The A to G polymorphism at -1082 of the interleukin-10 gene is rare in the Han Chinese population

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Abstract. Interleukin-10 (IL-10) is a multifunctional anti-inflammatory cytokine involved in various physiological and pathophysiological processes including cardiovascular disease. It has been reported that 50-75% of the variation in IL-10 production is genetically controlled. In the present study, the IL-10 -1082A/G (rs1800896) polymorphism was detected in 174 coronary artery disease (CAD) patients confirmed by selective coronary angiography and 176 age and gendermatched controls from the Jiangsu area (East China). The majority of the subjects (93.14%) carried the AA wild-type genotype, whereas only 0.29% carried the GG genotype. Our results suggest that IL-10 -1082A/G is rare and unlikely to be a significant contributory to disease susceptibility in the Han Chinese population.

Introduction

Interleukin-10 (IL-10) is a multifunctional anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses (1-4) whose effects are directed mainly against functions of mononuclear cells, T lymphocytes and polymorphonuclear leukocytes. IL-10 is also involved in the inhibition of cell adhesion molecules, monocyte chemotactic protein-1, tissue factor, fibrinogen, matrix metalloproteinase-9, T-lymphocyte granulocyte-macrophage

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colony-stimulating factor, inducible nitric oxide synthase and smooth muscle cell proliferation (5-8).

Recent studies have shown that IL-10 is associated with a variety of disease states, including cancer, Alzheimer's disease, systemic sclerosis, type 2 diabetes, ischemic stroke, atherosclerosis, cardiovascular disease, ankylosing spondylitis, asthma, rheumatoid arthritis and prostate hyperplasia (9-18).

It has been reported that 50-75% of the variation in IL-10 production is genetically controlled (19,20). The human IL-10 gene is located on chromosome 1 and has been mapped to the junction between 1q31 and 1q32 (21). Several polymorphic sites of the IL-10 gene have been identified. Three polymorphic sites (-1082A/G, rs1800896; -819C/T, rs1800871 and -592C/A, rs1800872) are located in the promoter region of the gene. Individuals homozygous for the -1082G allele have higher circulating IL-10, a higher expression of IL-10 mRNA and greater production of IL-10 following *in vitro* stimulation (8,22).

Based on these findings, the -1082A/G polymorphism was detected in the IL-10 gene promoter region in patients with coronary artery disease (CAD) and healthy individuals in the Jiangsu area (East China). The aim of the present study was to determine the prevalence of this IL-10 polymorphism and to examine its putative association with CAD in the southern Han Chinese population.

Patients and methods

Subjects. A total of 174 CAD patients confirmed by selective coronary angiography were eligible for this study. Subjects with congenital heart disease, cardiomyopathy, valvular disease and renal or hepatic disease were excluded. Coronary angiograms were analyzed by two experienced interventional cardiologists. CAD was defined as having angiographic coronary stenosis of at least 50% lumen reduction in at least one major epicardial coronary artery. A total of 176 age- and gender-matched individuals served as controls. The controls were judged to be free of CAD based on history, clinical examination, electrocardiography, ultrasonic echocardiogram

Population (Refs.)	Individuals investigated	Genotype frequency n (%)			Allele frequency, %	
		AA	AG	GG	A	G
Finnish (23) ^a	169	57 (33.73)	80 (47.34)	32 (18.93)	57.40	42.60
British (22) ^a	150	49 (32.67)	65 (43.33)	36 (24.00)	54.33	45.67
Italian (24) ^a	63	20 (31.75)	29 (46.03)	14 (22.22)	54.76	45.24
Spanish (25) ^a	100	21 (21.00)	50 (50.00)	29 (29.00)	46.00	54.00
African American (26) ^a	233	88 (37.77)	118 (50.64)	27 (11.59)	63.09	36.91
Asian Indian (27) ^a	140	73 (52.14)	61 (43.57)	6 (4.29)	73.93	26.07
Korean (3) ^b	495	435 (87.88)	56 (11.31)	4 (0.81)	93.54	6.46
Japanese (28) ^b	98	92 (93.88)	5 (5.10)	1 (1.02)	96.43	3.57
Chinese (our study)	350	326 (93.14)	23 (6.57)	1 (0.29)	96.43	3.57

Table I. Frequencies of the various IL-10 genotypes reported among various populations.

and the Rose questionnaire. Of the 176 controls, 146 subjects were confirmed by selective coronary angiography. All study participants were unrelated Han Ethnicity residents of the Jiangsu area (East China). The study was approved by the East China medical ethics committee and written informed consent was obtained from all participants.

