

Age-specific Relationship between CD14 and Atopy in a Cohort Assessed from Age 8 to 25 Years

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CD14 influences postnatal switching of T helper cell responses. CD14 C-159T has been associated with altered CD14 and IgE levels in cross-sectional studies. Identifying whether associations vary with age requires data from children of the same age followed longitudinally over many years. In this study, an unselected population with extensive longitudinal data was used to test the hypothesis that CD14 C-159T was associated with early-onset atopy. A total of 305 subjects were assessed on up to seven occasions between ages 8 and 25 years by questionnaire, histamine challenge, and skin prick test. For atopy, airway hyperresponsiveness (AHR), and wheeze, each subject was classified as having early onset, late onset, or no disease onset during follow-up. Compared with subjects with -159CT and -159TT, subjects with -159CC had an odds ratio of 2.2 ($p = 0.018$) for early-onset atopy and an odds ratio of 2.6 ($p = 0.019$) for early-onset AHR. Cross-sectional analysis showed increased prevalence of -159CC in subjects with atopy and AHR in childhood but not adulthood. These data suggest that the influence of CD14 -159C on the atopic phenotype may be age specific, exerting an effect during midchildhood, which is no longer apparent by early adulthood.

Keywords: longitudinal; gene; polymorphism; asthma

Atopic disorders are mediated by the T helper cell type 2 (Th2) cytokines interleukin 4 and interleukin 5 (1) and are characterized by an elevated and prolonged IgE response to common nonpathogenic allergens (2, 3). Th2 lymphocyte responses predominate in fetal life and infancy and then, possibly as a result of maturation of cellular immune function and exposure to allergens and bacterial infection, typically change to T helper cell type 1 (Th1) responses (4-6). Development of atopic disease may be associated with a failure of this immune system "switch" during early infancy (7-9).

LPS is a major constituent of the outer membrane of gram-negative bacteria (10-12). CD14 functions as a receptor for LPS, with binding of LPS enhancing production of interleukin 12, an obligatory signal in the maturation of naive T cells into Th1 cells (11). In the complex network of human adaptive immunity, CD14 may therefore play a crucial role in the postnatal switch from Th2 to Th1 responses. The promoter region of the CD14 gene contains a polymorphism (CD14 C-159T) that has been associated with altered serum-soluble CD14, total IgE levels, and skin prick test (SPT) responses in several populations (13-17).

Although atopic disorders are heterogeneous with distinct age-specific phenotypic differences between childhood- and

adulthood-onset disease (18, 19), the relationship between CD14 C-159T and atopy has only been studied using cross-sectional data (13-17, 20, 21). The CD14 C-159T polymorphism affects CD14 secretion and is likely to have an age-related role in the development of atopy (6, 10-12, 22); however, no studies have been reported that investigated age-related effects of the CD14 C-159T polymorphism on the development of atopy. Use of data from a cohort of children of the same or similar age who are followed longitudinally over many years ("age-specific longitudinal data") provides an optimal setting to investigate age-related factors in a disease, with more statistical power to analyze genotype-phenotype associations (23, 24). The aim of this study was to use an unselected population from which extensive age-specific longitudinal respiratory data has been collected to test the hypothesis that the CD14 -159C allele was associated with early-onset atopy.

Part of these data has been previously reported in the form of abstracts (25-27).

METHODS

Phenotype

In 1982, 718 8-year-olds were randomly recruited from schools in Belmont, New South Wales and reviewed on up to six occasions until 1999 (28-30). All seven assessments, performed at age 8, 10, 12, 14, 16, 18, and 25 years, included questionnaire, histamine challenge, and SPT. Blood was collected from consenting individuals for serum total IgE level at age 18 and 25 years and for DNA extraction at age 25 years. The self-administered questionnaire, completed by parents before 1990 and by subjects from 1990 onward, asked questions on recent wheeze, eczema, hay fever, and diagnosed asthma. Recent wheeze was defined by wheeze within the last 12 months. Diagnosed asthma was defined by a positive response to the question: "Has your child/Have you ever been diagnosed as having asthma by a doctor or at a hospital?" A subject was classified as having smoked ("ever smoked") if they had smoked regularly before completing the questionnaire, that is, at least one cigarette per day or one ounce of tobacco per month for at least a year. The study was approved by the King Edward Memorial and Princess Margaret Hospitals Ethics committee. Written informed consent was obtained from parents before 1990 and from subjects from 1990 onward.

