

The C–159T polymorphism in the *CD14* promoter is associated with serum total IgE concentration in atopic Chinese children

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Activation of macrophages through CD14 by microbes is crucial in inducing immunity by type 1 T helper cells. A C-to-T polymorphism at position –159 of *CD14* was associated with serum total IgE level in Caucasians but not in Japanese subjects. The objective of this study is to determine whether this polymorphic marker is associated with atopy and asthma phenotypes in Chinese children. Restriction fragment length polymorphism was used to characterize CD14/–159 genotypes. Microparticle immunoassay was used to measure serum total IgE level; fluorescent enzyme immunoassay was performed to measure serum concentrations of specific IgE to aeroallergens; and enzyme-linked immunosorbent assay was used to measure serum levels of soluble CD14 (sCD14). Lung function in asthmatics was assessed by spirometry. Two hundred and fifty-eight patients and 92 control children were recruited. Their mean serum total IgE concentrations were 331 and 74 kIU/l, respectively ($p < 0.0001$). Atopy, defined as the presence of at least one allergen-specific IgE in serum, was found in 220 (85%) patients and in 41 (45%) controls ($p < 0.0001$). Serum sCD14 levels were significantly associated with CD14/–159 genotypes ($p = 0.004$). Atopic subjects with CC genotype in CD14/–159 had the highest serum total IgE levels compared with CT and TT genotypes, with the respective mean values being 661, 427 and 380 kIU/l ($p = 0.015$). Similarly, a higher proportion of subjects with CC genotype had increased serum total IgE concentration ($p = 0.039$). This polymorphic marker was not associated with asthma or aeroallergen sensitization in our cohort. Our results suggest that the C–159T of *CD14* was associated with serum total IgE concentration in atopic Chinese children.

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Asthma is one of the most common chronic illnesses in childhood. The Phase II survey of the International Study of Asthma and Allergies in Childhood (ISAAC) found that 7.7% of 3110 10-year-old Hong Kong children suffered from asthma (1). Atopy, being a major component of asthma, is characterized by elevated serum IgE concentration (2). These conditions are caused by a complex interaction of genetic and environmental factors. Asthma and atopy were linked to

markers on chromosomes 5q31–33 in Caucasians (3, 4), Japanese (5) and Singaporean Chinese (6). This region contains a cluster of type 2 T lymphocyte (Th2)-related cytokine genes, including interleukin -4 (IL-4) and IL-13, which play important roles in the regulation of IgE synthesis (7, 8). Our research group has recently shown that R130Q in the coding region of *IL-13* located in this region was associated with serum total and allergen-specific IgE levels in Chinese children (9).

CD14, the pattern recognition receptor for lipopolysaccharide, is critically involved in innate immune responses (10). The gene encoding CD14 is located on chromosome 5q31.1 (11), and thus is one of the positional candidate genes for atopy. Baldini et al. (12) first described a C-to-T single nucleotide polymorphism at position -159 in the promoter of *CD14*. They found that children with TT genotype in the CD14/-159 had significantly higher soluble CD14 (sCD14) and lower total IgE concentrations in serum when compared with the CT and CC genotypes (12). In adult Dutch asthmatics, CD14/-159 was also linked to higher serum total IgE level and more self-reported allergic symptoms. Nonetheless, this polymorphism was not associated with asthma phenotypes (13). Gao et al. found that CD14/-159 was associated with serum total IgE levels in a sample of 300 British subjects (14). This polymorphic marker was found to be associated with skin test positivity to aeroallergens in a genome-wide screen of a founder population (15). The aim of this study was to investigate the relationship between CD14/-159 polymorphism and serum sCD14, total and allergen-specific IgE concentrations as well as asthma phenotypes in Chinese children.

Materials and methods

Study population

This study recruited unrelated children with asthma, diagnosed according to the American Thoracic Society (ATS) guideline (16), from the pediatric outpatient clinics of a university teaching hospital in Hong Kong. Both parents of our subjects were ethnic Chinese. Age- and sex-matched control subjects were selected among children attending the hospital for minor complaints, who did not have allergic or immunological disease, and did not require any drug treatment at the time of this study. All subjects were free from any self-reported symptom of infection for 4 weeks before study. Subjects or their parents gave written consent, and the Clinical Research Ethics Committee of our university approved this study.

