ORIGINAL RESEARCH

Racial differences in the association of CD14 polymorphisms with serum total IgE levels and allergen skin test reactivity

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Background: The CD14 C-159T single nucleotide polymorphism (SNP) has been investigated widely as a candidate genetic locus in patients with allergic disease. There are conflicting results for the association of the CD14 C-159T SNP with total serum immunoglobulin E (IgE) levels and atopy. There are limited data regarding the association of the CD14 C-159T SNP in subjects of African ancestry. The aim of the study was to determine whether the C-159T SNP and other CD14 SNPs (C1188G, C1341T) were associated with total serum IgE levels and with allergy skin test results in nonatopic and atopic subjects; as well as in Caucasian and African American subjects.
Methods: A total of 291 participants, 18–40 years old, were screened to determine whether they were atopic and/or asthmatic. Analyses were performed to determine the association between CD14 C-159T, C1188G, or C1341T genotypes with serum IgE levels and with the number of positive skin tests among Caucasian or African American subjects.
Results: We found no significant association of serum total IgE level with CD14 C-159T, C1188G, or C1341T genotypes within nonatopic or atopic subjects. Subjects with CD14-159 T alleles had significantly more positive allergen skin tests than subjects without CD14-159 T alleles ($P = 0.0388$). There was a significant association between the CD14 1188 G allele, but not the CD14 1341 T allele, with the number of positive skin-test results in Caucasians, but not in African Americans.
Conclusion: These results support a possible association between CD14 polymorphisms and atopy. CD14-159 T or CD14 1188 G alleles were associated with atopic disease. For subjects with CD14 1188 G alleles, the association with atopic disease was stronger in Caucasians compared to African Americans.
Keywords: total serum immunoglobulin E, IgE, skin prick test, SPT, CD14-159T, single nucleotide polymorphism, SNP, lipopolysaccharide, LPS, endotoxin

Introduction

Atopic diseases, such as asthma, hay fever, and allergic rhinitis, constitute a global health problem because of their high prevalence.[1–5] The prevalence of atopic diseases in children and adults has increased during the past two decades, especially in urban and industrialized countries,[6] and urban populations of African ancestry have been especially affected by atopic diseases.[7–9] Although drastic environmental changes such as global warming have been associated with an increased incidence of atopy,[10] there are also strong genetic predispositions for the development of allergic disease.[11] Recent studies have emphasized the interplay between environmental factors, such as lipopolysaccharide (LPS) exposure, and genetic differences in the development of allergic diseases.[12–14]

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CD14, a gene located on the cytokine gene cluster on chromosome 5q31.1,[15] is an essential receptor in the innate immune response to LPS[16] and a positional candidate gene for allergic diseases.[17,18] Genetic variations in CD14 may alter the structure of the CD14 receptor and may also alter CD14-LPS interactions. In 1999, Baldini et al[19] identified a single nucleotide polymorphism (SNP) in the 5' flanking region of the CD14 gene at position –159 from the transcription start site. The CD14-159 SNP is common in both Hispanic and non-Hispanic white subjects, with approximately half of all chromosomes carrying the T allele and half the C allele. In the Caucasian subset of a general population sample of 11-year-old children from the US (n = 314), Baldini et al[19] found that among skin-test-positive atopic children, when compared with the pooled group of subjects carrying CC and CT, homozygotes with the TT genotype had significantly higher levels of soluble CD14 (sCD14) than did either CC or CT genotype carriers. In this cohort, TT homozygotes also had significantly lower levels of total serum immunoglobulin E (IgE) plus a lower number of positive skin prick tests.[19]

The CD14 C-159T SNP has been investigated as a candidate genetic locus in other cohorts of subjects with asthma and allergic disease.[19–41] Most reports suggest that CD14-159 T allele homozygotes had higher sCD14 levels than subjects with other genotypes.[28,29,33,42] However, subsequent studies yielded conflicting results for the association of the CD14 C-159T SNP with total serum IgE levels in different populations. Some studies found the T allele was associated with lower IgE levels and/or a reduced risk for atopy,[20–24] while other studies found no association,[25–30] or an increased risk of atopy and higher IgE levels in subjects with the CD14-159 T allele.[38–41]

Importantly, gene-environment interactions play a role in the association between CD14 genotypes and allergies.[24,25,43–47] Epidemiological studies reported that asthma and allergic disorders are less common among children and adults who lived on a farm or in a rural area during early childhood.[48–52] Increased concentrations of house dust endotoxin were found in agricultural environments and urban households with indoor pets. It has been suggested that the time-dependent interactions between genetic determinants and environmental endotoxin exposure play a critical role in the development of IgE-mediated allergic disorders.[53] Evidence indicates that prenatal and/or childhood exposure to high levels of microbial agents such as endotoxin in farm environments decreases the risk of atopic sensitization in children and adults. These associations were more pronounced in individuals who lived on a farm during the first year of life compared with individuals who never lived on a farm.[24,47] In addition, levels of inhaled endotoxin during farming activities arguably surpass those in non-farming or urban living environments.[54]

