Association between gene polymorphism of CD14-159 (C/T) and allergic asthma

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Abstract. The objective of this study was to evaluate the possible association between the CD14-159 polymorphism and adult asthma in the Chinese population. A total of 188 asthmatic patients and 60 healthy adults were enrolled in the present study, and the CD14-159 polymorphism was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The results showed that the frequencies of CC, CT and TT genotypes were 12.2, 47.9, and 39.9%, respectively, in the asthma group, and 8.3, 50.0, and 41.7%, respectively, in healthy adults, with no statistical difference between the two groups (P>0.05). The frequencies of C and T allele were 36.2 and 63.8%, respectively, in the asthma group, 33.3 and 66.7%, respectively, in the healthy group, and no statistical difference was observed in the allele frequencies between the two groups (P>0.05). Our data suggest that the CD14-159 polymorphism is not associated with adult asthma in Chinese population.

Introduction

CD14 is expressed either on the surface of monocytes, macrophages and neutrophils, or may present in a soluble form in serum (1). Binding of CD14 to lipopolysaccharide (LPS) promotes the secretion of IL-12, which is an essential signal for differentiation and maturation of naïve T cells in Th1 cells (2).

Therefore, changes in the expression of CD14 affect the ratio of Th1 to Th2, thereby reducing IgE production (3,4). A functional single nucleotide polymorphism (C-159T) has been found in the promoter region of the CD14 gene, and this polymorphism is associated with the changes of CD14 and IgE levels in different ethnic groups (5-7). CD14 expression is up-regulated in asthma patients following stimulation with allergens and inhalation of LPS (3-7). In the present study, we characterized the genotypes of the CD14-159T gene polymorphism in adult asthma patients using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis in an attempt to determine the possible association of the CD14-159 polymorphism with asthma or asthma severity.

Patients and methods

Patients. A total of 188 asthma patients diagnosed in the Central Hospital of Lishui City, China, between October 2008 and October 2010, were assigned to the asthma group: 100 males and 88 females participated, aged 18-76 years, with an average age of 45.3 years. Diagnosis of asthma was established according to the criteria in 'Practical internal medicine' by Haozhu Chen (8). According to the grading standards for asthma, we identified 24 cases with intermittent asthma (grade I), 76 cases with mild persistent asthma (grade II), 68 cases with moderate persistent asthma (grade III), and 20 cases with severe persistent asthma (grade IV). The control group included 60 healthy adults aged 18-67 years with an average age of 43.1 years. These controls, including 36 males and 24 females, were randomly selected healthy volunteers without any personal or family history of atopy. They had no respiratory tract infections in the last month prior to enrolment, and had received no steroids or antihistamines in the last six months. Signed consent was obtained from all subjects. This study was approved by the Ethics Committee of the Medical Department of Lishui College, China.

Reagents. Reagents for PCR-RFLP, including TaqDNA polymerase, dNTP and DNA molecular weight markers, were purchased from Takara (Dalian, China). *Ava*II restriction enzyme was obtained from Promega (Madison, WI, USA).

Genomic DNA extraction. A total of 3 ml of peripheral venous blood was collected from asthma patients. Following anticoagulation with heparin, leucocytes were isolated and whole-genome DNA was extracted using a QiaAmp DNA blood mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Isolated DNA samples were then stored at -20˚C. Genomic DNA extraction from the control group followed the same procedures.

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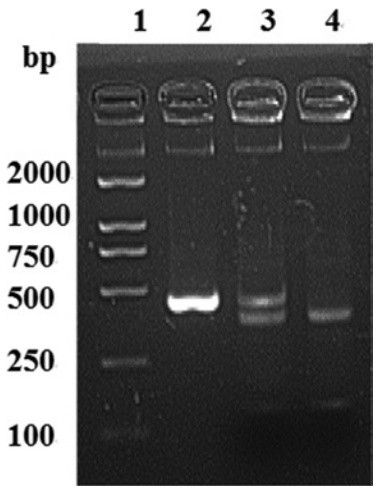

Figure 1. Agarose gel electrophoresis analysis of CD14 genotypes cleaved by *Ava*II enzyme. Note: Lane 1, DL2000 marker; Lane 2, wild-type genotype (C/C); Lane 3, heterozygous mutant genotype (C/T); Lane 4, homozygous mutant genotype (T/T).

