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ORIGINAL ARTICLE

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Association of CD14 promoter polymorphisms and soluble CD14 levels in mite allergen sensitization of children in Taiwan

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Abstract CD14 is responsible for environmental lipopolysaccharide recognition and is a positional candidate gene for allergy. We hypothesized that genetic polymorphisms in the promoter region of the CD14 gene may be associated with *Dermatophagoides pteronysinnus* (Der p) allergen sensitization in children. Three single nucleotide polymorphisms (SNPs) of the CD14 promoter region, C(−159)T, A(−1,145)G, and G(−1,359)T were genotyped, and analyzed in 240 randomized case–control school-age children in Taiwan. Serum concentrations of IgE and soluble CD14 (sCD14) were also assayed. We found a significant inverse correlation of sCD14 and total serum IgE levels in our study population. Moreover, sCD14 binds Der p allergen in vitro in a dose-dependent manner. The distribution of three SNPs genotypes was similar in asthmatic children and the control group. However, there was a significant difference in the distribution of genotype CD14 G(−1,359)T, but not C(−159)T, between mite-sensitive and non-sensitive children. Haplotype analysis showed strong linkage disequilibrium among these three SNPs in the CD14 promoter region. Carriers of the CD14−159C/−1,145A/−1,359T haplotype had the highest IgE and lowest sCD14 levels as compared to other haplotypes. Our results support the hypothesis that CD14 gene variants may play an important role in influencing allergen sensitization of children in Taiwan.

Keywords Asthma · Allergic disease · CD14 gene polymorphism

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Introduction

Allergic sensitization is defined by production of IgE against environmental antigens such as house dust mites, grass pollen, and animal proteins, and can lead to diseases that include asthma, rhinitis, and atopic dermatitis (Kay 2001). The prevalence of allergic diseases has increased significantly over the last few decades (Woolock and Peat 1997). Drastic environmental modifications and/or western-style living conditions have been targeted as the main culprits responsible for this rise. However, the variability in IgE-mediated allergic disease as well as the development of asthma in individuals depends on both environmental and genetic factors. Innate immunity genes, which operate at the interface between the immune system and pathogens such as mite allergens, are believed to play a critical role in the development of allergic asthma (Vercelli 2004). The gene encoding CD14, which is responsible for recognition of bacterial lipopolysaccharides (LPS) in innate immunity, is one of many genes that appear to contribute to the expression of the allergic phenotype, as it is localized on chromosome 5q31.1, a region linked to both asthma and total serum IgE concentration (Marsh et al. 1994; Meyers et al. 1994). The hygiene hypothesis suggests that early exposure to bacterial products, such as LPS,

may modulate IgE regulation, possibly by activating innate immune pathways that promote Th1 differentiation and/or suppress Th2-dependent IgE response and allergy development (Strachan 1989; Moffatt and Cookson 1999).

Although CD14 exists as a single-copy gene, its protein is found in two distinct forms: a 55-kDa membrane molecule (mCD14) expressed primarily on the surface of monocytes/macrophages, dendritic cells and neutrophils (Haziot et al. 1993), and a soluble form (sCD14) in serum (Durieux et al. 1994). Regulation of CD14 gene expression appears to be important in many diseases, and elevated serum levels of sCD14 have been found in several conditions, including atopic dermatitis (Wuthrich and Joller-Jemelka 1992). A genetic variant [CD14 C(−159)T] in the promoter region of the CD14 gene has been found to be associated with higher levels of sCD14 expression (Baldini et al. 1999), and inversely associated with levels of total serum IgE (Baldini et al. 1999; Gao et al. 1999; Koppelman et al. 2001). However, this association between total serum IgE levels and the CD14/−159 genotype reached statistical significance only among non-Hispanic white children (Baldini et al. 1999). This result seems to be consistent with reports from the Collaborative Study of the Genetics of Asthma, which indicated that linkage between markers in chromosome 5q and asthma was present among non-Hispanic whites but not Hispanic subjects (Ober et al. 1998). Similar studies were applied to many racial/ethnic groups, e.g., an association between IgE level and CD14/−159 polymorphism was shown in Czech (Buckova et al. 2003), Dutch (Koppelman et al. 2001), and Hong Kong Chinese (Leung et al. 2003) subjects, but not in German (Sengler et al. 2003) or Polish (Lis et al. 2001) subjects. Not surprisingly, these inconsistent data suggest that the CD14/−159 polymorphism may only be one of many mechanisms involve in IgE regulation. Recently, five single nucleotide polymorphisms (SNPs) (at positions −1,619, −1,359, −1,145, −809, and −159) were characterized in the promoter of the gene encoding CD14 (Vercelli et al. 2001a). Vercelli et al. (2001a) showed that carriers of the −1,359T/−1,145A/−159C haplotype had the highest levels of IgE, and the lowest levels of sCD14 and, conversely, carriers of the −1,359G/−1,145G/−159T haplotype had the highest levels of sCD14 and the lowest IgE values.