Recent studies have suggested that the association of CD14 polymorphisms and allergic sensitization were predicted by the level of environmental endotoxin exposure. Braun-Fahrländer et al[55] reported that, among children 6 to 13 years old who were living in rural areas of Germany, Austria, or Switzerland, the risk of allergic sensitization was inversely associated with the concentration of endotoxin in house dust. Martinez[42] further investigated this association in the same subjects in the study, and reported that, at low levels of LPS exposure, CD14-159 CC homozygotes had the highest risk for allergic sensitization, whereas, at high levels of LPS exposure, CD14-159 CC homozygotes were at the lowest risk for sensitization. This variable association with LPS exposure was supported by data from Eder et al,[25] showing that, in children 8–10 years old from rural Austria and Bavaria, the CD14-159 C allele was associated with higher levels of IgE and allergen-specific IgE in children who had regular contact with pets; the T allele was associated with higher IgE in children who had regular contact with farm animals kept in stables where there was a higher level of endotoxin exposure. Simpson et al[54] performed similar studies in children from Manchester, UK, and reported similar results; there was an inverse relation between house dust endotoxin exposure and allergic sensitization that was stronger in CC homozygotes as compared to the other two genotypes (CT or TT). In adult populations, Williams et al[56] found that, among new mothers enrolled in a newborn cohort in the Detroit urban area who had similar endotoxin exposures, subjects homozygous for the CD14-159 T allele were protected from atopy at low levels of endotoxin exposure and were at risk for atopy at high levels of endotoxin exposure. A similar finding was reported among workers in a large mouse repository in the US. In this cohort, the CD14-159 T allele was associated with higher IgE levels among workers with documented allergies to animals.[57] In a genetic linkage study[38] in the Hutterites from an isolated farming community in the American mid-west, the CD14-159 T allele was over-transmitted to subjects with positive skin tests. Because the Hutterites have an agricultural life style, they are likely exposed to high levels of endotoxin and this may be the reason that the CD14-159 T allele confers an increased atopic risk. Most studies of the CD14 C-159T SNP have focused on Caucasian subjects and there has been little study of subjects of African ancestry, despite the high prevalence of atopy and asthma in this population.

In addition to the C-159T SNP, other CD14 polymorphisms have been associated with atopic disease. In 2006, Buckova et al[58] found that the T allele of the +1341 G/T polymorphism was significantly associated with skin test reactivity to mites and molds, and the common −1619A/−1359G/−550C/+1188G/+1341T haplotype was associated with positive skin reactions to mites and molds in Czech patients. Therefore, the aim of this study was to investigate whether the T allele of the −159 CD14 SNP, the G allele of the +1188 CD14 SNP, and/or the T allele of the +1341 CD14 SNP were associated with total serum IgE levels and the number of positive allergen skin tests in a young adult population from central North Carolina that included a large percentage of subjects of African ancestry.

Material and methods
Study population
The study population consisted of 312 subjects from five asthma studies conducted at Duke University Medical Center from 2001 to 2008. One of these five studies was sponsored by the Sandler Program for Asthma Research and was designed to investigate the relationship between inducible nitric oxide synthase (NOS_2) genotypes and FeNO levels.[59] The other four studies were sponsored by the NIEHS and were studies of gene-environment interactions in the development of asthma. All research subjects were recruited from patients of or visitors to the Duke University Health System and from students or employees at four university campuses in central North Carolina. Informed consent was obtained as part of the protocols approved by the Duke University Institutional Review Board. All participants were screened to determine whether they were atopic and/or asthmatic and otherwise healthy using study questionnaires, a clinical history and physical exam, chest X-ray, electrocardiogram, pulmonary function tests, methacholine challenge, and skin prick testing (see sections below). Height, weight, and blood pressure were also measured. American Thoracic Society (ATS)[60] and Upper Respiratory Infection (URI)[61,62] questionnaires were administered (see sections below). A single blood sample by venipuncture was collected for measuring total serum IgE levels and for DNA isolation. Smokers and pregnant women were excluded from the studies. In the five studies outlined above, the recruited subjects were classified as atopic asthmatic, nonatopic asthmatic, and nonatopic non-asthmatics. In the current study, we reclassified the subjects as nonatopic and atopic subjects.

Among the 312 subjects in the five asthma studies, 12 subjects participated in more than two studies and nine subjects were older than 40 years. After excluding these 21 subjects, a total of 291 subjects ranging in age from 18–40 years were available for study. Samples and data from 275 subjects (139 nonatopic subjects and 136 atopic subjects) were analyzed after further excluding 16 subjects with missing data.

Allergy skin prick test
Skin prick testing was performed on all subjects using a battery of ten aeroallergens common in North Carolina: *Dermatophagoides pteronyssinus* (Greer Laboratories, Lenoir, NC, USA) and *Dermatophagoides farinae dust* mite allergen (Greer Laboratories), American cockroach (Greer Laboratories), *Alternaria tenuis* (Greer Laboratories), standardized cat hair (Greer Laboratories), dog mixed breeds (Greer Laboratories), ragweed (Greer Laboratories), *Ambrosia bidentata* (Greer Laboratories), 9 Southern grass mix (Greer Laboratories), Eastern 10 tree mix (Greer Laboratories), *Aspergillus fumigatus* (Greer Laboratories). Histamine (2.5 mg/mL) was used as a positive control and sterile saline was used as a negative control. Skin responses were measured 15 minutes after extracts were administered on the forearm by prick puncture using a disposable plastic prick device (DermaPIK, Greer Laboratories). Wheal sizes were determined by measuring the largest diameter of the wheal and by measuring the diameter at a 90° angle to the largest diameter. A wheal diameter ≥3 mm larger than the negative control was considered positive.[63] Atopy was defined as having a positive reaction to any one of the ten tested allergens. The number of positive tests was defined as the number of positive reactions among the ten tested allergens.