Spirometry was used to measure lung function. β -agonists were withheld for at least 6 hours beforehand. Forced expiratory maneuvers were repeated until two consistent readings of FEV₁ and FVC were obtained, of which the largest value was used in analyses (31). Airway responsiveness was tested by airway challenge to histamine using the rapid method (32). Dose-response to histamine was obtained by plotting percent fall in FEV₁ against the logarithm of the histamine dose administered. The dose of histamine causing a 20% fall in FEV₁ (PD₂₀FEV₁) was read by interpolation. Individuals with a baseline FEV₁/FVC less than 60% or with an FEV₁ less than 60% predicted for age and height received a bronchodilator challenge of 200 μ mol salbutamol through a Volumatic spacer rather than a histamine challenge. Subjects were classified as having airway hyperresponsiveness (AHR) if they had a PD₂₀FEV₁ less than 3.9 μ mol or a greater than 15% increase in FEV₁ in response to salbutamol.

SPT to *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat dander, ryegrass, plantain, and *Alternaria tenuis* (Hollister-Stier,

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Elkhart, IN) was performed on the forearm with histamine 10 mg/ml and glycerol used as controls (33). The long axis and the perpendicular of each wheal were measured and mean wheal size calculated. Consistent with previous studies showing an age-related change in SPT responses (33–35), a reaction was considered positive if mean wheal size was 3 mm or greater before 1986 and 4 mm or greater from 1986 onward. Individuals were classified as atopic if they had a positive reaction to any tested allergen. Serum total IgE was measured by the Quanticlone IgE method, a noncompetitive, two-site radioimmunoassay (36).

Longitudinal Categories

For each individual, data on atopy, AHR and recent wheeze were coded as binary variables (present or absent). Results were analyzed across the 17 years of follow-up and individuals classified into one of four longitudinal categories for the phenotypes of atopy, AHR, and wheeze. To be classified, subjects had to have been assessed at least once in each of the following age brackets: (1) 8 to 10 years, (2) 12 to 16 years, and (3) 18 to 25 years. Individuals unable to be confidently classified into a longitudinal disease category due to inadequate number of assessments, variation in characteristics, or intermittent characteristics were excluded from analyses. Longitudinal categories of disease were based on the above three age divisions as follows:

early persistent: present at age 8 and/or 10 years, then consistently present thereafter until the last visit at either 18 or 25 years;

early remittent: present at age 8 and/or 10 years as well as the next visit, then consistently absent until the last visit at either 18 or 25 years;

late onset: absent at age 8 and 10 years, then present on at least two subsequent visits, with no visits after the age of 10 years showing absent;

never: never present with subject assessed at least once between age 8 and 10 years, once between 12 and 16 years, and once between 18 and 25 years.

Genotype

Genomic DNA was extracted from peripheral blood mononuclear cells. Genotyping for CD14 C–159T was determined by polymerase chain reaction and restriction enzyme digestion using *AvaII* as reported previously (13).