Previously, we found that CD14 gene promoter polymorphism was associated with IgE levels in Taiwanese asthmatic children (Wang et al. 2005a). In this study, we hypothesized that CD14 may recognize aeroallergens, such as dust mites, and influence postnatal switching of the T helper cell response. Moreover, genetic polymorphisms in the promoter region of CD14 gene may determine the degree or susceptibility of allergen sensitization and association with the development of asthma. In this case–control study, primary school children in Taiwan, a highly homogenous population, were recruited to investigate the association of the three polymorphisms in the CD14 gene promoter region (at positions −159, −1,145, and −1,359) with the phenotypes of atopic status and asthma disease.

Materials and methods

Study populations

Our study population consisted of 301 primary school children randomly selected from an allergy and asthma questionnaire survey conducted in the ten primary schools in Tainan city, Taiwan, and divided into groups of study subjects as allergic asthmatics, non-allergic asthmatics, and controls. All participants or their guardians, after being well informed of the study protocol, signed consent forms and answered a modified "International Study of Asthma and Allergies in Childhood"—Chinese version (ISAAC-C) questionnaire, as well as additional questions pertinent to the diagnosis and assessment of asthma (Chen et al. 2005). The Ethical and Clinical Trial Committee of National Cheng-Kung University Hospital approved the study protocol. The definition of asthma included: (1) a history of two or more episodes of wheezing in the last 6 months, during or without concurrent respiratory infection; (2) chronic cough for more than 1 month and diagnosed by a physician regarding the presence of wheezing episode(s); and (3) confirmation of a positive response of an increase of 15% in forced expiratory volume in 1 s (FEV_1) using a bronchodilator test. Age-matched controls did not have any of the above conditions in their previous physical check-up and past history. Other evaluations included skin-prick tests for responsiveness to six common aeroallergens, a differential blood count (including total eosinophil count), and measures of total serum IgE, as well as IgE specific to house dust and mixed pollens using the Unicap system (Pharmacia Diagnostics, Uppsala, Sweden). A positive skin test was defined as the presence of ≥1 reaction with a weal diameter ≥5 mm. Total serum IgE was measured by solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics).

Sample preparation and genotyping at the CD14 promoter region by PCR-RFLP

Genomic DNA was extracted from peripheral blood leukocytes using a Blood and Tissue Genomic DNA extraction Miniprep system following the manufacturer's instructions (Viogene, Sunnyvale, CA). The isolated genomic DNA was quality checked by agarose gel electrophoresis analysis, quantified spectrophotometrically, and stored at −80°C. Genotyping was performed by polymerase chain reaction-fragment length polymorphism (PCR-RFLP) as follows. All PCRs were carried out in a volume of 25 μl containing 100 ng genomic DNA, 5 pmol of each primer, 2.5 μM deoxynucleotide triphosphates, and 2 U *Taq* polymerase

(Protech, Taipei, Taiwan). Cycling conditions were 94°C for 10 min, 35 cycles of 94°C for 1 min, 65°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 10 min. The polymorphism C(−159)T was typed using a sense (5′-ATCATCCTTTTCCCACACC-3′) and reverse (5′-AACTCTTCGGCTGCCTCT-3′) primer pair. Following PCR, the product was digested with 2 U *Hae*III (New England Biolabs, Frankfurt am Main, Germany) for 4 h and the fragments resolved on a 2% agarose gel. The PCR product was 295 bp in length. Digestion of the PCR product yielded bands of 295 bp in TT homozygotes, 140 and 155 bp in CC homozygotes, and all three bands in heterozygotes. The polymorphism A(−1,145)G was typed using a sense (5′-CTCAGGAATCTGAGGCAAGA-3′) and reverse (5′-AGTACAATCTCTGTGCCCTA-3′) primer pair. Following PCR, the product was digested with 1 U *Hpy*CH4 V (New England Biolabs) for 4 h and the fragments resolved on a 2% agarose gel. The PCR product was 371 bp in length. Digestion of the PCR product yielded bands of 300 bp in GG homozygotes, 230 bp in AA homozygotes, and two bands in heterozygotes. The polymorphism G(−1,359)T was typed using the same primer pair as for A(−1,145)G. After PCR, the product was digested with 2 U *Fok*I (New England Biolabs) for 4 h and the fragments resolved on a 2% agarose gel. The PCR product was 371 bp in length. Digestion of the PCR product yielded bands of 178 and 146 bp in GG homozygotes, 146, 112, and 66 bp in TT homozygotes, and all bands in heterozygotes. The genotyped results were further confirmed by auto-sequencing (Fig. 1).

Serum soluble CD14 concentration

Serum levels of soluble CD14 (sCD14) were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit supplied by R&D systems (Human sCD14 EIA kit, R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. The detection limit of this assay is ≤ 125 pg/ml.