ATS questionnaire
A modified ATS questionnaire[60] that included International Union against Tuberculosis and Lung Disease (IUATLD) standard questions for allergic symptoms was administered to the study subjects during the screening visit. The modified questionnaire collected information on smoking, cough, phlegm production, wheezing, dyspnea, asthma history and rhinitis, eczema, and other allergy symptoms. The questionnaire also determined whether subjects had occupational exposure to dusts (hay, grain, cotton, etc), fumes, and/or vapors.

Upper respiratory infection (URI) questionnaire
A validated URI questionnaire[61,62] was administered to participants at the time of enrollment. The purpose of this questionnaire was to determine if subjects had a recent respiratory infection that could affect study results. Self-report of a cold (with or without a runny nose) is highly correlated with a clinical diagnosis of URI. Answers from the questionnaire

were also used to validate subject self-reports of asthma, eczema, and allergic rhinitis symptoms.[59]

Total serum IgE measurement

Total serum IgE levels were measured by nephelometry using the Siemens Dade Behring BNII nephelometer (GMI, Inc, Ramsey, MN, USA) and levels were expressed as IU/mL.

Single nucleotide polymorphism (SNP) genotyping

Genomic DNA was extracted from whole blood by using PAXgene Blood DNA kits and tubes (QIAGEN Sciences Inc, Germantown, MD, USA) or extracted from serum samples using QIAamp DNA mini kits (QIAGEN). DNA extracted from serum samples was amplified by polymerase chain reaction (PCR) using the following: 100 mM dNTPs, 5x Q buffer (QIAGEN), 10x PCR Buffer (QIAGEN), 0.2 unit of Taq polymerase (HotStar Taq, QIAGEN), and 0.5 μM forward primer 5′- CCA ACT TCC TTT TCT TGA ACC TAA TTC −3′ and 0.5 μM reverse primer 5′- TCA CAC TTG TGA ACT CTT CGG −3′.

Genotyping of the CD14 C-159T polymorphism was performed according to the protocol described by Baldini et al.[19] PCR was performed in 25 μL reaction volumes consisting of 2.5 μL of DNA from whole blood or 2.5 μL of DNA from PCR amplification of serum samples, 100 mM dNTPs, 25 mM $MgCl_2$, 10x PCR Buffer (QIAGEN), 0.15 unit of Taq polymerase (HotStar Taq, QIAGEN), and 0.5 μM forward primer 5′ GTG CCA ACA GAT GAG GTT CAC 3′ and 0.5 μM reverse primer 5′ GCC TCT GAC AGT TTA TGT AAT C3′. The DNA was denatured at 95°C for 5 minutes and temperature cycling was set at 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 30 seconds for 30 cycles, followed by a final extension at 72°C for 5 minutes. PCR-amplified DNA was digested with 5 U AvaII in NEB4 buffer (New England Biolabs, Inc, Beverly, MA, USA) at 37°C for 2 hours. PCR products were electrophoresed on 2% agarose gels and visualized with ethidium bromide staining and ultraviolet illumination. The CD14 C-159T (rs2569190) genotype was recorded as homozygous C allele (−159 CC), heterozygous (−159 CT), and homozygous T allele (−159 TT).

The CD14 C+1188G and C+1341 A polymorphisms were detected using TaqMan assays according to previously described methods.[58] The CD14 C+1188G (rs4914) genotype was recorded as homozygous C allele (+1188 CC), heterozygous (+1188 CG), and homozygous G allele (+1188 GG). The CD14 C+1341 A (rs2563298) genotype was recorded as homozygous C allele (+1341 CC), heterozygous (+1341 CA), and homozygous A allele (+1341 AA).

Genotyping of all three CD14 SNPs was confirmed by analysis of duplicate samples.

Statistical analyses

All statistical analyses were performed using SAS Enterprise Guide Version 4.1 for Windows (SAS Institute, Cary, NC, USA). Two-sided P-values at the 0.05 level were used to determine statistical significance. Descriptive statistics including means (standard deviations) and medians (quartiles) for continuous variables and frequencies (proportions) for categorical variables were computed. t-tests were used for comparisons of two groups for continuous data and Chi-square tests were used for comparisons of two groups for categorical data. Data from continuous variables were examined to determine if they followed parametric normal distributions. If not, the data, eg, serum total IgE levels, were logarithmically transformed to obtain normally distributed data and geometric means were used as descriptive statistics. For comparisons of three groups, F-tests from one-way analysis of variance (ANOVA) were used for normally distributed continuous variables and non-parametric Kruskal–Wallis tests were used for non-normally distributed continuous variables. If the overall test among the groups was significant (based on either F-test or Kruskal–Wallis test), we examined all pair-wise group comparisons to determine if differences existed among any two of the three groups. Nonparametric Wilcoxon tests were used to compare the differences between non-normally distributed continuous variables from two groups in situations where the three groups were combined to form two groups.

Results
Demographic characteristics of subjects

Demographic characteristics of the 275 subjects are shown in Table 1. Three subjects had no total serum IgE data, and DNA samples were not available or SNP assays could not be performed for 40 subjects. Consistent with prior studies, there was a significantly increased percentage of male subjects ($P = 0.0041$) and significantly increased serum total IgE levels in the atopic group ($P = 0.0292$) as compared to the nonatopic group.