Statistical Analysis

CD14 C–159T genotype was recorded as homozygous C allele (–159CC), heterozygous (–159CT), and homozygous T allele (–159TT). Atopy, wheeze, diagnosed asthma, hay fever, and eczema were classified as binary variables. Genotypes were cross-tabulated with binary phenotypes, enabling comparison of genotype and allele frequency with phenotype. Significance was assessed using χ^2 analysis. Descriptive statistics were calculated, and analysis of variance was used to compare geometric mean IgE levels between genotypes. As the distribution of serum total IgE was skewed, it was log_e-transformed to approximate a normal distribution. Analysis of serum total IgE versus genotype was assessed at age 18 and 25 years and compared between subjects with and without atopy. The number of positive SPTs was analyzed using Kruskal–Wallis nonparametric testing. Longitudinal categories were analyzed as binary variables (present or absent), and specific pair-wise comparisons of one category with other categories performed for each phenotype, for example, early persistent atopy versus other longitudinal atopy categories combined. Significance was tested using χ^2 analyses. Logistic regression, adjusted for sex and, when appropriate, smoking history was used to further investigate the association of genotype with phenotype, for both longitudinal and cross-sectional analyses (37). Analyses were performed using Minitab for Windows version 12 software. For all analyses, statistical significance was defined at the 5% level.

RESULTS

Genotype

A total of 345 individuals consented to a blood sample: 180/718 (25%) at age 18 years, 345/718 (48%) at age 25 years, and 144/718 (20%) at both ages. Of the 345 individuals providing

blood for DNA, 305 were successfully genotyped for CD14 C–159T, with 79 (26%) genotype –159CC, 149 (49%) genotype –159CT, and 77 (25%) genotype –159TT. The frequency of allele –159C was 50%, in Hardy–Weinberg equilibrium, and consistent with studies of this polymorphism in other populations (13–15).

Phenotype

Of the initial 718 subjects, 74% had four or more assessments and 31% had all seven assessments. Of the 305 subjects with genotype results available, 86% had four or more assessments and 41% had all seven assessments. Characteristics of the subjects successfully genotyped are shown at visit one through seven in Table 1. All subjects were white. Of the 305 subjects, 205 could be classified into a longitudinal category for atopy, 228 for AHR, and 196 for wheeze. On average, 46% of the individuals in a longitudinal category were male and 41% current or past smokers. Characteristics of subjects classified into a longitudinal category were similar to those of subjects unable to be classified into a longitudinal category. For both cross-sectional and longitudinal analyses, genotyped subjects were representative of the original population of 718 in terms of sex, smoking history, and all atopic phenotypes, and there were no significant differences in study parameters between the subjects successfully genotyped and the 40 subjects unable to be genotyped.

Longitudinal Analyses

Atopy. CD14 genotype frequencies for the longitudinal categories of atopy are shown in Table 2. Early persistent atopy and early remittent atopy were initially classified together as early-onset atopy for analysis, due to low numbers (four subjects) in the early remittent atopy group. Early-onset atopy was compared with late-onset and never atopy combined using logistic regression. Compared with subjects with genotype –159TT, the odds ratio for early-onset atopy was 2.5 (95% confidence interval [CI] = 1.1–5.6; $p = 0.027$) for subjects with genotype –159CC and 1.2 (95% CI = 0.6–2.5) for subjects with genotype –159CT. Subjects with genotype –159CC had an odds ratio of 2.2 (95% CI = 1.2–4.2; $p = 0.018$) for early-onset atopy compared with subjects with genotype –159CT and –159TT. When early persistent atopy was compared with early remittent atopy, late atopy, and never atopy combined, subjects with genotype –159CC had an odds ratio of 2.0 (95% CI = 1.1–3.9; $p = 0.04$) for early persistent atopy compared with subjects with genotype –159CT and –159TT. Compared with subjects with genotype –159TT, subjects with genotype –159CC had an odds ratio of 2.1 (95% CI = 0.9–4.8) for early persistent atopy and subjects with genotype –159CT had an odds ratio of 1.1 (95% CI = 0.5–2.3).