Serum concentrations of total and allergen-specific IgE

Serum total IgE concentration was measured by microparticle immunoassay (IMx analyzer, Abbott Laboratories, Abbott Park, IL, USA) and specific IgE antibodies to crude *Dermatophagoides pteronyssinus*, cat, dog, mixed-cockroach (American and German) and mixed-mold

allergens were measured by fluorescent enzyme immunoassay (AutoCAP system, Pharmacia Diagnostics AB, Uppsala, Sweden). Results on serum total IgE levels were compared with the age-specific reference values in our laboratory. The grading of specific IgE into radioallergosorbent test (RAST) classes 1–6 was made according to manufacturer's instructions, with specific IgE concentration ≥ 0.35 kIU/l being positive. Serum total IgE levels were analysed as a quantitative trait following logarithmic transformation ($\text{IgE}_{\log}$). Children were defined as atopic if they were positive with at least one type of allergen-specific IgE antibody (9).

Serum sCD14 concentration

Serum sCD14 concentration was measured by enzyme-linked immunosorbent assay (ELISA) (IBL GmbH, Hamburg, Germany). Samples were diluted 100-fold prior to the measurement. The sensitivity of this kit for sCD14 is 0.24 ng/ml, and the intra- and interassay coefficients of variation were 5.5% and 7.6%, respectively.

Spirometry

Patients aged 5 years and above underwent spirometric assessment (COMPACT II, Vitalograph, Buckingham, England) to measure their forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) according to ATS standards (17). The measured FEV_1 and FVC results were then compared with local age- and sex-matched predicted values (18).

PCR assay for CD14/-159 polymorphism

Genomic DNA was extracted from peripheral blood using High Pure Viral Nucleic Acid Kit (Boehringer Mannheim GmbH, Mannheim, Germany). CD14/-159 genotypes were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The promoter region of *CD14* was amplified using the primers: 5'-GCC TCT GAC AGT TTA TGT AAT C-3' and 5'-GTG CCA ACA GAT GAG GTT CAC-3' according to an already published method (13). Briefly, PCR reactions were carried out in a total volume of 25 μl containing 60 ng of DNA, 10 mM Tris-HCl buffer (pH 8.3), 1.5 mM MgCl_2 , 200 μM of each deoxynucleotide triphosphate, 50 ng of each primer and 0.5 units of Taq DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany). Samples were denatured at 96°C for 30 s followed by 35 cycles of 94°C for 30 s, 58°C

hybridization temperature for 45 s and 72°C for 45 s, and a final extension for 7 min at 72°C. 10 µl of the 497-bp PCR fragment generated was then digested by adding 5 units of *Ava*II (New England Biolabs, Hitchin, Herts, UK) and incubated overnight at 37°C. This enzyme digested the PCR product into 144- and 353-bp fragments when the T allele was present. The digested products were visualized on 4% (w/v) agarose gel.

Eight DNA samples of known CD14/-159 genotypes were included in every PCR reaction, with restriction enzyme digestion as positive control together with 88 samples in 96-well plates. Finally, the results of this RFLP assay were confirmed by direct sequencing of the CD14/-159 polymorphism using BigDye Terminator Cycle sequencing kits with an ABI-310 autosequencer (Applied Biosystems, Foster City, CA, USA) in 30 randomly selected samples.

Statistical analysis

The primary outcome measure of this study is IgE_{log} whereas secondary outcomes include other atopy and asthma phenotypes. Caucasian children with CD14/-159 CC and TT genotypes were found to have a difference of 0.3 in their mean IgE_{log} (12). Assuming that the standard deviation in IgE_{log} in local Chinese children is 0.65 (9), the present study needs to recruit 75 subjects in each of these two groups in order to detect the above difference in mean IgE_{log} as calculated by unpaired *t*-test with 80% power and 5% alpha error. The clinical characteristics, serum sCD14 and total IgE level and results of allergen sensitization between different groups of subjects were compared using the Student *t*-test or Mann-Whitney *U*-test for numerical data and χ^2 statistics or Fisher's Exact test for categorical variables. The association between serum sCD14 and total IgE levels and spirometric variables was analysed using Spearman's correlation coefficients. Standard analysis of variance (ANOVA) for quantitative traits or Kruskal-Wallis test for nonparametric variables was used to compare serum concentrations of sCD14 and total and allergen-specific IgE as well as spirometric variables among subjects with CC, CT and TT genotypes in CD14/-159. All comparisons were made two-sided. *p*-Values of less than 0.05 were considered significant.