Association of CD14/−159, CD14/+1188 and CD14/+1341 with atopy

There were no statistically significant differences in the proportion of CD14/-159 TT homozygotes in the atopic group versus the nonatopic group ($P = 0.38$, Table 1). The CD14/−159 genotype frequency in the nonatopic group was 41.4% for CC homozygotes, 41.2% for CT heterozygotes, and 17.7% for TT homozygotes. In the atopic group, these

Table 1 Descriptive characteristics of the study population

Characteristics	Nonatopic (N = 139)	Atopic (N = 136)	P-values χ2 statistics
Male gender, N (%)	42 (30.22%)	64 (47.06%)	0.0041
Female gender, N (%)	97 (69.78%)	72 (52.94%)	
Age in years			0.3731
Mean (SD)	25.38 (5.99)	24.72 (5.31)	
Median	23.00	23.00	
Race			0.7887
African American	71 (51.08%)	62 (45.49%)	
Asian	7 (5.04%)	6 (4.41%)	
Caucasian	58 (41.73%)	65 (47.79%)	
Mixed	3 (2.16%)	3 (2.21%)	
Number of positive skin tests			<0.0001
Mean (SD)	0	3.54 (2.64)	
Median, range	0	3.0, 1–10	
Serum total IgE, IU/mL			0.0292
Number (missing)	138 (1)	134 (2)	
Mean (SD)	47.27 (83.5)	230.00 (278.93)	0.0292
Geometric mean (95% CI)	19.93 (16.03–24.77)	110.44 (87.80–138.90)	0.0292
CD14/−159 genotype	N = 119	N = 117	0.38
CC	49 (41.4%)	38 (32.5%)	
CT	49 (41.2%)	56 (47.9%)	
TT	21 (17.7%)	23 (19.7%)	
CD14/+1188 genotype	N = 122	N = 117	0.15
CC	90 (73.8%)	95 (81.2%)	
CG	27 (22.1%)	15 (12.8%)	
GG	5 (4.1%)	7 (6.0%)	
CD14/−159 genotype	N = 122	N = 113	0.47
AA	7 (5.7%)	5 (4.4%)	
CA	49 (40.2%)	38 (33.6%)	
CC	66 (54.1%)	70 (62.0%)	

Abbreviations: IgE, serum immunoglobulin E; SD, standard deviation.

frequencies were 32.5% for CC, 47.9% for CT, and 19.7% for TT. The C allele frequency was 61.5% in the skin test negative group and 56.4% in the skin test positive group. The C allele frequency was 50.9% in the Caucasian study population and was 69.3% in African American subjects, which is similar to published reference populations.[64]

The frequencies of the CD14/+1188 and CD14/+1341 genotypes in the atopic and nonatopic groups are shown in Table 1. The allele frequencies of CD14/+1188 and CD14/+1341 polymorphisms among the study population were not different from published reference populations.[64]

Racial differences in the frequencies of the CD14/−159, CD14/+1188 and CD14/+1341 genotypes

There was a significant difference in the frequency of CD14/−159 genotypes between African American and Caucasian subjects ($P = 0.0004$) such that African American subjects had a higher frequency of CC homozygotes (47.5% versus 28.2%) and a lower frequency of TT homozygotes (9.0%

versus 26.4%) than Caucasian subjects. The association of a higher frequency of CC homozygotes in African American subjects was significant among atopic subjects, but not nonatopic subjects. There were no significant genotype differences for the CD14/+1188 or CD14/+1341 SNPs between African American and Caucasian subjects (Table 2) or between atopic and nonatopic subgroups.

Association of CD14/−159, CD14/+1188 and CD14/+1341 with total serum IgE levels

In both nonatopic and atopic populations, CD14/−159 TT homozygotes had marginally higher IgE levels compared with CT heterozygotes and CC homozygotes. However, these differences were not statistically significant (Table 3; atopic population). There were also no significant differences in IgE levels between skin-test-positive subjects grouped on the basis of T allele expression, ie, the combined CD14/−159 TT homozygotes and CD14/−159 CT heterozygotes had geometric mean total serum IgE levels of 118 IU/mL

Table 2 Genotype frequency for CD14/−159, CD14/+1188, CD14/+1341 among African American and Caucasian study population

	African American	Caucasian	*P*-value
CD14/−159 genotype	N = 22	N = 110	0.0004
CC	58 (47.5%)	31 (28.2%)	
CT	53 (43.4%)	50 (45.5%)	
TT	11 (9.0%)	29 (26.4%)	
CD14/+1188 genotype	N = 121	N = 114	0.13
CC	96 (79.3%)	81 (71.1%)	
CG	22 (18.2%)	24 (21.1%)	
GG	3 (2.5%)	9 (7.9%)	
CD14/+1341 genotype	N = 22	N = 110	0.78
AA	58 (47.5%)	31 (28.2%)	
CA	53 (43.4%)	50 (45.5%)	
CC	11 (9.0%)	29 (26.4%)	

(CI, 89–156), and CC subjects had geometric mean total serum IgE levels of 82 IU/mL (CI, 52–130) (*P* = 0.1642).