AHR and wheeze. CD14 genotype frequencies for the longitudinal categories of AHR are shown in Table 3. Early-onset AHR was compared with late-onset and never AHR combined using logistic regression. Compared with subjects with genotype –159CT and –159TT, subjects with genotype –159CC had an odds ratio of 2.6 (95% CI = 1.2–5.6; $p = 0.019$) for early-onset AHR. When early persistent AHR was compared with early remittent, late-onset, and never AHR combined, subjects with genotype –159CC had an odds ratio of 4.6 (95% CI = 1.6–13.7; $p = 0.006$) for early persistent AHR compared with subjects with genotype –159CT and –159TT. The CD14 C–159T genotype was not associated with any longitudinal category of wheeze.

Cross-sectional Analyses

Atopy. Cross-sectional analysis showed increased prevalence of genotype –159CC in subjects with presence of atopy during childhood, becoming almost equal by 25 years of age (Table 4). Compared with subjects with genotype –159CT and –159TT, subjects with genotype –159CC had an odds ratio for atopy of

TABLE 1. PHENOTYPE AT EACH VISIT FROM AGE 8–25 YEARS FOR INDIVIDUALS SUCCESSFULLY GENOTYPED FOR CD14 C–159T

Phenotype	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Average age, yr	8	10	12	14	16	18	25
Individuals genotyped for CD14 C–159T, n	305	235	229	190	224	240	305
Male, %	47	45	49	48	46	47	47
Passive smoke exposure, %	*	*	60	58	*	*	*
Ever smoked, %	*	*	*	*	16	33	42
Atopy, %	22	33	41	39	42	43	43
Dust mite atopy, %	17	27	33	26	33	33	37
Number positive SPTs, mean	0.44	0.7	0.88	0.75	0.79	0.89	1
AHR, %	11	9	9	5	7	17	11
Asthma ever, %	10	14	15	22	21	23	28
Wheeze ever, %	20	20	25	28	25	30	47
Recent wheeze, %	11	12	13	14	19	28	38
Serum total IgE, geometric mean (n)	*	*	*	*	*	82.3 (117)	58.9 (302)

Definition of abbreviations: AHR = airway hyperresponsiveness; SPT = skin prick test.

* Data not collected.

2.0 (95% CI = 1.1–4.0), 1.6 (95% CI = 0.9–2.9), 2.5 (95% CI = 1.4–4.5), 2.1 (95% CI = 1.1–4.1), 1.8 (95% CI = 1.0–3.4), 1.9 (95% CI = 1.0–3.5), and 1.3 (95% CI = 0.7–2.2) at age 8, 10, 12, 14, 16, 18, and 25 years, respectively (Figure 1). This association was statistically significant at age 8 ($p = 0.021$), 12 ($p = 0.003$), 14 ($p = 0.041$), and 18 ($p = 0.041$) years. Subjects with genotype –159CC had a higher number of positive SPTs compared with subjects with genotype –159CT and –159TT. This was significant in early childhood but was not present by 25 years of age (Figure 2).

Total serum IgE. At age 18 years, compared with subjects with genotype –159CT and –159TT, subjects with genotype –159CC had a 2.1-fold (95% CI = 1.1–4.0; $p = 0.03$) higher geometric mean serum total IgE level overall and a 3.6-fold (95% CI = 1.2–11.3; $p = 0.032$) higher geometric mean serum total IgE level within SPT-negative subjects. There was no association between CD14 genotype and total serum IgE level at age 25 years.

Other atopic phenotypes. Compared with subjects with genotype –159CT and –159TT, subjects with genotype –159CC had evidence of a trend toward higher prevalence of AHR at all ages, which was significant at age 8 years (odds ratio = 2.4; 95% CI = 1.2–5.2; $p = 0.021$) (Table 5). No association was found between CD14 C–159T and diagnosed asthma, eczema, hay fever, or recent wheeze at any age.

