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Association Between *IRF-5* Polymorphisms and Risk of Acute Coronary Syndrome

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Previous studies suggested that genetic polymorphisms in interferon regulatory factor 5 (*IRF-5*) are implicated in the susceptibility to a range of autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease. Recently, IRF-5 has been implicated in inflammatory processes that are associated with excessive remodeling and atherosclerosis. Our purpose was to investigate the association between the *IRF-5* polymorphisms and the risk of acute coronary syndrome (ACS) in a Chinese population. The 5 bp indel (insertion/deletion) (CGGGG) polymorphism, located 64 bp upstream of the alternative exon 1a of *IRF-5* gene, and the deletion of 30 bp in exon 6 of *IRF-5* gene were analyzed among 148 patients with ACS and 246 controls in a Chinese population, using a polymerase chain reaction–restriction fragment length polymorphism strategy and direct sequencing. The frequencies of $(CGGGG)_3(CGGGG)_4$ genotype and $(CGGGG)_4$ allele in ACS patients were significantly higher than those in control subjects ($p = 0.018$, odds ratio [OR] = 1.76, 95% confidence interval [CI]: 1.10–2.81; $p = 0.028$, OR = 1.62, 95% CI: 1.05–2.50, respectively). However, no significant relationship between the 30 bp exon 6 polymorphism of the *IRF-5* gene and the risk of ACS was observed ($p = 0.770$, OR = 0.96, 95% CI: 0.72–1.28). The 5 bp indel (CGGGG) polymorphism of the *IRF-5* gene may be associated with susceptibility to ACS.

Introduction

As one kind of coronary artery diseases, acute coronary syndrome (ACS) is a clinical manifestation of preceding atherosclerosis, inflammation, and thrombosis (Hansson, 2005). Nowadays, there is evidence that blocking inflammation could lower thrombosis and thus acute coronary events. Previous studies have shown that environmental factors, such as smoking, hypertension, diabetes mellitus, hypercholesterolemia, obesity, and sedentary lifestyle, are related to the disease (Yano *et al.*, 1984; Kannel, 1985; Anderson *et al.*, 1991). Additionally, genetic factors, such as single-nucleotide polymorphisms (SNPs) of epidermal growth factor receptor (Gao *et al.*, 2008), interleukin (IL)-1B (Soylu *et al.*, 2008), and IL-6 (Antonicelli *et al.*, 2005), play important roles in the development of ACS. Although many gene SNPs have been reported to be associated with ACS (Antonicelli *et al.*, 2005; Gao *et al.*, 2008; Soylu *et al.*, 2008), it is a complex, multifactorial polygenetic disorder. Thus, it is of value to discover novel gene variants linked to disease risk.

The interferon regulatory factors (IRFs), transcriptional mediators of early inflammatory gene transcription in infected cells, play a crucial role in inflammatory and immune responses. IRF-5 is a member of the IRF family, involved in cell adhesion, apoptosis, cell cycle regulation, and early immune activation (Hu *et al.*, 2005). The *IRF-5* gene is located on human chromosome 7q32 (Lee and Nath, 2005), containing nine exons. Overexpression of IRF-5 induces interferon (*IFN*) gene expression, thus upregulates monocyte chemoattractants, and production of proinflammatory cytokines, such as IL-6 and IL-12 (Takaoka *et al.*, 2005). Variants of *IRF-5* gene may regulate the expression of IRF-5 mRNA in atherosclerotic tissue (Malarstig *et al.*, 2008). Heinemeyer *et al.* (1998) reported a 5 bp indel (CGGGG), located 64 bp upstream of the alternative exon 1a of *IRF-5* gene, which contains an additional binding site for the transcription factor SP1 and affects *IRF-5* gene expression. Several studies have shown that the indel (CGGGG) has moderately significant association with susceptibility to systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis (Dideberg *et al.*, 2007; Sigurdsson *et al.*, 2008; Shimane *et al.*, 2009). The other indel is a deletion of 30 bp in exon 6 that removes 10 amino acids from the IRF-5 protein; it has differential ability to initiate transcription of IRF-5 target genes (Barnes *et al.*, 2002, 2004; Mancl *et al.*, 2005; Graham *et al.*, 2007).