We compared IgE levels between atopic subjects grouped by genotype within race subgroups. There were no significant differences in serum total IgE levels between CD14/−159 genotypes for atopic African American subjects or atopic Caucasian subjects (Table 3).

Similarly, we did not find significant associations between the CD14/+1188 or CD14/+1341 genotypes and total serum IgE levels among atopic African American subjects or atopic Caucasian subjects (Tables 4 and 5).

Association of CD14 polymorphisms with the number of positive skin tests in atopic subjects

The atopic subjects with homozygous CD14/−159 TT had a higher median number of positive skin tests than both the CT heterozygote and the CC homozygote groups

Table 3 Geometric mean (SD) serum total IgE levels (IU/mL) based on CD14/−159 genotypes in atopic subjects

IgE	CD14/−159 genotype			*P*-value
	CC	CT	TT	
N (all subjects)	37	56	23	0.1948
Geometric mean (SD)	82.27 (3.91)	105.89 (3.59)	153.6 (3.33)	
N (African American)	24	26	3	0.3473
Geometric mean (SD)	65.93 (3.86)	89.45 (3.09)	180.20 (1.24)	
N (Caucasian)	12	28	16	0.7440
Geometric mean (SD)	117.97 (4.02)	111.43 (4.00)	153.37 (3.39)	

Abbreviations: IgE, serum immunoglobulin E; SD, standard deviation.

Table 4 Geometric mean (SD) serum total IgE levels (IU/mL) based on CD14/+1188 genotypes in atopic subjects

IgE	CD14/+1188 genotype			*P*-value
	CC	CG	GG	
N (all subjects)	94	15	7	0.9268
Geometric mean (SD)	102.84 (3.68)	114.98 (4.28)	118.59 (3.95)	
N (African American)	42	7	2	0.2271
Geometric mean (SD)	94.96 (3.62)	47.71 (2.96)	30.98 (2.79)	
N (Caucasian)	45	8	5	0.1234
Geometric mean (SD)	99.27 (3.65)	248.26 (3.77)	202.86 (3.14)	

Abbreviations: IgE, serum immunoglobulin E; SD, standard deviation.

(Table 6). A comparison of these groups using a Kruskal–Wallis test yielded a two-tailed *P*-value of 0.0598. The difference in the number of positive skin tests between CD14/−159 CC homozygous subjects and subjects with T alleles (combined CT and TT subjects) was statistically significant (*P* = 0.0388). Caucasian subjects had a higher frequency of CD14/−159 T alleles than African American subjects (Table 2), suggesting that the association of positive skin tests with CD14/−159 T alleles was attributed to Caucasian subjects. When divided into subsets by race, we found that differences in the median number of positive skin tests between CD14/−159 genotypes was larger in Caucasian versus African American subjects despite the fact that significant associations were not observed for CD14/−159 genotypes and the number of positive skin tests within each race group due to the reduced sample size (Table 6). When CD14/−159 CC homozygous subjects were compared to subjects with T alleles (combined CT and TT subjects), the *P*-values were 0.65 and 0.057 for African American and Caucasian groups, respectively.

Table 5 Geometric mean (SD) serum total IgE levels (IU/mL) based on CD14/+1341 genotypes in atopic subjects

IgE	CD14/+1341 genotype			*P*-value
	AA	CA	CC	
N (all subjects)	5	38	69	0.1492
Geometric mean (SD)	274.31 (3.16)	83.15 (4.90)	108.13 (3.16)	
N (African American)	1	19	30	0.6699
Geometric mean (SD)	69.0 (NA)	66.19 (4.51)	92.43 (3.05)	
N (Caucasian)	4	18	34	0.1551
Geometric mean (SD)	387.34 (2.62)	93.09 (5.18)	117.67 (3.12)	

Abbreviations: IgE, serum immunoglobulin E; SD, standard deviation.

Table 6 Mean (SD) and median (lower and upper quartiles) based on CD14/−159 genotypes in atopic subjects

Number of positive skin tests	CD14/−159 genotype			P-value
	CC	**CT**	**TT**	
N* (All subjects)	38	56	23	
Mean (SD)	2.89 (2.51)	3.61 (2.73)	4.04 (2.31)	
Median	2.0	3.0	3.0	0.0598
Lower quartile, upper quartile	1.0, 3.0	1.5, 5.5	2.0, 6.0	
N (African Americans only)	25	26	3	
Mean (SD)	2.64 (2.18)	2.96 (2.39)	2.33 (0.58)	
Median	2.0	2.0	2.0	0.8896
Lower quartile, upper quartile	1.0, 3.0	1.0, 4.0	2.0, 3.0	
N (Caucasians only)	12	28	16	
Mean (SD)	3.08 (3.03)	4.21(3.02)	4.81(2.34)	
Median	2.0	3.0	5.0	0.1121
Lower quartile, upper quartile	1.0, 4.0	2.0, 7.0	2.5, 6.5	

Note: *Subjects with single nucleotide polymorphism assay results.
Abbreviation: SD, standard deviation.