Binding of sCD14 to *Dermatophagoides pteronyssinus* and LPS

Lyophilized house dust mite (*Dermatophagoides pteronyssinus*, Der p) was purchased from Allergon (Engelholm, Sweden). The crude mite preparation was prepared as previously described (Wang et al. 2001). In brief, after dialysis with deionized water, the mite extract was lyophilized and dissolved in pyrogen-free isotonic saline (YF Chemical, Taipei, Taiwan) and filtered through a 0.2 μm-filter (Microgen, Laguna Hills, CA) before use. The Der p extract was treated with polymyxin B to remove endotoxin contamination. To determine the direct interaction between Der p extract and LPS (Lipopolysaccharides, Sigma-Aldrich, St. Louis, MO) to CD14, a sandwich enzyme immunoassay was used. Der p and LPS are pre-coated onto a 96-well ELISA plate overnight. After blocking with 5% skim milk, various concentrations of recombinant CD14 are added to the wells. Later, a monoclonal antibody specific for CD14 is added and incubated for 2 h, followed by addition of enzyme-linked anti-mouse IgG. Binding was evaluated by measuring the color developed under 450 nm wavelength light using the substrate and substrate solution.

Statistical analysis

The association between serum sCD14 and asthma phenotype or mite-sensitization, and CD14 genotype

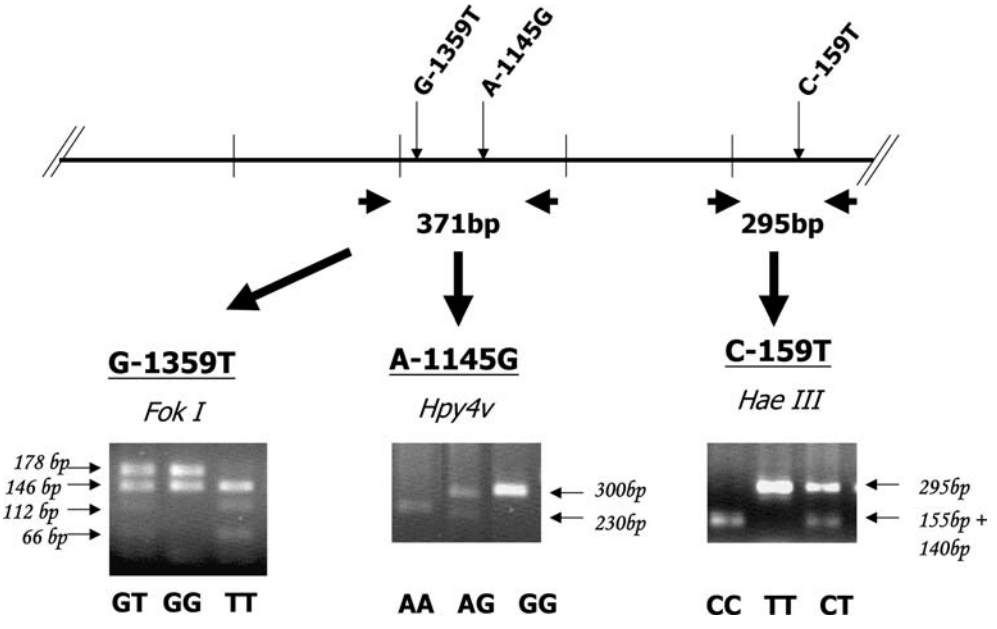

Fig. 1 Representative results of polymerase chain reaction-fragment length polymorphism (PCR-RFLP) analysis and genotyping for C(−159)T, A(−1,145)G, and G(−1,359)T single nucleotide polymorphisms (SNPs) in the promoter region of the CD14 gene. For details of procedures, see Materials and methods

was analyzed using Spearman's correlation coefficients. Standard analysis of variance (ANOVA) for quantitative trials or Kruskal–Wallis test for non-parametric variables was used to compare serum concentrations of sCD14 and asthma phenotype or mite sensitization, as well as the sCD14 variable among CD14 genotypes of three different SNP sites. The CD14 alleles and genotype frequencies were determined, and comparisons of the frequencies were performed by the χ^2-test using 2×2 or 2×3 contingency tables, respectively. Allelic odds ratio (OR) and confidence intervals (CI) were computed. *P*-values less than 0.05 were considered to be significant. The software used for the analyses was SPSS 10.0 for Windows. In addition, the extent of linkage disequilibrium between SNP and haplotype frequencies was calculated using Pypop win32.20.5 and Popgene ver1.31.

