Study of the relationship between *IL-10* polymorphism and serum lipoprotein levels in Han Chinese individuals

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ABSTRACT. Previous studies have shown that cytokines can affect serum lipoprotein concentrations. The aim of this study was to examine the association between *IL-10* gene polymorphisms and serum lipoprotein levels of Han Chinese individuals. A total of 359 Han Chinese people were enrolled in this investigation. *IL-10* -592, -819, and -1082 genotypes were established using polymerase chain reaction-restriction fragment length polymorphism analysis. An automatic biochemistry analyzer was used to determine serum concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) in each individual. We observed that the three *IL-10* polymorphisms did not significantly differ in terms of age or age of carrier ($P > 0.05$), and the -592 and -819 variants did not significantly affect serum lipoprotein levels ($P > 0.05$). HDL concentrations were higher and TG levels were lower in carriers of the -1082 GA genotype compared to those with the AA genotype, and these differences were statistically significant ($P < 0.05$). However, TC, VLDL, and LDL levels were unaffected

by this sequence variation (P > 0.05). Our results suggest that the polymorphism at position -1082 in the promoter region of *IL-10* may affect serum HDL and TG concentrations, while other variants of this gene appear to have no relationship with serum lipoprotein levels.

Key words: Interleukin-10 Serum lipoprotein; Genetic polymorphism

INTRODUCTION

Plasma lipoproteins and lipids have been shown to be very important contributors to the pathophysiology of atherosclerotic vascular disease, and high levels of cholesterol, low-density lipoprotein (LDL), and triglycerides (TG) are considered major cardiovascular disease risk factors (Ross, 1999; Go et al., 2013). In addition, plasma levels of high-density lipoprotein (HDL) are inversely correlated with atherosclerosis risk.

A previous study has revealed that inflammatory cytokines can induce changes in lipid metabolism and lead to hyperlipidemia (Feingold et al., 1998). In contrast, metabolic disorder may affect the production, secretion, and effects of cytokines. For example, lipoproteins acting as pro-inflammatory mediators can induce the synthesis and release of inflammatory cytokines including interleukin (IL)-6, IL-1, IL-10, and NF-κB (Lynn et al., 2001).

According to their effects in immune and inflammatory responses, the cytokines are divided into pro- and anti-inflammatory categories. The former include IL-1β, TNF-β, and IL-6, among others (Hashizume and Mihara, 2011). IL-6 is a key regulatory factor in inflammatory cell differentiation, and participates in the metabolism of lipids, influencing TG and LDL levels (Haddy et al., 2003; Shen et al., 2008). Therefore, it may play an important role in the inflammatory response, atherosclerosis, and thrombus formation (Haddy et al., 2003). *IL-6* promoter polymorphisms might contribute to the differences between individuals in transcription of this gene and expression of the encoded protein, thus influencing its effects (Fishman et al., 1998).

The anti-inflammatory cytokine IL-10 is produced by Th2 cells and can inhibit cellular immunity and the inflammatory response (Pajkrt et al., 1997). Moreover, pro-and anti-inflammatory cytokines are capable of affecting each other. They play an important role in abnormal lipid metabolism (Nunes et al., 2008), in which they demonstrate a reciprocal relationship, and exert an effect pathophysiological processes (Huang, 2010).

Despite much evidence connecting inflammatory cytokines to lipid metabolism disorders, their relationship and the mechanism responsible remain unclear. However, cytokine promoter polymorphisms provide a novel research perspective. Therefore, this study aimed to investigate whether *IL-10* gene polymorphisms are associated with variation in plasma lipoprotein levels.

MATERIAL AND METHODS

Subjects

A total of 359 individuals aged 23-68 years old (with an average age of 37.34 ± 6.89 years), comprising 195 men and 164 women examined in our hospital between June 2014 and June 2015, were enrolled in this study. All subjects were medically assessed by systematic physical examination, and blood pressure, liver and kidney function, and blood routine tests.

In addition, their medical histories, including details of hypertension, diabetes, heart disease, and smoking and drinking habits were inspected. Subjects with significant hepatocellular, renal, or heart failure, malignant diseases, severe infection, or potential symptoms of infection for more than 4 weeks, rheumatic heart disease, or history of surgery or injury were excluded. Participants had no heredofamilial history of disease and were not genetically related. This study was approved by the relevant ethics committee, and written informed consent was obtained from all individuals.