Table 7 Mean (SD) and median (lower and upper quartiles) based on CD14/+1188 genotypes in atopic subjects

Number of positive skin tests	CD14/1188 genotype			P-value
	CC	**CG**	**GG**	
N* (All subjects)	95	15	7	
Mean (SD)	3.16 (2.35)	3.80 (2.81)	6.57 (3.26)	
Median	2.0	3.0	8.0	0.0190
Lower quartile, upper quartile	1.0, 4.0	1.0, 6.0	3.0, 10.0	
N (African Americans only)	43	7	2	
Mean (SD)	2.47 (1.89)	4.43 (3.26)	2.5 (0.71)	
Median	2	3	2.5	0.2786
Lower quartile, upper quartile	1.0, 3.0	1.0, 8.0	2.0, 3.0	
N (Caucasians only)	45	8	5	
Mean (SD)	3.8 (2.65)	3.25 (2.43)	8.2 (2.05)	
Median	3.0	2.5	8.0	0.0098
Lower quartile, upper quartile	2.0, 6.0	1.0, 5.5	8.0, 10.0	

Note: *Subjects with single nucleotide polymorphism assay results.
Abbreviation: SD, standard deviation.

In the atopic group, there were significant differences between CD14/+1188 genotypes ($P = 0.0190$) in the median number of positive skin tests where CD14/+1188 GG homozygotes had a higher median number of positive skin tests than CD14/+1188 CG heterozygotes and CD14/+1188 CC homozygotes. When divided into subsets based on race, there was a significant difference in the number of positive tests between CD14/+1188 genotypes in the Caucasian subjects ($P = 0.0098$) but not in the African American subjects ($P = 0.2786$) (Table 7), although the number of GG African American subjects was small (n = 2).

There was no association between CD14/+1341 genotypes and the number of positive skin tests. Likewise, there was no association between CD14/+1341 genotypes and the number of positive skin tests when subjects were grouped based on race (Table 8).

Discussion

Our study supported a possible association between CD14 polymorphisms and atopy. In particular, we found associations of the CD14-159 T and CD14 1188G alleles with atopic disease. Importantly, we found that the associations of these CD14 polymorphisms with atopic disease were stronger in Caucasians compared to African American subjects, suggesting that there may be important racial differences between CD14 and its relationship to the development of atopy.

The first report of the CD14/C-159T allele indicated an association of the T allele with lower IgE levels among non-Hispanic Caucasian children.[19] Since that report, there have been over 200 subsequent published studies on the CD14/C-159T locus among different ethnic groups. Many studies found a similar association of the CD14/-159 T allele with lower IgE levels among subjects that were British,[20] French,[24] Australian children,[21] Czech,[23] and Chinese.[29] However, no association of the CD14/-159 locus was observed in Polish children,[31] two German populations,[32,33] Taiwanese children with asthma,[36] two Japanese cohorts[20,37] and a general population in Barbados of African descent.[34,35] Conversely, the CD14-159T allele was found to be over

Table 8 Mean (SD) and median (lower and upper quartiles) based on CD14/+1341 genotypes in atopic subjects

CD14/1341 genotype	CC	CA	AA	P-value
Overall number	70	38	5	
Mean (SD)	3.30 (2.36)	3.45 (2.72)	5.20 (3.83)	
Median	2.5	3.0	5.0	0.5584
Lower quartile, upper quartile	2.0, 4.0	1.0, 6.0	2.0, 8.0	
African American (N)	31	19	1	
Mean (SD)	2.42 (1.95)	3.21 (2.30)	2.00 (−)	
Median	2.0	3.0	2.0	0.4738
Lower quartile, upper quartile	1.0, 3.0	1.0, 5.0	2.0, 2.0	
Caucasian (N)	34	18	4	
Mean (SD)	4.21 (2.53)	3.72 (3.21)	6.0 (3.92)	
Median	3.5	2.0	6.5	0.3381
Lower quartile, upper quartile	2.0, 6.0	1.0, 6.0	3.0, 9.0	

Abbreviation: SD, standard deviation.

transmitted to atopic subjects in an inbred population of Hutterites,[38] new mothers living in the Detroit urban area,[56] Caucasians living in the St Paul (MN, USA) urban area,[39] Tunisian children,[41] and among atopic Australian white adults.[40] The published studies listed in Table 9 summarize these results from a wide range of subjects that vary by age, location, and ethnicity, and who likely had significantly different environmental exposures. From the studies listed in Table 9, the minor allele (CD14/−159T) frequencies ranged from 47% to 52% in Caucasian study populations, 55% to 60% in Asian populations, and were 35% in subjects of African descent in one study from Barbados. We found similar allele frequencies in our Caucasian and African American subjects as reported in Table 9. Because CD14/−159 allele frequencies among different ethnic groups vary significantly, it is important to compare genetic association studies with others of the same ethnic group.

Extrapolating reports from past studies, it appears that in populations with low levels of LPS exposure, CD14/−159 CC homozygotes may have the highest risk for allergic sensitization when compared to subjects with CT and TT genotypes. However, in populations with high levels of LPS exposure, individuals with TT genotypes may have the highest risk for allergic sensitization. All our subjects were recruited from the Raleigh-Durham-Chapel Hill (NC, USA) urban area, and half of these subjects were of African ancestry. More information about the subjects' home environment, such as pet exposures, endotoxin levels, and their early childhood environment, would be needed to better understand associations of environmental endotoxin exposure with our genotype results.