Results

Demographics of study population

The demographic characteristics of the study population are shown in Table 1. In total, 301 school children were enrolled, comprising 72 allergic asthmatics, 58 non-allergic asthmatics, 75 non-asthma with allergic symptoms, and 96 age-matched controls. There were no differences in the mean age or sex ratio among these groups. Spirometric assessment was performed on the total study population, revealing the lower predicted percentage (%) of FEV_1 and the ratio of FEV_1/FVC in asthmatic children as compared to values obtained with non-asthmatics groups (88.5 ± 10.2 vs 105.7 ± 11.2%, and 91.2 ± 12.3 vs 110.6 ± 11.2%, $P < 0.01$, respectively). Results of skin prick tests of Der p as well as measurements of total serum IgE and allergen-specific IgE levels are given in Table 1. A total of 97.3% of allergic asthmatics and 46.6% of allergic non-asthmatics showed positive skin tests to Der p, as confirmed by in vitro allergen-specific tests (UniCAP, Pharmacia Diagnostics). Moreover, more than one-half of allergic asthmatics ($n = 46$) and one-third ($n = 27$) of allergic non-asthmatics were sensitive to more than one allergen. Sensitization to Der p, cockroach, and mixed molds were significant risk factors for allergic diseases (OR 5.20 and 95% CI 3.04–9.82, $P < 0.001$). Five patients with non-allergic asthma have minor reactivity (0.35 kU) to Der p in UniCap tests but have negative skin tests to Der p extracts, while in control group, seven children had positive skin tests but without allergen-specific IgE antibodies to Der p.

Soluble CD14 and IgE

Total serum IgE levels exhibited large differences among the groups studied, with higher IgE concentrations in allergic asthma and allergic non-asthma subjects as compared to non-allergic asthma and controls (Table 1). On the contrary, the level of sCD14 was significantly lower in allergic asthmatics (2,136.6 ± 128.1 ng/ml) as compared to non-allergic asthmatics (2,985.1 ± 193.4 ng/ml) ($P < 0.01$), and in allergic non-asthmatics (1,936.9 ± 121.8 ng/ml) as compared to control groups (2753.4 ± 135.0 ng/ml) ($P < 0.0001$) (Fig. 2a). After stratification of our study subjects, we also found that there was no difference of sCD14 level between asthma and non-asthma children (2,461.6 ± 115.5 and 2,355.7 ± 98.4 ng/ml, respectively; $P = 0.13$), while sCD14 level was significantly lower in Der p-sensitive (2,037.6 ± 88.5 ng/ml) as compared to Der p non-sensitive children (2,840.3 ± 111.3 ng/ml) ($P < 0.0001$) (Fig. 2b). Figure 3 shows the inverse correlation of sCD14 and log-transformed serum IgE levels ($r = 0.0189$, $P < 0.0001$).

Genotype frequency of the CD14 promoter region

Using restriction enzyme-based RFLP genotyping, we simultaneously determined the frequencies of a C to T transition at nucleotide position −159 C(−159)T, an A to G at position−1,145 A(−1,145)G, and a G to T at position−1,359 G(−1,359)T from the transcription start site in the promoter region of the CD14 gene in each of the study subjects (Fig. 1). The allele frequencies for each SNP genotype in our study population are in agreement with the predicted Hardy–Weinberg equilibrium when calculated using the DE Finetti program (http://ihg.gsf.de/cgi-bin/hw/hwal.pl). We found

Table 1 Baseline characteristics of the study population. *FEV_1* Forced expiratory volume in 1 s, *Der p Dermatophagoides pteronyssinus*

Characteristic	Allergic asthmatics ($n = 72$)	Non-allergic asthmatics ($n = 58$)	Allergic non-asthma ($n = 75$)	Controls ($n = 96$)
Age (years)	8.5 ± 1.2	9.8 ± 1.3	8.7 ± 1.1	7.9 ± 1.6
Male (%)	43 (59.7)	30 (51.7)	42 (56.0)	51 (53.1)
Predicted FEV_1 (%)	88.2 ± 12.2	91.3 ± 11.7	103.0 ± 10.5	114.3 ± 13.2
FEV_1/FVC (%)[a]	85.3 ± 9.8	92.7 ± 7.6	108.7 ± 8.7	112.8 ± 9.8
Asthma (doctor's diagnosis, %)	100	100	0	0
Skin test positive for Der p extracts	68 (94.4%)	0	35 (61.3%)	7 (7.3%)
Serum total IgE (IU)	1,005.5 ± 635.5	271.4 ± 86.9	407.3 ± 86.9	43.7 ± 24.3
sCD14 (ng/ml)	2,136.6 ± 975.5	2,985.1 ± 1140.2	1,936.6 ± 910.2	2,753.4 ± 1045.9

[a]Results shown as mean ± SD or *n* (%)