Methods

Reagents and instruments

The following were used in this investigation: total blood DNA extraction kit (TIANGEN Biotechnology Company, Beijing, China), *Taq* DNA polymerase, 10X polymerase chain reaction (PCR) mix, restriction endonucleases (Thermo Fisher, Waltham, MA, USA), automatic biochemical analyzer (Beckman LX20; Beckman Coulter, Brea, CA, USA).

Sample preparation

Blood samples were obtained from all subjects in the morning following an overnight fasting. Venous blood (5 mL) was withdrawn from the vein into Vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, USA) containing ethylenediaminetetraacetic acid as an anticoagulant. To separate plasma, blood samples were centrifuged at 3000 rpm for 10 min. The supernatant was used for biochemical measurements, while the buffy coat and red blood cell pellet were used for DNA extraction.

Biochemical analyses

Serum glucose, TG, total cholesterol (TC), and LDL, HDL, and very low-density lipoprotein (VLDL) cholesterol levels were measured by the standard enzymatic method, using the automatic biochemical analyzer mentioned above.

Genotyping

Genomic DNA was amplified by PCR using *IL-10* (-592, -819, and -1082)-specific primers (Table 1). The PCR conditions used for each polymorphism are shown in Table 2. Restriction enzyme digestion was employed to determine *IL-10* variant genotypes (Table 1), with the resulting fragments being analyzed on 2-2.5% agarose gels.

Table 1. Primer sequences and methods used for detection of *IL-10* gene polymorphisms.

Primer	Sequences (5'-3')	Method
IL-10 -592	Forward: 5'-CAACTTCTTCCACCCCATCTTT-3' Reverse: 5'-GTGGGCTAAATATCCTCAAAGTT-3'	*Rsa*I-based RFLP
IL-10 -819	Forward: 5'-CAACTTCTTCCACCCCATCTTT-3' Reverse: 5'-GTGGGCTAAATATCCTCAAAGTT-3'	*Mae*III-based RFLP
IL-10 -1082	Forward: 5'-CCAAGACAACACTACTAAGGCTCCTTT-3' Reverse: 5'-GCTTCTTATATGCTAGTCAGGTA-3'	*Xag*I-based RFLP

RFLP: restriction fragment length polymorphism.

Table 2. Polymerase chain reaction (PCR) conditions for amplification of *IL-10* loci.

Locus	PCR conditions
IL-10 -592	94°C (5 min); 35 cycles: 94°C (45 s), 57°C (45 s), 72°C (1 min); 72°C (10 min)
IL-10 -819	94°C (5 min); 35 cycles: 94°C (45 s), 57°C (45 s), 72°C (1 min); 72°C (10 min)
IL-10 -1082	94°C (5 min); 35 cycles: 94°C (45 s), 60°C (45 s), 72°C (1 min); 72°C (10 min)

Statistical analysis

All single nucleotide polymorphism (SNP) data were evaluated for Hardy-Weinberg equilibrium. Data are reported as medians and ranges or means and standard deviations, and were analyzed using the chi-square test and Fisher's exact test, as appropriate. Differences in the frequencies of polymorphisms between groups were compared using logistic regression analysis, while analysis of variance was used to compare serum lipid levels. All tests were two-tailed, with a confidence interval of 95%. Statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA), and P values < 0.05 were considered statistically significant.

RESULTS

IL-10 gene polymorphism

The results of *IL-10* polymorphism genotyping are shown in Table 3. We found that the genotype frequencies of the three *IL-10* variants examined conformed to Hardy-Weinberg equilibrium. Each had two alleles (-592C/A, -819C/T, and -1082G/A), and a strong allelic association between sites -592 and -819 was discerned, in that the combinations -592CC and -819CC, -592CA and -819TC, and -592AA and -819TT always occurred at the same time. The -1082 GG genotype was not observed, which is consistent with a previous study (Fan et al., 2004). There were no significant differences regarding sex and age of carrier between the genotypes of the three polymorphisms (P > 0.05).

Table 3. Genotype frequencies of polymorphisms in the *IL-10* gene promoter region.

