The Association with the –159C/T Polymorphism in the Promoter Region of the CD14 Gene and Juvenile Idiopathic Arthritis in a Chinese Han Population

HUA SONG ZENG, XIANG YUAN CHEN, and XIAO PING LUO

ABSTRACT. ***Objective.*** Juvenile idiopathic arthritis (JIA) is generally considered to be caused by interaction of genetic and environmental factors. We investigated the association of a C-to-T transition in the promoter region of the CD14 gene on chromosome 5q31.1 and JIA in a Chinese Han population.

Methods. One hundred sixty-three children with JIA and 281 healthy children (age- and sex-matched to JIA group) were studied. Polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) was used for analysis of the genotypes. (Trial registration number ChiCTR-CCC-00000312.)

Results. CD14 promoter-159 genotype frequencies of CC, CT, and TT were 11.48%, 49.18%, and 39.34%, respectively, in the systemic onset JIA group; 21.62%, 43.24%, and 35.14%, in the polyarticular JIA group; 16.67%, 50%, and 33.33%, in the oligoarticular JIA group; 6.9%, 75.86%, and 17.24%, in the group with other types of JIA; and 37.01%, 46.98%, and 16.01%, in the control group. Genotype frequency and allele frequency distribution were in accord with Hardy-Weinberg equilibrium. There were statistically significant differences in frequencies of genotype and allele in CD14 C-159T polymorphism between JIA group and control group (genotype: chi-squared = 33.168, $p < 0.05$, CT vs CC, OR 2.946, 95% CI 1.739-4.990; TT vs CC, OR 5.426, 95% CI 2.977-9.891. Allele: chi-squared = 33.168, $p < 0.05$, T vs C, OR 2.251, 95% CI 1.704-2.973). The T allele frequencies of boys and girls were significantly higher than those in the control group ($p < 0.001$ of both).

Conclusion. CD14 gene promoter C-159T polymorphism is significantly correlated with JIA in the Chinese Han population. The T allele of the C-159T polymorphism of CD14 gene may be a genetic risk factor for JIA. (J Rheumatol First Release July 15 2009; doi:10.3899/jrheum.081093)

Key Indexing Terms:
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Juvenile idiopathic arthritis (JIA) is a clinically heterogeneous disease with unknown etiology. Certain genetic and environmental factors are believed to predispose the host to the development of JIA. Linkage studies and association studies have been carried out to delineate the factors involved in various rheumatic diseases. There is much evidence that patients with JIA have an altered cytokine profile, a possible cause for pathogenic chronic inflammation. A number of cytokine candidate gene associations to JIA have been found. These include an association with interleukin 1 (IL-1) gene family polymorphisms, IL-6 gene polymorphisms, IL-10 gene polymorphisms, tumor necrosis factor- (TNF-α), macrophage migration inhibitory factor gene polymorphism, and CC chemokine receptor 5[1-7].

CD14 is a 55-kDa glycosyl phosphatidylinositol–anchored glycoprotein expressed on the surface of monocytes, macrophages, and polymorphonuclear leukocytes[8]. CD14 has been reported to bind to the lipopolysaccharide, leading to nuclear factor-κB activation and cytokine expression mediated by the TLR4/MD2 complex[9]. Soluble CD14 (sCD14) is produced by enzymatically cleaved membrane CD14 (mCD14), mediated mainly by phospholipase C, and via secretion of CD14[10]. sCD14 is considered to be an acute-phase protein, and its concentration in serum has been found to increase in several clinical pathologies, such as rheumatoid arthritis (RA)[11], systemic lupus erythemato-

From the Pediatric Department, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan; and the Department of Allergy, Immunology and Rheumatology, Guangzhou Children's Hospital, Guangzhou, China.

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H.S. Zeng, MD, PhD, Pediatric Department, Tongji Hospital (currently Department of Allergy, Immunology, and Rheumatology, Guangzhou Children's Hospital); X.Y. Chen, MD, Department of Allergy, Immunology and Rheumatology, Guangzhou Children's Hospital; X.P. Luo, MD, PhD, Professor, Pediatric Department, Tongji Hospital.

Address correspondence to Prof. X.P. Luo, No. 318 Renminzhong Road, Guangzhou Children's Hospital, Guangzhou City, Guangdong Province, China, 510120. E-mail: xpluo@tjh.edu.cn

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sus[12], brucellosis[13], and tuberculosis[14]. A common single-nucleotide polymorphism (SNP) is found at position –159 in the CD14 promoter, where a C→T change occurs. Some studies have shown an association between the CD14-159TT genotype and chronic periodontitis[15], myocardial infarction[16], Crohn disease[17], brucellosis[18], and pulmonary tuberculosis[19]. Other studies show no association between CD14-159 and RA[20,21]. In this study, we used polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) to investigate whether this SNP has any association with cases of JIA in a Chinese Han population.