Considering the critical role of IRF-5 in inflammatory diseases, *IRF-5* polymorphisms might be another genetic factor

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Table 1. The Characteristics of the Study Population

	Control	*ACS*	
	n = *246 (%)*	n = *148 (%)*	p[a]
Sex (M/F)	154/92 (62.6/37.4)	89/59 (60.1/39.9)	NS
Age (mean ± SD)	59.4 ± 11.6	59.1 ± 11.1	NS
UA/NSTEMI/STEMI		75/39/34 (50.7/26.4/23.0)	
Hypertension		62 (41.9)	
Diabetes mellitus		26 (17.6)	

[a]ACS versus controls by the Student's *t*-test or χ^2 test.

ACS, acute coronary syndrome; UA, unstable angina; NSTEMI, non-ST–segment elevation myocardial infarction; STEMI, ST-segment elevation myocardial infarction; NS, not significant; SD, standard deviation.

in the development of ACS. However, so far, no studies have reported the association between *IRF*-5 polymorphisms and ACS. The purpose of this study was to investigate whether the CGGGG indel and the deletion of 30 bp in exon 6 polymorphisms of the *IRF-5* gene play a role in ACS in a Chinese population.

Materials and Methods

Study population

In all, 148 unrelated ACS patients (mean age 59.1 ± 11.1) of West China Hospital, from September 2005 to September 2007, were enrolled in this study (Table 1). The diagnosis of ACS was based on patient's history, physical examination, electrocardiogram, and echocardiogram according to the World Health Organization criteria [1]. The control group consisted of 246 healthy subjects (mean age 59.4 ± 11.6) from a routine health survey. All subjects were Han population living in Sichuan province of southwestern China. Patients with a history of hypertension, coronary heart disease, cardiac valve disease, tachyarrhythmia, heavy alcohol intake, acute viral myocarditis, systemic diseases of putative autoimmune origin, or skeletal myopathies were intentionally excluded. The study was approved by the hospital ethics committee of West China Hospital, and all subjects gave informed consent.

Determination of genotypes

Genomic DNA of each individual was extracted from a tube of 200 μL EDTA-anticoagulated peripheral blood sample by a DNA isolation kit from Bioteke (Beijing, China). The procedure was performed according to the instruction manual. The polymerase chain reaction (PCR)–polyacrylamide gel electrophoresis method was used to genotype the CGGGG indel and exon 6 deletion polymorphisms of *IRF-5*. DNA fragments containing the polymorphisms were amplified by PCR using two primer pairs. The primer sequences were CGGGG-F: 5′-CGGGGCCCGGAGTGGATTC-3′ and CGGGG-R: 5′-GGGCACTTCCGCGTCTT G-3′; exon 6-F: 5′-CCCCACATGA CACCCTATTC-3′ and exon 6-R: 5′-GGCTGGGGTCTGGA GCAG-3′. PCR was performed in a total volume of 25 μL, including 2.5 μL 10×PCR buffer, 1.5 mM $MgCl_2$, 0.15 mM dNTPs, 10 μM each primer, 100 ng of genomic DNA, and 1 U of Taq DNA polymerase. The PCR conditions were 94°C for 4 min, followed by 35 cycles of 30 s at 94°C, 30 s at 62.5°C for -CGGGG-; 30 s at 60.8°C for exon 6, and 30 s at 72°C, with a final elongation at 72°C for 10 min. Three microliters of PCR products were separated by an 8% polyacrylamide gel and staining with 1 g/L argent nitrate. For the CGGGG polymorphism, allele $(CGGGG)_3$ yields a 102 bp band and allele $(CGGGG)_4$ yields a 107 bp band (Fig. 2); for the exon 6 polymorphism, allele insertion yields a 145 bp band and allele deletion yields a 115 bp band (Fig. 1). To confirm the genotyping results, PCR-amplified DNA samples were examined by DNA sequencing and the results were 100% concordant.

Statistical analysis

All data analyses were carried out using SPSS 13.0 statistical software (Chicago, IL). Allele and genotype frequencies of *IRF-5* gene polymorphisms were obtained by directed counting, and Hardy–Weinberg equilibrium was evaluated by the χ^2 test. Odds ratio (OR) and respective 95% confidence intervals (CIs) were reported to evaluate the effects of any difference between alleles and genotypes. A $p \leq 0.05$ was regarded as statistically significant in ACS patients compared with healthy controls.