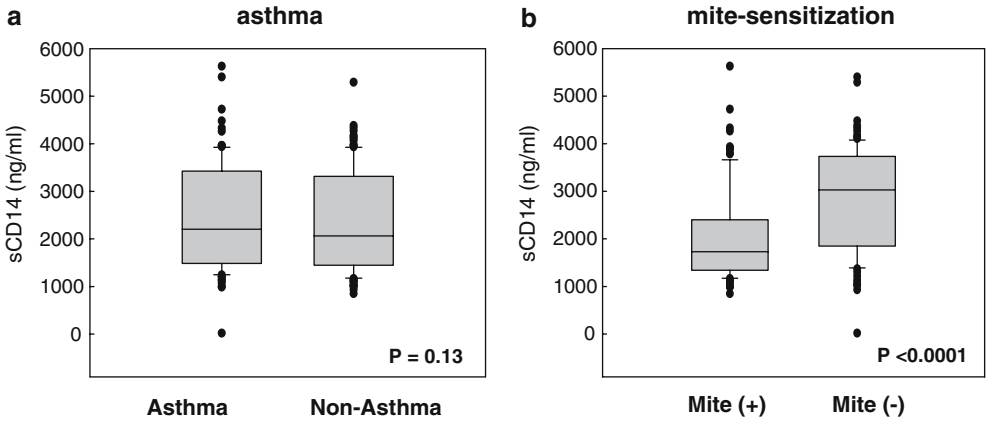

Fig. 2 Box plots of serum levels of soluble CD14 (sCD14; ng/ml) in asthmatics vs controls (**a**), and children with mite sensitivity vs controls (**b**), respectively. Box plots illustrate median values and interquartile distances

that the genotype distributions and allele frequencies for these genotypes of the CD14 promoter region were not significantly different among the four groups of our study population, or between asthmatic and non-asthmatic children (Table 2). When the study population was divided according to sensitivity to dust mite (Der p), there was a significant difference of genotype distributions at G(−1,359)T genotypes between Der p-sensitive and non-sensitive children. The homozygote GG genotype (67.5 vs 45.8%, OR = 2.455, 95% CI 1.453–4.146, $P < 0.001$) and G alleles at CD14−1,359 (OR = 1.62, 95% CI = 1.062–2.483, $P < 0.05$) was predominant in mite-sensitive as well as non-sensitive children (Table 3). In this analysis, the genotype results of non-sensitization controls were not in Hardy–Weinberg equilibrium, which may be due to random events in our sample.

The association of CD14 promoter region genotypes with serum sCD14 and IgE levels

Figure 4a shows the association between each SNP genotype in the promoter region of CD14 and serum levels of sCD14. There were no significant differences in serum sCD14 concentrations between the SNP genotypes of C(−159)T or A(−1,145)G. A trend for lowered sCD14 levels in carriers of CD14/−1,359 TT homozygotes was noted as compared to GT and GG genotypes of CD14/G(−1,359)T, but this did not reach statistical significance in our study population. Geometric mean values of serum IgE levels were also tested for associations with SNP genotypes of the CD14 promoter region (Fig. 4b). As with serum sCD14 levels, no differences in serum IgE levels were detected in C(−159)T or A(−1,145)G genotypes. With the G(−1,345)T genotype, there was a significant increase in total serum IgE levels in carriers of TT genotype (517.0 ± 122.2 IU/ml) compared to the GT (260.2 ± 37.7 IU/ml) and GG (410.8 ± 56.6 IU/ml) genotypes ($P < 0.05$, ANOVA).

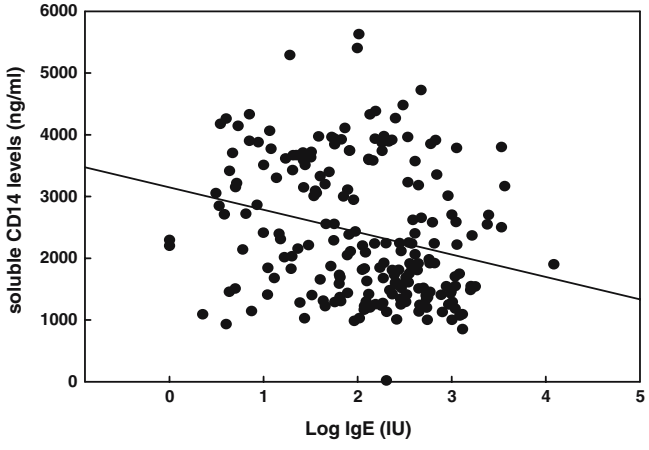

Fig. 3 Linear correlation plotted with serum levels of sCD14 (ng/ml) against log values of total serum IgE (IU) in all study populations

Linkage disequilibrium and haplotype analysis of SNP genotypes of CD14 promoter regions

The three SNPs in the CD14 promoter region are located within a fragment 1.5 kb in length. To determine the linkage disequilibrium (LD) between these SNPs, the PopGene 1.32 program and Pypop win32-0.52 (http://allele5.biol.berkeley.edu/pypop) were used to calculate LD between the two loci. The D' values between C(−159)T and A(−1,145)G, A(−1,145)G and G(−1,359)T, and C(−159)T and G(−1,359)T were 0.93, 0.72, and 0.65, respectively, with all P-values below 0.05. As expected, there was strong LD among these three SNP genotypes: 70 individuals carried the most common homozygote haplotype of CD14−159T/−1,145G/−1,359G, whereas 5 subjects were CD14−159C/−1,145A/−1,359G, and 5 were CD14−159C/−1,145A/−1,359T. Table 4 shows that there were significant differences in total serum IgE levels ($P < 0.01$), but not sCD14 levels, among subjects homozygous for three major CD14 haplotypes. We also found that carriers of the −159C/−1,145A/−1,359T haplotype had the highest IgE and lowest sCD14 levels.