DISCUSSION

Although many studies have reported associations between various genotypes and asthma and asthma-related phenotypes, virtually none have considered the temporal evolution of features in their analyses. This is the first study to report an age-related

association by using defined cohorts according to multiple assessments of a population from childhood to adulthood. Data from this unique, age-specific, community-based cohort with repeated, accurate longitudinal assessments suggests that CD14 –159C has an effect on the development of atopy in early childhood.

In assessing the association between genotype and asthma-related phenotypes as related to age, previous studies would have been limited by their design and their recruitment of research subjects. In particular, the inhomogeneity of cohorts containing subjects of different ages and too few individuals of any specific age would have mitigated against ascertaining these relationships. Few comparable data sets are available. A recent study on a large population of German children evaluated atopy-related phenotypes annually from birth until 10 years of age and investigated the role of CD14 C–159T in the presentation of the atopic phenotype (20). They reported no association between CD14 genotypes and atopy; however, their study differed from ours in that children were younger and followed over a shorter period of time, and the analyses were cross-sectional in nature and not based on longitudinal categories of disease. Only one published study has used longitudinal analyses to investigate the effect of age, in particular, transition from childhood to adult life, on associations between genotype and the asthma phenotype (38). Children were assessed once between the age of 8 and 12 years and again 10 years later, with subjects classified and then analyzed as childhood onset, adult onset, persistent, or never onset of phenotype. The protective effect against asthma of the CCR5Δ32 allele was shown to be present in childhood but lost by adulthood. These results highlight the importance of considering age as a confounder in genetic association studies. However, although

TABLE 2. CD14 C–159T GENOTYPE DISTRIBUTION IN LONGITUDINAL CATEGORIES OF ATOPY

Atopy Category	CD14 C–159T Genotype			Total n
	n (% of total)			
	–159CC	–159CT	–159TT	
Early persistent	22 (34)	27 (42)	15 (24)	64
Early remittent	2 (50)	2 (50)	0	4
Late onset	6 (20)	12 (40)	12 (40)	30
Never	21 (20)	56 (52)	30 (28)	107

TABLE 3. CD14 C–159T GENOTYPE DISTRIBUTION IN LONGITUDINAL CATEGORIES OF AIRWAY HYPERRESPONSIVENESS

AHR Category	CD14 C–159T Genotype			Total n
	n (% of total)			
	–159CC	–159CT	–159TT	
Early persistent	9 (60)	2 (13)	4 (27)	15
Early remittent	5 (31)	5 (31)	6 (38)	16
Late onset	0 (0)	5 (62)	3 (38)	8
Never	47 (25)	96 (51)	46 (24)	189

Definition of abbreviation: AHR = airway hyperresponsiveness.

TABLE 4. CD14 C-159T GENOTYPE DISTRIBUTION IN SUBJECTS WITH AND WITHOUT ATOPY FROM AGE 8 TO 25 YEARS

Age, yr	With Atopy			Without Atopy			χ^2 p Value
	n (%)			n (%)			
	CD14 –159CC	CD14 –159CT	CD14 –159TT	CD14 –159CC	CD14 –159CT	CD14 –159TT	
8	25 (37)	30 (45)	12 (18)	54 (23)	119 (50)	65 (27)	0.04
10	26 (33)	36 (46)	16 (21)	37 (24)	78 (50)	42 (26)	NS
12	35 (37)	41 (44)	18 (19)	26 (19)	66 (49)	43 (32)	0.005
14	23 (32)	31 (43)	18 (25)	21 (18)	64 (56)	30 (26)	NS
16	30 (33)	37 (41)	24 (26)	26 (21)	62 (50)	36 (29)	NS
18	31 (32)	40 (42)	25 (26)	26 (20)	68 (53)	35 (27)	NS
25	35 (28)	58 (47)	31 (25)	38 (24)	82 (51)	42 (25)	NS

Definition of abbreviation: NS = not significant.

detecting age relationships requires extensive, precise data from a cohort of young children of very similar age followed over an extended period, it also requires repeated assessments of these subjects over the data collection period. The longitudinal, community-based cohort used in our study was ideal for detecting age-related genetic associations. Comprehensive and standardized data were collected from the same individuals on repeated occasions over 17 years of follow-up from childhood into adulthood allowing numerous cross-sectional analyses as well as a large number of individuals to be accurately classified into a longitudinal category of disease. The long duration and frequency of follow-up also minimized misclassification due to transient natural variation in responses. The effect of genotype on the natural history of disease and age-related changes in disease could therefore be assessed.