Results

Genotype distributions had no deviation from Hardy–Weinberg equilibrium in both case and control groups. Allele and genotype frequencies of both polymorphisms for 148 ACS patients and 246 healthy controls are shown in Table 2. The frequencies of $(CGGGG)_3(CGGGG)_4$ genotype and $(CGGGG)_4$ allele in ACS patients were significantly higher than those in control subjects ($p = 0.018$, OR = 1.76, 95% CI: 1.10–2.81 and $p = 0.028$, OR = 1.62, 95% CI: 1.05–2.50, respectively). However, no significant relationship between the 30 bp in exon 6 polymorphism of the *IRF-5* gene and the risk of ACS was observed ($p = 0.770$, OR = 0.96, 95% CI: 0.72–1.28).

Discussion

To our knowledge, this is the first study to investigate the association between ACS and the CGGGG indel and the deletion of 30 bp in exon 6 polymorphisms of *IRF-5* gene in a Chinese population. In the current study, we found that CGGGG indel polymorphism may be associated with the susceptibility to ACS.

Table 2. The Genotype and Allele Distribution of Interferon Regulatory Factor-5 Polymorphisms in Acute Coronary Syndrome Patients and Controls

Polymorphism	*Control n = 246 (%)*	*ACS n = 148 (%)*	*OR (95% CI)*	p
CGGGG insertion/deletion				
Genotypes				
$(CGGGG)_3(CGGGG)_3$	197 (80.1)	103 (69.6)	1.00 (Ref.)	
$(CGGGG)_3(CGGGG)_4$	49 (19.9)	45 (30.4)	1.76 (1.10–2.81)	0.018
Alleles				
$(CGGGG)_3$	443 (90.0)	251 (84.8)	1.00 (Ref.)	
$(CGGGG)_4$	49 (10.0)	45 (15.2)	1.62 (1.05–2.50)	0.028
30 bp insertion/deletion				
Genotypes				
deletion/deletion	65 (26.4)	32 (21.6)	1.00 (Ref.)	
insertion/deletion	129 (52.4)	95 (64.2)	1.50 (0.91–2.47)	0.113
insertion/insertion	52 (21.1)	21 (14.2)	0.82 (0.42–1.59)	0.556
Alleles				
deletion	259 (52.6)	159 (53.7)	1.00 (Ref.)	
insertion	233 (47.4)	137 (46.3)	0.96 (0.72–1.28)	0.770

OR, odds ratio; CI, confidence interval.

Our study had 82% (CGGGG) and 84% (30 bp) power to detect an effect with a relative risk of 2.0 in the group of patients and healthy controls under a dominant genetic model.

Previous studies have shown that longer allele of CGGGG repeats contains an additional binding site for the transcription factor SP1 and affects *IRF-5* gene expression (Shimane *et al.*, 2009). It has not only conferred increased risk of systemic lupus erythema fosus (SLE) and inflammatory bowel disease (IBD) but also increased expression of IRF-5 through creating an additional SP1-binding site (Shimane *et al.*, 2009), and thus we hypothesize that it may be one of the factors leading to ACS. Our data confirmed this hypothesis and provided evidence that CGGGG indel polymorphism is associated with ACS. However, our data are in disagreement with Malarstig's reports that found IRF-5 was not associated with unstable coronary artery disease in a Scandinavian population (Malarstig *et al.*, 2008). Although it is difficult to decipher the reasons for these discrepancies, two possibilities should be considered. On the one hand, *IRF-5* polymorphism is distinct in different ethnicities. On the other hand, ACS is a multifactorial disease; different environmental factors, such as smoking and sedentary lifestyle, might have caused the different results.

The impact of allele-specific mRNA expression on mRNA translation into functional IRF-5 protein remains unknown. IRF-5 has a critical function in downstream signaling of toll-like receptors (TLRs) and the subsequent production of inflammatory cytokines tumor necrosis factor-α, IL-6, and IL-12 in IRF-5 null mice (Barnes *et al.*, 2003; Takaoka *et al.*, 2005). IRF-5 increases the expression of many genes, such as genes coding signaling molecules, proteins involved in cell signaling, apoptosis, cell-cycle regulation, and early immune response (Sigurdsson *et al.*, 2005). Meanwhile, Barro and Patton