Methods. DNA was extracted from peripheral blood using a commercially available kit. Sequence amplification was performed using polymerase chain reaction (PCR). The primers were 5'-TTCCCCAGGTAGAGCAACACT-3' (sense) and 5'-GATGGGGTGGAAGAAGTTGAA-3' (antisense). This set encompasses the region of interest in the IL-10 promoter and generates a 238-bp product. The reaction volume was 25 μ l in each well of a 96-well plate, with final reaction component concentrations of 0.3 mmol/l for each of the four dNTPs, 1.5 mmol/l MgCl₂, 0.2 pmol/l for each of the primers, 0.1 mg/l for genomic DNA and 1.25 units Taq polymerase. The PCR program consisted of initial denaturation at 94°C for 5 min and 38 cycles of denaturation at 94°C for 45 sec, annealing at 52°C for 38 sec, extension at 72°C for 1.5 min and a final extension at 72°C for 10 min. The genotypes were resolved using restriction endonuclease digestion. Amplification products (15 μ l) were digested with 2 units MnII (New England Biolabs Ltd., Beijing, China) at 37°C for over 4 h to detect allele A (238 bp) and allele G (136 bp + 102 bp). The size of the digestion products were then determined by electrophoresis on 2% agarose gel stained with ethidium bromide, and positive and negative controls were used to ensure reliability. Twenty-five amplification products from patients with CAD and 25 from controls were sent for direct DNA sequencing.

Results and Discussion

The frequencies of -1082A/G genotypes of IL-10 in our population are shown in Table I. Among the 174 CAD patients and 176 controls studied, 326 carried the AA wild-type, 23 carried the AG genotype and only one GG genotype was detected. The 50 samples sent to direct DNA sequencing were consistent with the results from PCR-RFLP. Due to this distribution, the original case-control analysis was not pursued.

Human linkage studies have attempted to associate polymorphisms of certain cytokines with CAD. IL-10 plays a significant role in the inhibition of macrophage function, including cytotoxic activity and cytokine synthesis, suggesting that IL-10 may arrest and reverse the chronic inflammatory response in established atherosclerosis, as well as limit thrombotic complications (29,30). Previously, a large number of studies have focused on shedding light on the possible association between the IL-10 polymorphisms and the risk of CAD, but have yielded conflicting results (2,31-34).

The correlation between CAD and the IL-10 polymorphisms has not previously been studied in the Han Chinese population. In the present study, the results showed that 93.14% of the subjects carried the AA wild-type and only 0.29% carried the GG genotype. Our findings are consistent with a recent study in another Han Chinese population, which identified no -1082GG genotype out of 110 ankylosing spondylitis patients and 120 ethnicity-matched healthy controls (11).

As shown in Table I, the distribution of the frequencies of IL-10 -1082A/G genotypes in the Chinese population are similar to those from other studies in eastern Asian populations, apart from a slight variation (3,28). Of 98 Japanese subjects, only one GG homozygous geneotype was detected, while in the Korean population, the frequency of the GG genotype was only 0.81%. By contrast, the genotype distributions of IL-10 -1082A/G in several Caucasian groups were similar, although the A allele frequency in European Caucasians increased with higher latitudes, with the highest demonstrated in a Finnish population (22-25). The Asian Indian population (27) had an A allele frequency that was intermediate to those of the Caucasian and Eastern Asian populations. These data confirm previous studies with regard to evolutionary trees, suggesting that European and Southwest Asian (Indian) populations diverged from the Chinese population, with an estimated genetic distance of at least 20,000 years (35).

In summary, the IL-10 polymorphism at -1082 bp is rare and unlikely to be a significant contributory factor to disease susceptibility in the Han Chinese population.

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