In terms of study compliance, an excellent response rate was maintained in this cohort, with large numbers of children assessed at each visit. This allowed repeated, large, cross-sectional analyses to be done that included every individual studied on that occasion. As all subjects were the same age as one other at each visit, age-related effects could be investigated, not by each individual cross-sectional analysis, but instead by evaluating the series of cross-sectional analyses thereby using all data. Thus, this study has enabled examination of the effect of age on geno-

type-phenotype correlations in two ways: longitudinal analysis including individuals who were assessed on each occasion as well as repeated cross-sectional analyses that allowed age to be assessed by considering the pattern of changes between cross-sectional analyses. Potential for bias exists in longitudinal studies analyzing subjects consenting to ongoing follow-up as it might be difficult to distinguish between groups enriched by subjects with persistent, severe, or adult-onset disease and the natural increase in some phenotypes with increasing age. This does not appear to be the situation in our study as the frequency of specific phenotypes in the adult ages is not significantly higher than those seen in the general population. As with all longitudinal studies, the number of subjects genotyped and followed at different ages is inconsistent and may have affected the phenotype studied and therefore may be responsible for the association differences shown between the various ages when assessed cross sectionally. However, longitudinal analyses of the subjects who had been assessed on at least three occasions from before the age of 12 years to after the age of 18 years adds further support to these apparent age-related effects.

Genotype distribution in this population was consistent with studies of the polymorphism in other populations (13–15, 20). Not unexpectedly, genotyping was incomplete as specimens were not available from all the initially recruited population and some