MATERIALS AND METHODS

Patients. We included 163 unrelated patients, 106 boys and 57 girls, including 61 with systemic onset JIA, 63.93% (39) boys and 36.07% (21) girls; 37 with polyarticular JIA, 67.57% (25) boys and 32.43% (12) girls; 36 with oligoarticular JIA, 55.56% (20) boys and 44.44% (16) girls; and 29 with other types of JIA, 75.86% (22) boys and 24.14% (7) girls. The mean age of the patients with JIA was 7.25 ± 3.46 years [± standard deviation (SD)]. Patients were diagnosed according to the revised International League of Associations for Rheumatology (ILAR) classification criteria for JIA[22]. A group of 281 randomly selected, unrelated, healthy subjects was compared with the JIA group as controls (157 boys, 124 girls aged 4.62 ± 0.91 years). Written informed consent was obtained from all patients and parents from whom data were collected. The trial registration number was ChiCTR-CCC-00000312.

DNA analysis. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Genotyping of the CD14/–159 polymorphism was performed according to the protocol described by Kedda, *et al*[23]. A PCR fragment of about 500 base pairs of the CD14 gene promoter was amplified with the forward primer: 5'-GTG CCA ACA GAT GAG GTT CAC-3' and the reverse primer: P2: 5'-GCC TCT GAC AGT TTA TGT AAT C-3'. PCR was performed with 250 ng DNA in 25 ml reactions containing GoTap Green Master Mix 2x (Promega, Madison, WI, USA), 12.5 ml, 15 pmol of each primer. The PCR conditions were denaturation at 94˚C for 5 min, then 30 cycles for denaturation at 94˚C for 30 s, annealing at 57˚C for 30 s, and extension at 72˚C for 30 s, followed by final extension at 72˚C for 5 min. The PCR products was then digested by AvaII (New England BioLabs, Beverly, MA, USA) for 16 h at 37˚C, including 8 μl template in a total volume of 20 ml containing 0.5 ml AvaII and 2 ml B4-buffer. The product was applied to a 1.5% agarose gel. DNA was visualized using a single-intensity transilluminator (300 nm) and photographed with a Gel-Doc system (Bio-Rad Laboratories, Hercules, CA, USA). AvaII digests the PCR product only when the T allele is present. The uncut product is 497 bp, whereas the digested products are 144 and 353 bp. The results of this RFLP assay were confirmed by direct sequencing of the –159 promoter region of the CD14 gene in 10% of patients and controls.

Hardy-Weinberg equilibrium (HWE). HWE analysis was performed for the research subjects by comparing the detected distribution of allele frequencies with the theoretical distribution estimated on the basis of the SNP allelic frequencies. $p > 0.05$ (chi-squared statistics) was considered to indicate equilibrium.

Statistical analysis. All data were analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The comparisons of genotype and allele frequencies were made using chi-squared test. A p value < 0.05 was considered significant. The odd ratios (OR) with 95% confidence interval (CI) were used to estimate the risk of an association between the outcome and each variable.

RESULTS

The observed CD14-159 restriction fragment assay is shown in Figure 1. The CC homozygote has only 1 band, CT heterozygote has 3 bands, and TT homozygote has 2 bands. No deviation from HWE was detected in patients with JIA or healthy controls (Table 1); this indicates that the groups are already in equilibrium and can be used to do further genetic research.

CD14 frequencies of genotype and allele. The results from the assessment of CD14-159 genotype frequencies for the subjects in both groups are reported in Table 2. The distribution of the different genotypes within the test group was different from that in the controls. The homozygous TT genotype was detected in 33.13% (54 of 163) in the JIA group and in 16.01% (45 of 281) in the control group. The OR of TT genotype to CC genotype was 5.426 (95% CI 2.977–9.891). The homozygous CC genotype was detected similarly between the JIA subjects and control groups. Similarly, the distribution of genotypes within the fraction of patients with JIA as a whole or subset, respectively, differed significantly from the controls.

The T allele frequency in the JIA group was 59.51%. The corresponding figure for the control group was 39.5%. This difference was statistically significant ($p < 0.05$) between the JIA group and controls. The OR of T genotype carriage to C genotype carriage was 2.251 (95% CI 1.704–2.973) among subjects with JIA. This indicated that T allele predisposed to JIA compared with the controls.

Sex differences in genotype and allele frequencies between the patients with JIA and controls are shown in Table 3. Significant differences existed between them in both boys and girls.

