6 G-174C has been confirmed to be an important risk biomarker for some diseases such as stroke, cancer, mental retardation and ankylosing

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Table 1. IL-6 genotypes and allele distribution among two ethnic groups.

Parameter	Han (n = 200)	Uyghur (n = 100)	P Value
Age(years)	63.20 ± 10.35	63.18 ± 10.19	>0.05
Sex(M/F)	105/95	52/48	>0.05
IL-6 (-174)			
GG	199	98	
GC	1	2	>0.05
CC	0	0	
IL-6 alleles			
G	399	198	
C	1	2	>0.05
MAF	0.25 %	1 .00%	

MAF, Minor allele frequency.

spondylitis (Pola et al., 2003; Jenny et al., 2002; Gangwar et al., 2009; Resch et al., 2009; Collado-Escobar et al., 2000). We detected this important polymorphism in Chinese Han and Uyghur populations, which are the first and sixth largest ethnic populations in China, respectively. The goal of the study was to determine whether the IL-6 G-174C polymorphism differ in various ethnic population in different countries.

MATERIALS AND METHODS

Study subjects

This study was carried out with prior approval from the local Ethics Committee. Three hundred healthy subjects examined in hospitals with detailed medical routine check-up report were enrolled from Shenzhen and Xinjiang. All subjects were given informed consents. The health condition conformed to the national stipulation physical examination standard. Subjects with a history or family history of hypertension, diabetes, stroke, cancer and chronic inflammatory disorders were excluded from the study.

Sample processing

After obtaining informed consent from these groups, 5 ml blood samples were taken in ethylenediamine tetraacetic acid (EDTA) and plain vials. Genomic DNA was extracted from the peripheral blood leukocyte pellet using a DNA extraction kit (AXYGEN, California, USA). DNA samples were stored at -80°C before use.

Genotyping of the IL-6 promoter genomic variants

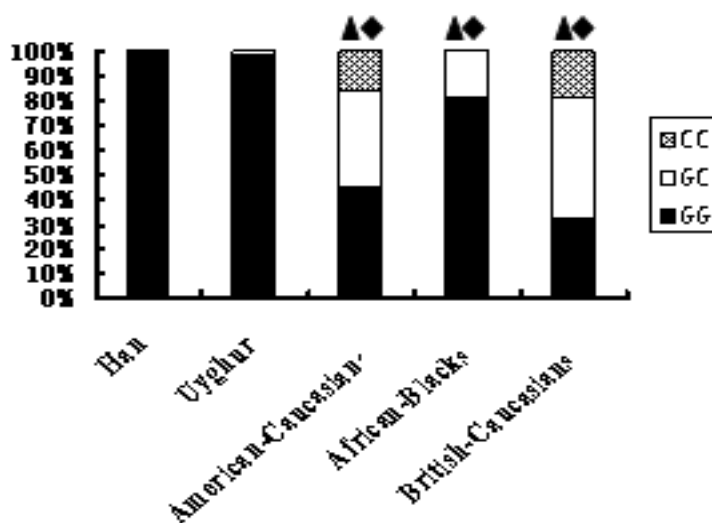
The polymorphisms of IL-6 G-174C were determined using a TaqMan single nucleotide polymorphism (SNP) genotyping technique. The IL-6 -174G/C primers were forward, 5'-AGCCTCAATGACGACCTAAG-3' and reverse, 5'-GGGCTGATTGGAAACCTTA-3'. The TaqMan minor groove binding (MGB) probes for detection of G/C polymorphism (rs1800795) were FAM-AGTTGTGTCTTGCGATGC-MGB and HEX- AGTTGTGTCTTGCCATGC-MGB. The primers and probes

were commercially supplied by Shanghai GeneCore Biotechnologies. Thermal cyclings were performed on, and allele frequencies were determined by Stratagene Mx4000 systems (Stratagene, La Jolla, CA, USA).