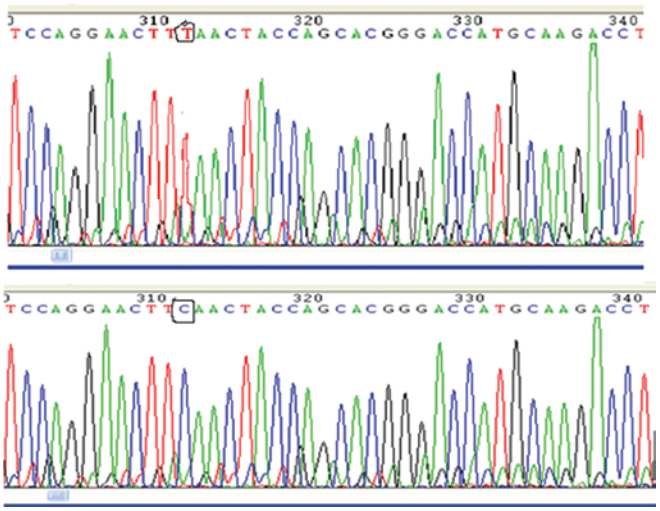

Figure 2. Gene sequencing results for CTTCAA (wild-type CC genotype) and CTTTAA (mutant TT genotype).

Table I. CD14 C-159T genotype and allele frequencies in adult asthma patients.

Group	Subgroup (Grade)	n	Genotype frequency (%)[a]			Allele frequency (%)[b]	
			CC	CT	TT	C	T
Asthma group	I	24	3 (12.5)	11 (45.8)	10 (41.7)	35.4	64.6
	II	76	10 (13.2)	37 (48.7)	28 (38.2)	37.5	62.5
	III	68	8 (11.8)	32 (47.1)	28 (41.2)	35.3	64.7
	IV	20	2 (10.0)	10 (50.0)	8 (40.0)	35.0	65.0
Sub-total		188	23 (12.2)	90 (47.9)	75 (39.9)	36.2	63.8
Control group		60	5 (8.3)	30 (50.0)	25 (41.7)	33.3	66.7
Total		248	28 (11.3)	120 (48.4)	100 (40.3)	35.5	64.5

Group, [a]χ^2= 0.691 (P=0.708) and [b]χ^2= 0.320 (P=0.572); subgroup, [a]χ^2= 1.014 (P=0.998) and [b]χ^2= 0.519 (P=0.972).

PCR amplification. The primers, previously described (4) and synthesized by Takara (China), used were: forward 5'-GTGCCAACAGATGAGGTTCAC-3' and reverse 5'-GCC TCTGACAGTTTTATGTAATC-3'. The PCR reaction was performed in a total volume of 50 µl containing 5 µl of 10X buffer (15 mM $MgCl_2$), 4 µl of dNTP (200 µmnol/l), 1 µl of each primer (25 pmol/µl), 0.5 µl of TaqDNA polymerase (5 U/µl), 4 µl of DNA template, and sufficiently distilled water to a final volume of 50 µl. PCR amplification was performed at an initial denaturation step at 94˚C for 5 min, followed by 35 cycles consisting of 94˚C for 30 sec, 64˚C for 30 sec and 72˚C for 1 min, and a final extension step at 72˚C for 10 min. After amplification, PCR products were separated by 2% agarose gel containing 0.5 mg/ml ethidium bromide (EB) electrophoresis.

PCR-RFLP. A total of 10 µl of each PCR amplification product was digested by *Ava*II in 10 x restriction buffer with $MgCl_2$, bovine serum albumin (1µg/µl) (Promega, Madison, WI, USA) and 1 unit *Ava*II (Promega) and sterile water to a final volume of 20 µl. Digestion was carried out in a water bath at 37˚C for 3 h. After restriction digestion, restricted products were subject to 2% agarose gel (containing EB) electrophoresis. Subsequently, genotypes were analyzed in a gel image analysis system. Bands with appropriate sizes showing different genotypes were as follows: CC: (497 bp), CT (497, 353, and 144 bp), TT (353 and 144 bp).

Gene sequencing. Each randomly selected sample of PCR products identified to harbor the CC or TT genotype by RFLP analysis was sent to Shanghai Sangon Biological Engineering Technology and Services Co., Ltd for purification and sequencing.

Statistical analysis. Genotype and allele frequencies in the asthma and control groups were calculated using the gene counting method. After being tested using the Hardy-Weinberg equilibrium, the data were analyzed by the statistical software

SPSS version 12.0. Genotype and allele frequencies in the two groups were tested using the χ^2 test.