Age is an important variable in population genetic studies. O'Donnell et al[21] reported that CD14/−159 CC subjects had a significantly higher number of positive skin prick tests as compared to CD14/−159 CT and TT subjects in early childhood, but the significant association was not present by age 25. In the same study population, O'Donnell reported that CD14/−159 CC subjects had higher total serum IgE levels at age 18, and again, the association of CD14/−159 CC genotypes was not present when the same subjects were older (age 25).[21] These data were consistent with reports on subjects with a similar age and ethnic background among non-Hispanic Caucasian children living in the USA[19] and among Austrian and German children with regular contact with pets.[25] The average age in our study populations was 25 years and we did not find an association between CD14 and total serum IgE level in either Caucasian or African American subgroups. These reports suggest that the influence of CD14 polymorphisms on the atopic phenotype may be age specific, and future studies to follow different racial groups longitudinally may better define the association of age and gene expression on atopic disease among children and adults.

Gene analyses are also complicated by the fact that different genes and their combinations are involved in the regulation of total IgE and allergen specific responses. Importantly, the haplotype background of each polymorphism may also affect the results of these studies. Recent studies have suggested that CD14 polymorphisms and haplotype markers are associated with allergic disease and interact with environmental exposures to affect the development of atopy. Wang et al[36] reported that, among Taiwanese children with asthma, the CD14/−159 SNP was only associated with total IgE levels when the T allele was part of a haplotype containing a specific D5S2011 E allele (143 bp). Ober et al[38] reported that the CD14-159T allele was over-transmitted to atopic Hutterites only when the allele was on a haplotype with the D5S642 marker allele (D5S642, 185-bp) but not those with other D5S642 markers. Walley et al[65] reported a linkage but not association between CD14/−159 polymorphisms and atopy. Most recently, Bruce et al[66] studied 3113 European children growing up on farms and reported that the effect of farm animal contact on the development of allergic symptoms in children was strongly modified by the neuropeptide S (NPS) receptor 1 (NPSR1) genetic background. These reports suggest that the reason for the inconsistent results among different study populations was probably because the CD14/−159 SNP and the putative susceptibility variant are in the same LD block in some study subjects but not in all populations. It is also possible that an association with specific haplotypes[67] or a combination of genotypes affect serum total IgE concentrations.[68] Unfortunately, in our study we did not identify an association of CD14 polymorphisms and IgE expression.

It should be noted that seemingly incompatible results in atopic population studies might at least be partly explained by methodological factors such as questionnaire phrasing, different definitions of atopy and/or asthma, the skin prick testing technique (method of measuring the wheal size, varied allergens for skin testing, etc), or the type of assay for the measurement of specific IgE (Table 9). For example, the methods of identifying positive skin tests varied among studies. When comparing eight studies using the same method as ours, three studies[33–35] found no association of the CD14/−159T allele with allergen sensitization and five studies including ours[38–41] found that the CD14/−159T allele was positively associated with allergen sensitization.

Table 9 Characteristics of study subjects in studies of CD14/−159 SNP and association with IgE levels and skin allergen testing

Year	First author	Subject characteristics			T allele frequency %	Atopy definition
		Age (y)	Race/ethnicity	Location		
Association of CD14/−159 C allele with allergen sensitization						
1999	Baldini et al[19]	10–11	White (Hispanics or non-Hispanic)	US, Arizona; non rural area	48.6	Positive skin test: at least one skin test with diameter sums >3 mm
1999	Gao et al[20]	Adult	British white, Japanese	Great Britain and Japan; non-rural area	British 52 Japanese 60	Presence of high serum total IgE (>120 IU/mL in British, >400 IU/mL in Japanese) and/or positive specific IgE titer
2001	Koppelman et al[22]	34–76	Dutch white	Netherlands; non-rural area	Probands 44 spouse 53.5	Positive skin test: the largest wheal diameter ≥5 mm
2001	Vercelli et al[68]	11	Caucasian, Hispanics	US, Tucson; non rural area	48.6	Positive skin test: at least one skin test with diameter sums >3 mm
2003	Buckova et al[23]	15–36	Czech	Czechoslovakia	Atopic asthmatic 47, normal 44.2	Specific IgE ≥3.5 IU/mL and/or a positive skin prick test to any of the allergens tested
2003	O'Donnell et al[21]	8–25	Australian white	Australia; non rural area	49.5	Positive skin test: mean wheal size ≥3 mm before 1986 and ≥4 mm from 1986 onward; atopic: positive reaction to any tested allergen
2006	Leynaert et al[24]	20–44	French	France; urban and farm	49	Specific IgE ≥3.5 IU/mL to any of the allergens tested
2007	Smit et al[26]	16–26	Danish whites	Denmark; farm living	Controls 43.8 asthma 42.5	Positive skin prick test to one or more common inhaled allergens
2003	Leung et al[29]	5–15	Chinese	China, Hong Kong; non rural area	56	Presence of at least one allergen-specific IgE antibody
2006	Tan et al[28]	6–12	Taiwanese	Taiwan; non rural area	Asthma 62.5 controls 57	Positive skin test: the presence of ≥1 reaction; a wheal diameter ≥5 mm
2005	Takeuchi et al[30]	20–74	Japanese	Japan; non rural area	55	Specified allergic rhinitis criteria
2008	Kowal et al[27]	23–26	Polish	Poland	Asthmatic 55 healthy 47	HDM skin test positive and HDM specified IgE
Association of CD14/−159 C or T allele with allergen sensitization						
2005	Eder et al[25]	8–10	Austrian, German	Austria and Bavaria (Germany); farm or non-farm living	49	Atopic: specific IgE ≥3.5 IU/mL to any of the allergens tested
2005	Simpson et al[54]	0–5	British	Britain	48.5	Allergic sensitization: specific IgE >0.2 kU/L to at least one of seven allergens or skin prick weal diameter ≥negative control
2006	William et al[56]	Expectant mothers	African American, White, other	US Detroit; non rural area	No race specified 41	NA
2008	Bottema et al[69]	Fetus–8	Dutch white	Netherlands	48	Specific IgE ≥0.35 IU/mL against food allergens (milk or egg) at 1 and 2 year and indoor allergens (house dust mite, cat and dog) at 4 and 8 years
No association of CD14/−159T alleles with allergen sensitization						
2001	Lis et al[31]	13–14	Polish	Poland	Asthmatic 36 non-asthmatic 38	Asthma: wheezing in the last year, serum IgE level >150 kIU/L, positive bronchial challenge test
2003	Sengler et al[32]	0–10	German	Germany	Asthmatic 47 atopic 51 healthy 49	Allergic sensitization: specific IgE ≥0.7 kU/L against at least one out of nine food and inhalant allergens
2004	Kabesch et al[33]	6–11, 18–67	German	Germany	47–49	Allergic sensitization: wheal reaction ≥3 mm than negative control
2005	Nishimura et al[37]	Children	Japanese	Japan; non rural area	51.5	NA