Comparisons between Han and Uyghur populations were made with the Fisher's exact test (nominal data). The statistical analysis was carried out using SPSS software package SPSS12.0 (SPSS Inc., Chicago, Illinois, USA). A meta-analysis was done to test heterogeneity between different populations. The Q statistic test was used to assess heterogeneity, in which a P value greater than 0.05 suggested a lack of heterogeneity. All statistic analyses were done with Stata Statistical Package (version 10.0). To improve the genotyping quality and validate our results, a random selection of 10% of the samples were re-genotyped and ten randomly selected samples were re-sequenced by laboratory personnel not otherwise involved in the study, and the results were found to be reproducible with no discrepancies noted.

RESULTS AND DISCUSSION

Table 1 includes the demographic and clinical characteristics of the patients and controls from the Han and Uyghur populations. The ratio of males to females was 105:95 in the Han subjects and 52:48 in the Uyghur subjects. There were no significant differences in sex between two populations ($P>0.05$). In addition, Table 1 also shows the distributions of the genotypes and allelic frequencies of IL-6 -174G/C polymorphisms in the Chinese Han and Uyghur population. There were no statistically significant differences in the distribution of IL-6 G-174C polymorphism between two either ethnic group ($P>0.05$). The C allele frequencies at the -174 promoter region of IL-6 were extremely low in both the Chinese Han and Uyghur populations. Figure 1 compares the IL-6 G-174C genotype frequency distribution between various populations, including Asian population, Caucasians and Blacks from American, African, Gujarat and Spanish (Cox et al., 2001; Collado-Escobar et al., 2000). The results among Han and Uyghur are very different from Caucasians and Blacks ($P<0.05$). The results of meta-



IL-6 -174G/C genotype frequency distribution between various populations.

Compared with Han population; $P < 0.05$.

Compared with Uyghur population; $P < 0.05$.

analysis were also featured with strong heterogeneity between Asians, Caucasians and Blacks (Total $I^2 = 0.81$, $P < 0.001$).

IL-6, one of the most important mediators in *in vivo* inflammatory reactions, is implicated in the development of many inflammatory diseases (Jang et al., 2004; Nagabhushanam et al., 2003; Boulware et al., 2011; Sobti et al., 2010; Ruff et al., 2007; Newsom-Davis et al., 2004; Poudrier et al., 2001; Pola et al., 2003; Lalouschek et al., 2006; Hojo et al., 2002). There are also mounting evidences confirming that IL-6 is a key regulator of inflammatory mechanisms that play an important role in the pathophysiology and development of autoimmune disease (Maddur et al., 2010). A part of the single-nucleotide polymorphisms (SNPs) identified in the IL-6 gene, especially within the non-coding promoter sequence has been shown to have a powerful influence on the expression of the gene (Fishman et al., 1998).

Our study shows the distribution of a significant SNP (G-174C) of IL-6 in Chinese Han and Uyghur population. The Uyghur population, a minority group originated from Turkish nomads living in Xinjiang having language, religious beliefs and lifestyles that may be very different from either Han population or American/European populations, is a special population presenting the typical admixture of Eastern and Western anthropometric traits in china. A study has shown that the Uyghur population has 60% European ancestry and 40% East Asian ancestry (Xu et al., 2008).

Some previous studies have found a unique association between the CC genotype of the G-174C IL-6 polymorphism and many important diseases (Pola et al.,

2003; Jenny et al., 2002). Recently, the G-174C promoter polymorphism has been linked with increased risk of cervical cancer and developmental delay (Gangwar et al., 2009; Resch et al., 2009). However, we found the distribution of this functional polymorphism of G-174C in the IL-6 is different from other populations. Interestingly, we found that the C allele frequency at the -174 promoter region of IL-6 was extremely low in the both Han and Uyghur population. The meta-analysis findings also suggest to us that there may be genetic backgrounds variations between different countries that may affect the risk of diseases. However, the reason for the rarity of C allele in the Chinese population is unclear and needs further ethnicity-specific studies.

From the current study, we conclude that the C allele frequency at the -174 promoter region of IL-6 is extremely low in the Chinese Han and Uyghur population, which are very different from Caucasians and Blacks (Cox et al., 2001; Collado-Escobar et al., 2000). These findings may provide some evidences for future associated research.

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