Locus	Genotype frequency (N)		
-592	AA 0.40 (144)	AC 0.41 (147)	CC 0.19 (68)
-819	CC 0.19 (68)	TC 0.41 (147)	TT 0.40 (144)
-1082	AA 0.92 (329)	AG 0.08 (30)	GG 0 (0)

Relationship between *IL-10* polymorphisms and serum lipoprotein levels

Our analysis of the relationship between the *IL-10* gene polymorphisms under investigation and serum lipoprotein levels is shown in Table 4. No statistically significant difference was found between the -592 and -819 genotypes in terms of serum lipoprotein levels (P > 0.05). However, HDL levels were higher and TG concentrations lower in carriers of the -1082 GA genotype than in those with an AA genotype, and these differences were statistically significant (P < 0.05). Nonetheless, TC, VLDL, and LDL levels were unaffected by variation at this site.

Table 4. Serum concentrations of triglycerides and lipoproteins in carriers of various *IL-10* polymorphism genotypes (means ± standard deviation, mmol/L.

Locus	Gt	TC	TG	HDL	LDL	VLDL
-1082	AA	4.698 ± 0.815	1.703 ± 1.823*	1.265 ± 0.347*	2.406 ± 0.582	1.034 ± 0.354
	GA	4.973 ± 0.915	0.987 ± 0.316*	1.524 ± 0.498*	2.459 ± 0.671	0.983 ± 0.138
	GG	0	0	0	0	0
-819/-592	CT/CA	4.883 ± 0.819	1.899 ± 2.394	1.327 ± 0.229	2.527 ± 0.652	1.139 ± 0.335
	CC/CC	4.812 ± 0.429	1.294 ± 0.548	1.321 ± 0.213	2.535 ± 0.447	0.957 ± 0.211
	TT/AA	4.674 ± 0.721	1.684 ± 0.802	1.254 ± 0.309	2.409 ± 0.582	1.042 ± 0.274

*Statistically significant, $P < 0.05$. Gt = genotype, TC = total cholesterol, TG = triglycerides, HDL = high-density lipoprotein, LDL = low-density lipoprotein, VLDL = very low-density lipoprotein.

DISCUSSION

Abnormal lipid metabolism involves a complex pathology, being influenced by factors such as diet, age, occupation, environment, and metabolism (Saito, 1997). Following the development of studies in molecular genetics, the genetic basis of abnormal lipid metabolism has attracted widespread interest. The mechanism responsible for clinical cases of this disease is especially complicated, often involving gene-gene or gene-environment interactions (Kirillova, 2012).

Many studies have demonstrated that inflammatory cytokines have a close relationship with abnormal lipid metabolism (Haas and Mooradian, 2010). However, the mechanism behind this association is unclear, and prior investigations have principally concentrated on signal transduction, immune regulation, and protein expression level. In this study, healthy Han Chinese individuals were tested, and the relationship between *IL-10* gene polymorphisms and lipid levels was investigated. Our results revealed that nucleotide variations at the -1082 site affected serum lipoprotein and lipid concentrations. Specifically, HDL and TG levels were higher and lower, respectively, in individuals with the -1082 GA genotype than in those carrying the AA genotype, implying a relationship between IL-10 and concentrations of these factors. Besides, age and sex had no significant effect on HDL or TG level. Turner et al. (1997) reported that the G allele of SNP -1082, which lies in the *IL-10* promoter, is highly correlated with elevated IL-10 secretion.

Although our results suggest that the -592 polymorphism has no significant effect on lipid and lipoprotein levels, the TG, TC, LDL, and VLDL serum concentrations of -592 A allele carriers were higher than those of individuals harboring the C allele. Previous studies have also suggested that IL-10 may be involved in lipid metabolism. For instance, Mizia-Stec et al. (1999) reported that IL-10 concentration is significantly increased in patients with hypercholesterolemia ($P < 0.05$). Moreover, in a study of the effect of IFN-γ and IL-10 on blood lipids and glucose, Ma et al. (2004) found that IL-10 level was positively related to LDL concentration in patients with coronary diseases ($r = 6.56$, $P = 0.03$). These results reveal a relationship between IL-10 and lipid levels.

In conclusion, the *IL-10* -1082 polymorphism demonstrates a close relationship with the serum levels of certain lipoproteins and lipids, and inflammatory cytokine gene sequence variations may constitute the genetic basis of abnormal lipid metabolism and related diseases. The effects of interactions between inflammatory cytokines, cytokine polymorphisms, and lipid metabolism disorder merit further investigation.

Conflicts of interest

The authors declare no conflict of interest.

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