Results

Clinical and laboratory characteristics

Two hundred and fifty-eight asthmatic children and 92 controls were enrolled. The mean (SD)

ages at evaluation of patients and control children were 9.8 (4.2) and 10.3 (4.7) years, respectively (*p* = 0.385), and 64% of patients and 55% of controls were males (*p* = 0.169). Spirometric assessment was performed on 214 patients, with their median (interquartile range, IQR) FEV₁ and FVC results being 96 (83–109)% and 114 (101–132)% of predicted, respectively. Only 19% of our asthmatics had FEV₁ ≤ 80%. Table 1 describes results on the measurement of serum total and allergen-specific IgE levels. Two hundred and twenty (85%) patients and 41 (45%) controls in this study were atopic (odds ratio 7.20 and 95%CI 4.07–12.78, *p* < 0.0001). Sensitization to *D. pteronyssinus*, cat and mixed molds were significant risk factors for asthma diagnosis.

CD14 C-159T and serum sCD14 concentration

The median (IQR) serum sCD14 concentrations in our asthmatics and controls were 5491 (4836–6068) ng/ml and 5480 (4866–5997) ng/ml, respectively (*p* = 0.771). Figure 1 illustrates the distribution of sCD14 with different CD14 C-159T genotypes. Subjects homozygous for the T allele had the highest serum sCD14 levels (median: 5269 ng/ml for C/C, 5441 ng/ml for C/T and 5774 ng/ml for T/T; *p* = 0.004). No correlation was found between serum sCD14 concentrations and serum total IgE levels and FEV₁ and age of our patients (results not shown).

CD14/-159 polymorphism as predictors of atopy and asthma

The frequencies for C and T alleles of CD14/-159 in our controls were 0.44 (81/184) and 0.56 (103/184), respectively. The distributions of genotypes and alleles for this polymorphism were in

Table 1. Serum total IgE concentrations and pattern of allergen sensitization

Characteristics	Asthmatics	Controls
Serum total IgE concentration*	331 ± 4†	74 ± 6
Number of subjects with increased serum total IgE level (%)	196 (76)†	38 (41)
Number of subjects with positive allergen-specific IgE in serum**		
<i>Dermatophagoides pteronyssinus</i> (%)	216 (84)†	39 (42)
Cockroaches (%)	67 (26)	15 (16)
Cat (%)	37 (14)§	2 (2)
Mixed molds (%)	20 (8)‡	1 (1)
Dog (%)	6 (2)	0

*Expressed as means ± standard deviations; †*p* < 0.0001; ‡*p* < 0.05; §*p* < 0.005 between two groups, by Student *t*-test or Fisher Exact test. **Odds ratios (95%CI) for asthma diagnosis were 6.99 (3.98–12.30), for *Dermatophagoides pteronyssinus*, 7.53 (1.72–46.19), for cat and 7.65 (1.07–155.19), for mixed molds.

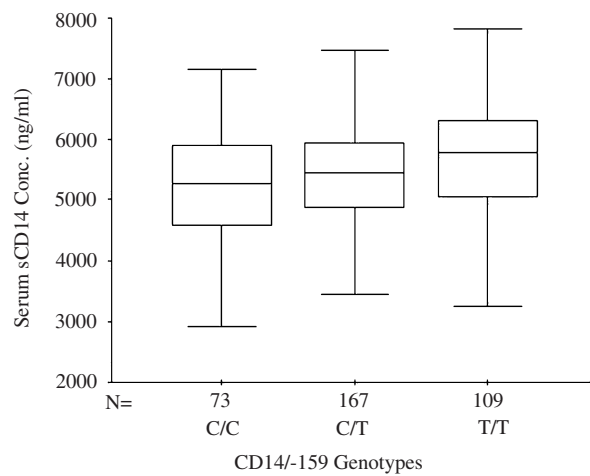


Fig. 1. Serum levels of sCD14 by CD14/–159 genotypes. Box plots illustrate median values and interquartile ranges ($p = 0.004$ by Kruskal–Wallis test). Serum sample was not available from one subject for this measurement.