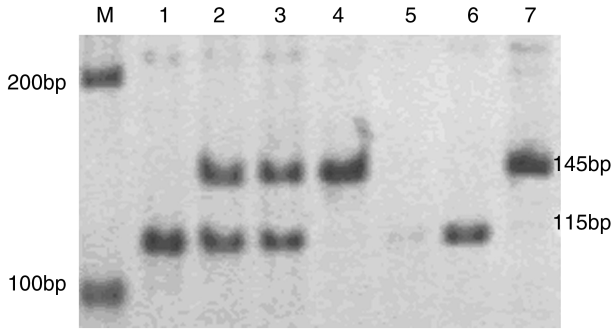

FIG. 1. Polyacrylamide gel electrophoresis analysis of 30 bp insertion/deletion interferon regulatory factor 5 (*IRF-5*) polymorphism. The deletion allele corresponds to the 115 bp fragment, and the insertion allele corresponds to the 145 bp fragment. The polymerase chain reaction products after electrophoresis in 8% polyacrylamide gel are shown. Lanes 1, 5, and 6, homozygous (deletion/deletion); lanes 2 and 3, heterozygous (insertion/deletion); lanes 4 and 7, homozygous (insertion/insertion); M, DNA marker.

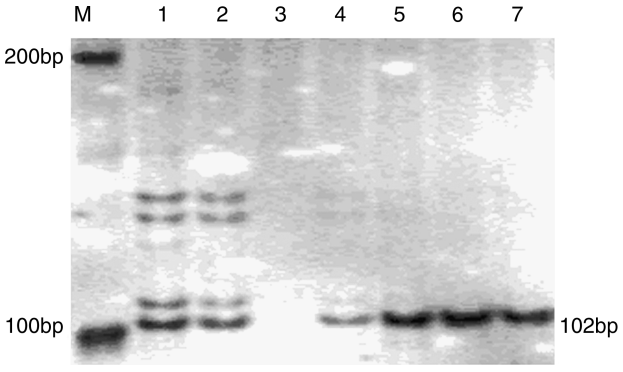

FIG. 2. Polyacrylamide gel electrophoresis analysis of CGG GG insertion/deletion *IRF-5* polymorphism. The deletion allele corresponds to the 102 bp fragment and the insertion allele corresponds to the 107 bp fragment. The polymerase chain reaction products after electrophoresis in 8% polyacrylamide gel are shown. Lanes 1, 2, and 4, heterozygous ($(CGGGG)_3$ $(CGGGG)_4$); lanes 5–7, homozygous ($(CGGGG)_3(CGGGG)_3$); lane 3, negative control; M, DNA marker.

(2007) reported that NSP1 used same proteasome-mediated mechanism to induce the degradation of IRF-5 that it used in inducing the degradation of IRF-3 and IRF-7. Barnes *et al.* found that although IRF-5 and IRF-7 can form heterodimers, it is not cooperative and does not result in an inhibition of *IFNA* gene transcription. The presence of IRF-5/IRF-7 heterodimers could be detected in infected but not uninfected cells, which means clearly that IRF-5 and other IRF stimulated a large number of common genes that make the complicated network (Barnes *et al.*, 2003). Moreover, in human primary plasmacytoid dendritic cells, *IRF-5* is transcribed into four distinct alternatively spliced isoforms (V1, V2, V3, and V4), whereas in human primary peripheral blood mononuclear cells, two additional new isoforms (V5 and V6) were identified. The *IRF-5* V1, V2, and V3 transcripts have different noncoding first exons and distinct insertion/deletion patterns in exon 6. In addition to V5 and V6, there are three more alternatively spliced IRF-5 isoforms (V7, V8, and V9) (Mancl *et al.*, 2005) that may demonstrate the existence of multiple IRF-5–spliced isoforms with distinct cell type–specific expression, cellular localization, differential regulation, and dissimilar functions. Additionally, Malarstig reported that IRF-5 mRNA expression levels in atherosclerotic tissue and control tissue may reflect that expression of IRF-5 mRNA in a local and systemic state of chronic inflammation is enhanced, and IRF-5 mRNA is expressed in human arotid plaques. IRF-5 expression in atherosclerotic tissue is influenced by several SNPs within the *IRF-5* gene. In the present study, we found that $(CGGGG)_3(CGGGG)_4$ genotype and $(CGGGG)_4$ allele were associated with a significantly increased risk of ACS compared with $(CGGGG)_3(CGGGG)_3$ genotype and $(CGGGG)_3$ allele.

In conclusion, we found that the CGGGG indel polymorphism in the *IRF-5* gene is associated with susceptibility to ACS. These data indicate that the observed effects of SNPs on *IRF-5* gene expression may influence the disease traits investigated or that IRF-5 expression is a casual factor in ACS development and progression in a Chinese population.

Disclosure Statement

No competing financial interests exist.

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