Results

PCR products and results of AvaII digestion. The PCR product was 497 bp in length (Fig. 1). When a wild-type cytosine nucleotide was present at position 159 in the CD14 gene, *Ava*II was not able to cleave this gene and therefore generated a fragment of 497 bp alone (CC genotype). In contrast, when the position 159 contained a mutant thymine nucleotide, *Ava*II cleaved the CD14 gene into two fragments with 353 and 144 bp, respectively (TT genotype). Similarly, the heterozygous genotype (CT genotype) generated three fragments of 497, 353 and 144 bp, respectively (Fig. 1).

Hardy-Weinberg genetic equilibrium test. The CD14 genotypes in the asthma and control groups were subject to the Hardy-Weinberg genetic equilibrium test. The genotypes of the two groups were in Hardy-Weinberg genetic equilibrium, suggesting a good genetic representation of the source population (P>0.05).

Comparison of CD14-159 genotype and allele frequencies between the asthma and control groups. The frequencies of the three genotypes CC, CT and TT were 12.2, 47.9 and 39.9%, respectively, in the asthma group and 8.3, 50.0 and 41.7%, respectively, in the control group. No statistical difference of genotype distribution between the two groups was observed (P>0.5). The C and T allele frequencies were 36.2 and 63.8%, respectively, in the asthma group and 33.3 and 66.7%, respectively, in the control group. No significant difference was found between the two groups (P>0.5, Table I).

Gene sequencing. Following gene sequencing, the obtained DNA sequences were compared with the human CD14-159 gene sequences retrieved from GenBank database. Comparison results were confirmed by gene sequencing as shown in Fig. 2.

Discussion

CD14 gene is mapped to 5q31, a region shown to be linked to Th2 cell functions and responsible for the increase of serum IgE levels in different populations (8). Various studies have addressed the polymorphisms of the CD14 gene, and the C-159T gene polymorphism localized in the promoter region of the CD14 gene is most closely related to allergic diseases (10,11). The *In vitro* transient transfection of monocytes with the CD14 gene demonstrates that the C-159T gene polymorphism increases CD14 transcription by lowering the affinity of CD14 regulatory regions to Sp3, a factor known to inhibit the activity in most promoters (12). Additionally, the C-159T TT genotype is also associated with the elevation of sCD14 in circulation (10,11). A low level of sCD14 means a low number of positive skin tests and a reduced level of serum IgE, which is linked to non-allergic asthma and food allergy in different ethnic groups (5,10,11). Conversely, the CC genotype increases serum IgE levels and the number of positive skin tests (5,10,11). Therefore, the C allele is associated with allergy.

The C-159T gene polymorphism in the allergic mechanisms may be age-related (13). At present, the correlation between an increased expression of CD14 and the C-159T gene polymorphism has only been found in the serum of children, but not in that of adults (13). Furthermore, the CC genotype is only associated with early allergic reactions and early airway hyperresponsiveness in individuals aged 8-25 years. Therefore, allergic reactions to the -159C genotype were also found to be age-specific (13).

Studies have also shown that the associations between the C-159T gene polymorphism and allergy are not always consistent (14-16). For example, the gene polymorphism in the CD14 gene was associated with elevated levels of soluble CD14, but not with IgE or allergic diseases, including bronchial asthma (14-16). Notably, a high degree of linkage disequilibrium in the CD14 promoter region has offered an explanation for the association of CD14, or other genes on chromosome 5q, including the C-159T polymorphism with asthma (17). Presumably, the over-expression of the CD14-159T allele expressed in Spanish and Portuguese individuals with near relations may be due to the presence of linkage disequilibrium of the CD14-159T allele in the mutant chromosome 5q (18). However, whether or not an association between the C-159T gene polymorphism and allergy in different ethnic populations exists, may depend on environmental factors (5,10,11).

Allergic asthma is an IgE-mediated type I allergic disease characterized by pulmonary eosinophil accumulation, excessive mucus secretion and airway hyperresponsiveness. No study has as yet reported the association of the C-159T gene polymorphism with allergic asthma. The present study found that the CD14-159 gene polymorphism was not associated with asthmatic adults and asthma severity. Although the CD14C-159T gene polymorphism may regulate airway remodeling in asthma, it is likely that separate genes on chromosome 5q may regulate total serum IgE levels and bronchial airway responsiveness.

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