Table 2. Association between CD14/–159 genotypes and serum total IgE concentration

Subjects	n	Serum IgE _{log} for Different CD14/–159 Genotypes*			p-values†
		CC	CT	TT	
All subjects	350	2.44 ± 0.81	2.32 ± 0.75	2.33 ± 0.70	0.525
Asthmatics	258	2.54 ± 0.81	2.49 ± 0.64	2.55 ± 0.56	0.793
Atopic asthmatics	220	2.83 ± 0.47	2.67 ± 0.47	2.65 ± 0.47	0.084
Controls	92	2.12 ± 0.75	1.87 ± 0.84	1.73 ± 0.73	0.266
RAST-positive subjects	261	2.82 ± 0.46	2.63 ± 0.49	2.58 ± 0.52	0.015
RAST-negative subjects	89	1.34 ± 0.57	1.49 ± 0.70	1.48 ± 0.57	0.671

*Results expressed as means ± standard deviations for different CD14/–159 genotypes; †Compared using ANOVA. RAST: radioallergosorbent test.

Hardy–Weinberg equilibrium for patients and controls. Table 2 described the association between IgE_{log} and CD14/–159 genotypes. This genetic marker was not associated with IgE_{log} in either the asthmatics or the controls. Nonetheless, among the subgroup of atopic subjects (i.e. more than one allergen-specific IgE in serum), those with CC genotype had the highest IgE_{log} as compared with subjects with CT and TT genotypes ($p = 0.015$). A higher proportion of atopic subjects with CC genotype in CD14/–159 also had elevated serum total IgE levels as compared with those with CT or TT (CC: 94%, CT: 81%, TT: 79%; $p = 0.039$). This relationship was not observed in RAST-negative subjects. On the other hand, the ages of our subjects did not exert any effect on the association between IgE_{log} and CD14/–159. There was also no significant association between CD14/–159 polymorphism

Table 3. Association between CD14/–159 genotypes and asthma and atopy-related phenotypes

Phenotypes	n	CD14/–159 genotypes			p-values*
		CC (%)	CT (%)	TT (%)	
All physician-diagnosed asthma	258	55 (21)	123 (48)	80 (31)	0.938
with control children	92	18 (20)	45 (49)	29 (32)	
Atopic subjects	261	54 (21)	123 (47)	84 (32)	0.766
with nonatopic subjects	89	19 (21)	45 (51)	25 (28)	
Atopic asthma	220	46 (21)	103 (47)	71 (32)	0.572
with nonatopic asthma	38	9 (24)	20 (53)	9 (24)	
with control children	92	18 (20)	45 (49)	29 (32)	
Sensitization to aeroallergens					0.941
<i>Dermatophagoides pteronyssinus</i> -IgE present	255	54 (21)	121 (47)	80 (31)	
<i>Dermatophagoides pteronyssinus</i> -IgE absent	95	19 (20)	47 (49)	29 (31)	0.614
Cockroaches-IgE present	82	20 (24)	39 (48)	23 (28)	
Cockroaches-IgE absent	268	53 (20)	129 (48)	86 (32)	0.247
Cat-IgE present	39	11 (28)	14 (36)	14 (36)	
Cat-IgE absent	311	62 (20)	154 (50)	95 (31)	0.668
Molds-IgE present	21	6 (29)	9 (43)	6 (29)	
Molds-IgE absent	329	67 (20)	159 (48)	103 (31)	0.301
Dog-IgE present	6	2 (33)	1 (17)	3 (50)	
Dog-IgE absent	344	71 (21)	167 (49)	106 (31)	

*Analysed by Pearson χ^2 test.

and the diagnosis of asthma or atopy-related phenotypes (Table 3) and spirometric indicators of asthma severity (results not shown).

Discussion

The *CD14* gene is located on chromosome 5q31.1 (11), and polymorphisms in this gene were linked to asthma and atopy in several populations (12–15). Among these genetic markers, the C-to-T substitution at position –159 of the *CD14* promoter was first reported to be associated with serum sCD14 and total IgE level (12). In accordance with the literature, we have also found serum sCD14 levels to be the highest in subjects homozygous for the T allele. Skin-test positive Caucasian children with CC genotype had higher serum total IgE levels and larger mean number of positive skin tests to aeroallergens (12). CD14/–159 was also linked to higher serum total IgE level and more self-reported allergic symptoms in Caucasian adult asthmatics (13). Among the skin test positive individuals in this study, those with CC genotype had a larger number of skin prick tests being positive compared with CT and TT genotypes. Nonetheless, the authors could not find any association between CD14/–159 and various asthma phenotypes. When serum allergen-specific IgE was used to characterize the atopic status of our subjects, we found similarly that C–159T of *CD14* was