When the study population was stratified into asthma and non-asthma groups, there was no significant differ-

Table 2 Case–control analysis of asthmatic vs non-asthmatic controls. *OR* Odds ratio, *CI* confidence interval, *SNP* single nucleotide polymorphism

SNP	Number of samples		*P*-value	OR	95% CI
	Asthma	Controls			
CD14/−159					
CC	17	24			
CT	56	55	0.446		
TT	47	41			
C	90	103	0.226	0.8	0.554–1.150
T	150	137			
CD14/−1,145					
AA	5	9			
AG	60	63	0.429		
GG	55	48			
A	70	81	0.280	0.81	0.549–1.189
G	170	159			
CD14/−1,359					
GG	70	66			
GT	43	50	0.481		
TT	7	4			
G	183	182	0.915	1.02	0.376–1.556
T	57	58			

ence in haplotype frequencies of CD14/−159/−1,145/−1,359 genotypes between these two groups (data not shown). On the contrary, the allele frequencies of these haplotypes revealed that carriers of CAG (16 vs 8%) and CGG (10 vs 5%) of CD14/−159/−1,145/−1,359 haplotypes appeared predominantly in mite-sensitive children, while carriers of CAT (22 vs 14%) and TGT (5 vs 2%) haplotypes were more frequently seen in the mite non-sensitive group. These findings are consistent with the result that carriers of the homozygote GG genotype at CD14/−1,359 have a greater chance of becoming mite-sensitive (Table 3).

Table 3 Case–control analysis of subjects with mite sensitization vs non-sensitization controls

SNP	No. of samples		*P*-value	OR	95% CI
	Mite (+)	Controls			
CD14/−159					
CC	22	19			
CT	59	52	0.407		
TT	39	49			
C	103	89	0.192	1.28	0.885–1.839
T	137	151			
CD14/−1,145					
AA	10	4			
AG	56	67	0.150		
GG	54	49			
A	76	75	0.922	1.02	0.694–1.499
G	164	165			
CD14/−1,359					
GG	81	55			
GT	31	62	< 0.0001		
TT	8	3			
G	193	172	0.025	1.62	1.062–2.483
T	47	68			

Binding of sCD14 to Der p

Regarding the biological significance of the association of CD14 SNP genotypes and Der p sensitization in asthmatic children, one possibility is that CD14 may influence the sensitization pathway via direct binding to Der p. To test this hypothesis, the binding of soluble CD14 to dust mite extract was tested in a solid-phase ELISA assay. As shown in Fig. 5, sCD14 was found to bind to immobilized Der p extracts and LPS, but not to bovine serum albumin, in a dose-dependent manner.

Discussion

In this study, we have genotyped three SNPs, C(−159)T, A(−1,145)G, and G(−1,359)T,, which were in strong linkage disequilibrium, in the promoter region of the CD14 gene in a large population of Taiwanese school children. Although the genotype results of the A(−1,145)G and G(−1,359)T in the non-sensitization samples were not in the Hardy–Weinberg equilibrium, which may be due to the bias of random events in our sample population, a statistically significant association between CD14 gene G(−1,359)T polymorphism and Der p-sensitive children, but not with asthmatics, was nevertheless observed. Furthermore, we also found lower levels of sCD14 in CD14/−1,359 TT homozygotes, albeit not reaching statistical significance, as compared to the GT and GG genotypes of CD14/−1,359, while serum IgE levels in CD14/−1,359 TT homozygotes were significantly higher than other CD14/−1,359 genotypes. Although we could not find any association between CD14 promoter polymorphisms at C(−159)T and A(−1,145)G with asthma or sensitivity to Der p, haplotype analysis of these three SNPs in the promoter region of CD14 gene clearly showed strong linkage disequilibrium among these three genotypes. In our study population, carriers of the −159C/−1,145A/−1,359T haplotype had the highest levels of IgE, and the lowest level of sCD14. This finding was in agreement with the report of Vercelli et al. (2001a).

The results of association studies of the CD14 gene with allergic asthma in our populations further emphasize the importance of environmental exposure in the development of allergen sensitization in children. Previously, we have found that D5S2011 (near CD14 genes) differs highly significantly between allergy and non-allergic asthma (Wang et al. 2005b). In this study, we have shown that the increase in soluble CD14 levels is inversely correlated with total serum IgE concentrations in our study population. Previous studies in different populations have reported associations between the CD14/−159C allele and elevated IgE levels (Baldini et al. 1999; Koppelman et al. 2001; Leung et al. 2003).