(Continued)

Table 9 (*Continued*)

Year	First author	Subject characteristics			T allele frequency %	Atopy definition
		Age (y)	Race/ethnicity	Location		
2005	Wang et al[36]	1–16	Chinese	Taiwan; non rural area	Not reported	High IgE group: IgE >1000 IU/mL normal IgE group: IgE <200 IU/mL
2003	Zambelli-Weiner[34]	Children	African descent	Barbados; rural area	35	Positive skin test an average wheal size ≥3 mm than saline control
2005	Zambelli-Weiner et al[35]	11–55	African descent	Barbados; rural area	Asthmatic 29.5, non-asthmatic 34	Positive skin test an average wheal size ≥3 mm than saline control
Association of CD14/−159 T allele with allergen sensitization						
2000	Ober et al[38]	6–89	Hutterites	US South Dakota; farm living	Not reported	Skin prick test wheal ≥3 mm than negative control
2005	Kedda et al[40]	Adult	Australian white	Australia	44–51	Skin prick test wheal >3 mm than to at least one of the five aeroallergens tested
2006	Jackola et al[39]	6–75	White	US, St Paul; non rural area	47.3	Positive skin test: wheal area ≥9 mm² above the negative control
2008	Lachheb et al[41]	5–16	Tunisian (African white Caucasian)	Tunisia	Asthmatic 56.5, controls 68	Skin prick test wheal ≥3 mm and with total IgE ≥200 IU/mL
2009	Our study	18–40	African American, White, other	US, Central North Carolina; non rural area	African American 31, Whites 49	Skin prick test wheal ≥3 mm than negative control

Abbreviations: HDM, house dust mites; IgE, serum immunoglobulin E; SNP, single nucleotide polymorphism.

We identified a significant increase in the median number of positive skin tests for Caucasian subjects with the G allele of the CD14/+1188 SNP. Bucková et al[58] found that the common −1619A/−1359G/−550C/+1188G/+1341T haplotype was associated with a positive reaction to mites and molds in Czech patients. They also reported that the T allele of the CD14/+1341 SNP was significantly associated with positive reactivity to mites and molds. Unfortunately, there are no published reports regarding the association of the CD14/+1188 SNP with skin prick test using the same method that was used in our study.

There are several potential limitations of our study. First, the study data was pooled from five cohort studies. However in our five cohort studies, all study subjects were recruited from the same general population and identical study methods were used and consistently administered by the same study team. The emphasis in all five studies was on younger adult subjects with limited medication use and exclusion of tobacco users. The frequencies of minor alleles and genotypes of CD14 genes were similar to each other across each of the five study cohorts (data not shown).

The interaction of CD14 with environmental LPS appears to be an important modifier of allergic disease. As such, a second potential weakness of our study was the lack of detailed information that was collected on environmental exposures (such as early life farm exposure, pet exposure, house dust endotoxin levels, number of siblings, etc). Finally, another weakness of our study was the low number of subjects who were CD14/1188 GG homozygous. This significantly limits the generalizability of our findings on this SNP due to the small number of subjects that were analyzed.

In summary, our study demonstrated that there was a significant association of the CD14/−159 T allele with a higher number of positive skin tests among skin-test-positive subjects from a cohort of young adults living in the urban area of central North Carolina. The CD14/+1188G allele was also associated with a significantly higher number of positive skin tests, especially in Caucasian subjects, although interpretation of these latter results is complicated by the low numbers of subjects in some of the analyses. The association of the CD14 gene polymorphism with atopic disease may be strongly associated with race. Our future studies will need to focus on measuring environmental exposures to allergens and LPS to interpret our results optimally. The eventual identification of more atopic genes will provide us with better insight into the pathophysiological mechanisms of atopic diseases, and will build the foundation for new and more effective immunotherapy strategies, early diagnosis methods for individuals at risk of atopy, and more insight into pharmacogenetics.

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Disclosure

The authors report no conflict of interests in this work.

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