associated with serum total IgE concentration in RAST-positive Chinese children. On the other hand, Gao et al. reported *CD14* -159C to be associated with increased serum total IgE levels only in British subjects with negative RAST (14). To complicate this issue, the T allele instead of the C allele in *CD14*/-159 was associated with atopy, as indicated by positive skin prick tests in the genome screen of Hutterites (15). Some investigators could not find any association between C-159T and atopy. Sengler et al. could not find any association between C-159T of *CD14* and asthma, atopic dermatitis or asthma-related traits such as BHR and serum total IgE levels in 872 children from the German Multi-center Allergy Study cohort (19). Studies in Japanese and Africans also could not confirm previous findings between *CD14*/-159 polymorphism and serum total IgE level or soluble *CD14* concentration (14,20).

It is important to note that the present study and some earlier reports have found an association between serum IgE levels and the C allele of *CD14*/-159 (12, 13), whereas others detected this association with -159T (15). Besides, two of the above positive studies for *CD14*/-159 (12, 13), as well as the present one, found this marker to be selectively associated with serum IgE levels in skin test or RAST-positive subjects, whereas one study reported this association in RAST-negative Caucasian subjects (14). These apparently inconsistent results may be explained by the presence of a gene-environment interaction in which *CD14*/-159 acts as a basic modifier of atopy in individuals with different degrees of environmental endotoxin (ET) exposure (14). Gereda et al. showed a direct relation between endotoxin (ET) exposure and the proportion of Th cells producing INF- γ , suggesting a pattern of Th1-skewed cytokine production that drives our immune system away from atopy development (21). The effects of *CD14*/-159 genotypes on serum total IgE level might be more evident in allergen-sensitized subjects with presumably low exposure to the protective factor of environmental ET during early childhood. Unfortunately, none of the above genetic association studies of *CD14* measured environmental ET exposure in their subjects. The small numbers of subjects included in the above case-control studies may indeed make sampling variation another plausible explanation for the above inconsistencies. Besides, the immunomodulatory effects of *CD14* C-159T polymorphism on serum total IgE level in children may be different from those in adults. The present study has recruited a pediatric population whereas previous studies variably used

adults and children. This developmental difference in the effects of *CD14*/-159 on atopy and asthma possibly explain some of the inconsistencies. Future prospective studies on the effects of C-159T in *CD14* on asthma and atopy should focus on a larger sample of children and measure the environmental ET exposure to elucidate more clearly the interaction between *CD14* genetics and environmental factors.

The above studies found significant interethnic differences in allele frequencies of *CD14*/-159, with the frequency of the T allele being 35% in Afro-Caribbeans, 49% to 54% in Caucasians and 60% in Japanese (12-14). The figure for Chinese children was similar to those of the latter two groups, with -159T being found in 56% of our controls. On the contrary, the allele frequencies of *IL-13* R130Q in Chinese children were different from the respective figures in Japanese or Caucasian populations (9, 22, 23). Because of this variation in gene frequencies between different ethnic groups, it is crucial to repeat genetic association studies in each group rather than to generalize these findings when we try to establish the relationship between a polymorphic marker and atopy or serum IgE levels.

Following the proposal of the hygiene hypothesis in 1989 (24), a number of population studies in developed countries reported a negative association between 'bronchitis' in early childhood and the later development of asthma and atopy (25-27). The ISAAC survey also found early day care attendance and a larger number of siblings in the families of Chinese children to be protective against the development of asthma (unpublished data). A greater exposure to infectious agents might be the 'protective' factor. An inverse relationship was recently observed between farm animal contact during early life and the risk of having allergic sensitization and asthma in children (28). A higher exposure to ET in the homes of farmers' children might protect them against atopy and asthma (21, 29). As *CD14*/-159 is intimately involved in the regulation of *CD14* production (12, 30), this marker probably modifies serum total IgE level and allergen sensitization through interacting with ET which is ubiquitous in our environment. Further studies should try to delineate the effects of environmental factors on the association between childhood atopy and C-159T of *CD14*.

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