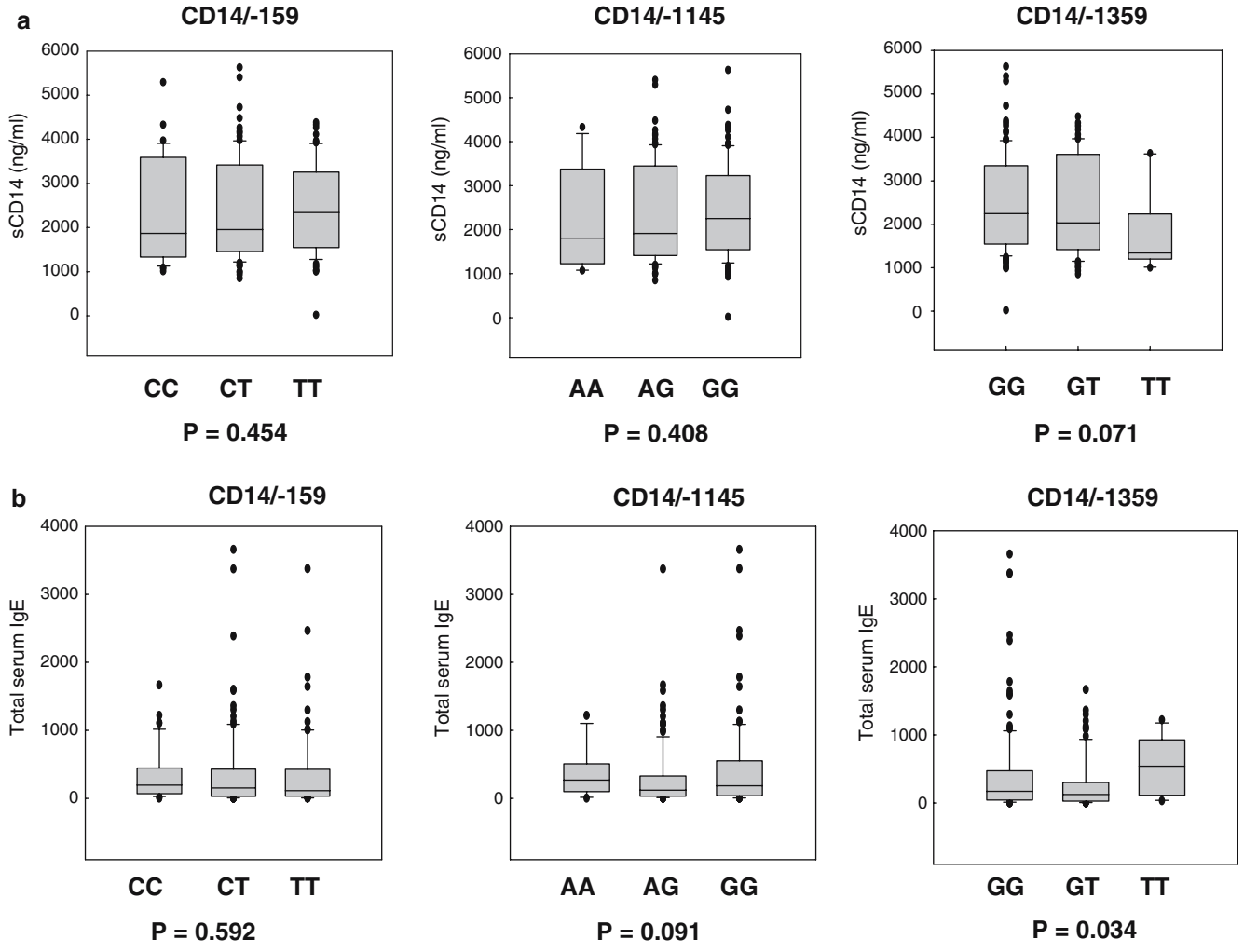

Fig. 4 Box blots of serum values of sCD14 in ng/ml (**a**), and total IgE levels in IU/ml (**b**) associated with three SNPs in the promoter region of the CD14 gene: C(−159)T, A(−1,145)G, and G(−1,359)T. Box plots illustrate median values and interquartile distances

O'Donnell et al. (2004) in Australia investigated the age-related effect of CD14/C(−159)T polymorphism on CD14 secretion and in the development of atopy. Moreover, their findings are in agreement with our results that the effect of CD14 on serum total IgE values is only apparent among subjects sensitized to allergen (any skin test reactivity results). There was no apparent influence on IgE values for children whose skin prick tests were negative, implicating CD14 in the predisposition to allergen sensitization rather than in regulation of IgE production.