DISCUSSION

JIA is one of the most common systemic and chronic autoimmune diseases in childhood, with an incidence of

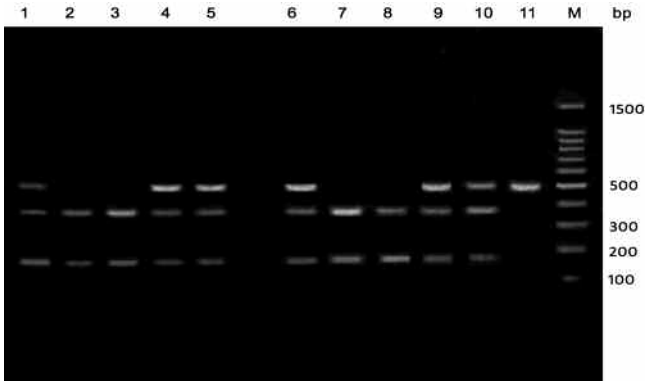

Figure 1. CD14-159 restriction fragment assay. Electrophoresis gel showing size markers on lane M. One band is visible for homozygotes C (lane 11); there are 2 bands for homozygotes T (lanes 2, 3, 7, and 8); and 3 bands for CT heterozygote (lanes 1, 4, 5, 6, 9, and 10).

Table 1. Hardy-Weinberg equilibrium test.

Factor	JIA Patients CC	JIA Patients CT	JIA Patients TT	Controls CC	Controls CT	Controls TT
Observed frequency (O)	23	86	54	104	132	45
Expected frequency (E)	26.724	78.552	57.724	55.3707	121.2507	66.3786
$(O - E)^2/E$	0.519	0.706	0.24	0.0128	0.0395	0.0305
Sum chi-square		1.465			0.0828	
		$p > 0.05$			$p > 0.05$	

JIA: juvenile idiopathic arthritis.

Table 2. CD14-159C/T dimorphisms in JIA patients and controls. Data are n (%).

		Control	JIA	sJIA	oJIA	pJIA	Other*
Genotype frequencies	CC	104 (37.01)	23 (14.11)	7 (11.48)	6 (16.67)	8 (21.62)	2 (6.90)
	CT	132 (46.98)	86 (52.76)	30 (49.18)	18 (50)	16 (43.24)	22 (75.86)
	TT	45 (16.01)	54 (33.13)	24 (39.34)	12 (33.33)	13 (35.14)	5 (17.24)
Allele frequencies	C	340 (60.50)	132 (40.49)	44 (36.07)	30 (41.67)	32 (43.24)	26 (44.83)
	T	222 (39.50)	194 (59.51)	78 (63.93)	42 (58.33)	42 (56.76)	32 (55.17)
OR† (95% CI)			2.25 (1.74–2.97)	2.72 (1.81–4.08)	2.14 (1.30–3.53)	2.01 (1.23–3.28)	1.89 (1.09–3.28)

* Psoriatic JIA, enthesitis-related JIA, and undefined JIA. † OR of allele T vs C of JIA group and control group. sJIA: systemic JIA; oJIA: oligoarticular JIA; pJIA: polyarticular JIA.

Table 3. Sex comparison in patients and controls, n (%).

		Control		JIA		p	
		Male (157)	Female (124)	Male (106)	Female (57)	Male	Female
Genotype frequencies	CC	63 (40.13)	41 (33.06)	14 (13.21)	9 (15.79)		
	CT	74 (47.13)	58 (46.78)	59 (55.66)	27 (47.37)	< 0.001*	0.0035**
	TT	20 (12.74)	25 (20.16)	33 (31.13)	21 (36.84)		
Allele frequencies	C	200 (63.69)	140 (56.45)	87 (41.04)	45 (39.47)		
	T	114 (36.31)	108 (43.55)	125 (58.96)	69 (60.53)	< 0.001†	0.0027††

* Genetic type TT vs CC in male JIA group compared with control. ** Genetic type TT vs CC in female JIA group compared with control. † Allele T vs C in male JIA group compared with control. †† Allele T vs C in female JIA group compared with control.

10–20 per 100,000 children[24]. It arises before 16 years of age and is accompanied by arthritis lasting more than 6 weeks. JIA is classified into 7 subclasses including systemic JIA and the articular type, which also includes oligoarticlar and polyarticular JIA. We assessed the frequency of the –159C/T polymorphism in the CD14 gene in JIA. Although this polymorphism has been previously assessed in RA, this is the first case-control study in JIA. As the CD14 molecule seems to be involved in several inflammatory disorders, CD14 represents a candidate gene for JIA. In our study, CD14-159 polymorphisms may play a role in the pathogenesis of at least distinct JIA subgroups, particularly in systemic JIA. Our data suggest that the CD14-159T allele seems to be associated with JIA since it was found more frequently in these patients compared to healthy controls.

CD14 gene is located on chromosome 5q31.1. A C-to-T exchange at position –159 in the promotor region of the CD14 gene (5q31) leads to higher sCD14 levels, and carriers of the TT genotype showed an increased CD14 expression. Higher CD14 levels are associated with increased levels of cytokines, such as TNF-α, IL-1, IL-6, and other proinflammatory cytokines[25], the latter triggering and maintaining inflammatory processes. Some studies show that patients with JIA have a higher level of these cytokines expressed in the blood and synovial fluid in the joints[26,27].

According to our data, the CD14/C-159T polymorphism is associated with a predisposition to JIA. However, these results are preliminary and will need confirmation by further observation, since the total number of patients in several JIA subgroups was small.

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