However, in Hutterites, a highly inbred population, CD14/−159T was shown to be associated with atopy only when the CD14/−159T allele shared the same haplotype with the D5S642 185 bp allele (Ober et al. 2000). Furthermore, linkage, but not association, between the CD14/−159 polymorphism and atopy has been reported (Walley et al. 2001). These conflicting results suggest that CD14/−159 and the putative susceptibility variant are in the same LD block in some study subjects but not in all populations. In our previous report, we also found that CD14/−159 was associated with IgE levels only when the −159T allele was part of a haplotype containing a specific D5S2011 allele (D5S2011, 143 bp) but not other D5S2011 alleles (Wang et al. 2005a). Another possible explanation for inconsistent results from

Table 4 Serum IgE and sCD14 levels of carriers with three homozygous haplotypes in the promoter region of the CD14 gene

−159	−1,145	−1,359	Number	Total serum IgE	sCD14
CC	AA	GG	15	310.6 ± 64.4	2,708.6 ± 498.3
CC	AA	TT	15	632.4 ± 201.6	1,679.8 ± 474.6
TT	GG	GG	90	536.8 ± 182.6	2,448.8 ± 988.7
P-value for ANOVA test				0.0075	0.1599

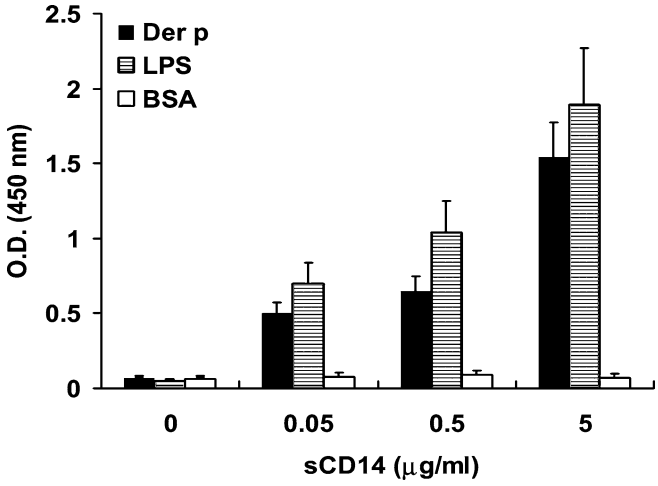

Fig. 5 Binding of sCD14 to Der p allergen extract and lipopolysaccharide (LPS). Serial dilutions of sCD14 were incubated with solid-phase immobilized Der p extract (10 μg/ml), LPS (1 μg/ml) or bovine serum albumin (BSA) as a control. The average and range of three separate experiments are shown

different studies is that association with IgE level should be a specific haplotype (Baldini et al. 2000; Vercelli et al. 2001b) or combination of genotypes (Vercelli et al. 2001a). That is, not just one SNP but a combination of more than one SNP in the CD14 promoter region affects serum IgE concentration. This hypothesis is in agreement with the findings our haplotype analysis suggests, i.e., that the CD14 G(−1,359)T polymorphism was associated with IgE level in Taiwanese school children while the CD14 C(−159)T polymorphism, the SNP of a previous report on non-Hispanic whites, was not associated with IgE level in our study. The CD14 C(−159)T polymorphism was associated with IgE level only as part of a haplotype containing a specific CD14 G(−1,359)T allele.

It is interesting to consider how genetic variation in CD14 genes could influence sensitization of Der p in children. Although the detailed mechanisms of interaction between CD14 and LPS or Der p allergen are not known at present, in this study we have demonstrated that sCD14 was able to bind Der p devoid of any LPS contamination, and this binding was not disrupted by adding LPS to the reaction buffer. At the cellular and molecular levels, the interplay between LPS-dependent signals, CD14 engagement, and IgE regulation appear to be complex. Vercelli et al. (2001b) have shown that addition of LPS to peripheral blood mononuclear cells or B cells with exogenous IL-4 in vitro results in a marked up-regulation of IgE production. While engagement of CD14 on monocytes inhibits synthesis of IgE, under the same conditions, exogenous IL-4 could downregulate expression of CD14 in normal human monocytes (Jabara and Vercelli 1994). Therefore, the interaction between LPS exposure and genetic variants in the LPS response pathway may have different consequences depending on the stage of the disease(s) at which exposure occurs. Whether binding of CD14 and mite allergen, as demonstrated in our study, has any biological relevance in influencing allergic sensitization, or whether genetic factors that modify LPS responsiveness may influence susceptibility to developing allergy and/or asthma are questions that remain to be answered.

In summary, we have shown that there is association of CD14 gene promoter polymorphisms at G−1,359T with high serum IgE—with a trend towards lower sCD14—and with Der p-sensitive children in our population. We also found that sCD14 can bind to Der p in a dose-dependent manner. These results suggest that the CD14 gene plays an important role in the determination of sensitization to environmental allergens as well as in innate immunity against pathogens.

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