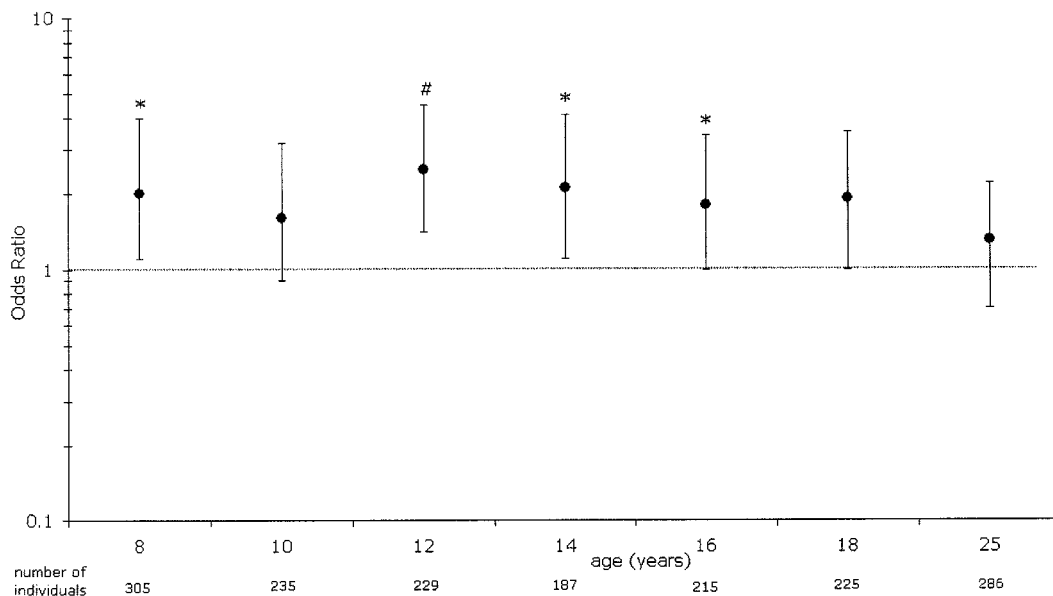


Figure 1. Odds ratio for atopy associated with genotype CD14 -159CC compared with CD14 -159CT and -159TT from age 8 to 25 years. Compared with subjects with genotype -159CT and -159TT, subjects with genotype -159CC had an odds ratio for atopy of 2.0 (95% confidence interval [CI] = 1.1–4.0; $p = 0.021$), 1.6 (95% CI = 0.9–2.9), 2.5 (95% CI = 1.4–4.5; $p = 0.003$), 2.1 (95% CI = 1.1–4.1; $p = 0.041$), 1.8 (95% CI = 1.0–3.4), 1.9 (95% CI = 1.0–3.5; $p = 0.041$), and 1.3 (95% CI = 0.7–2.2) at age 8, 10, 12, 14, 16, 18, and 25 years, respectively. Error bars represent 95% CI. # Represents p values less than 0.01, and * represents p values less than 0.05.

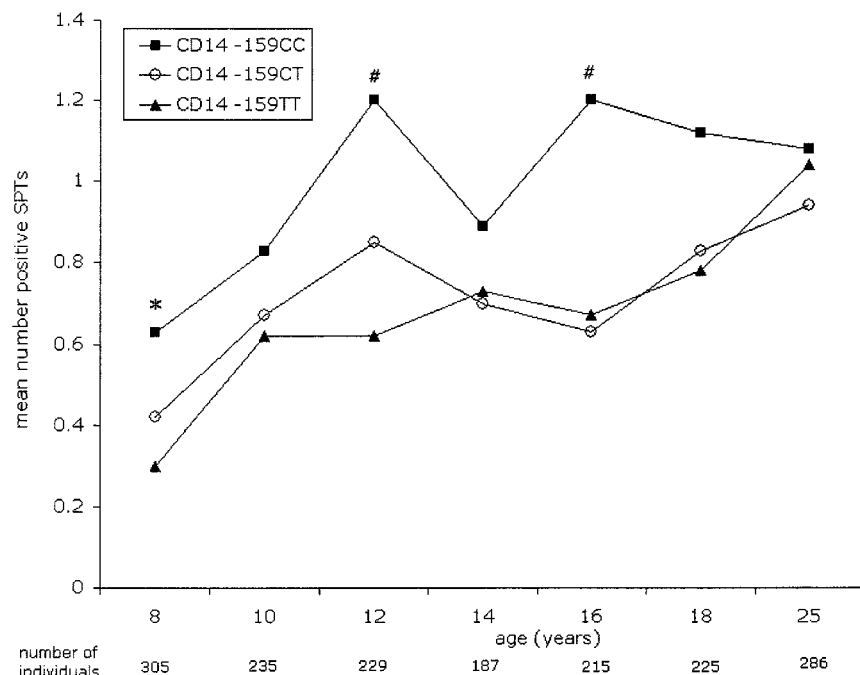


Figure 2. Number of positive skin prick tests (SPTs) with genotype CD14 C-159T from age 8 to 25 years. Using Kruskal-Wallis nonparametric testing, subjects with genotype CD14 -159CC had a higher number of positive SPTs compared with subjects with genotype CD14 -159CT and -159TT combined. This was significant at 8 ($p = 0.016$), 12 ($p = 0.004$), and 16 ($p = 0.009$) years of age. # Represents p values less than 0.01, and * represents p values less than 0.05.

were not successfully genotyped for the CD14 C-159T polymorphism. Analysis of only those subjects successfully genotyped may have potentially limited power, introduced bias, and influenced results. Nonetheless, phenotype was not found to be statistically different between the subjects successfully genotyped and those not able to be genotyped or not providing DNA, suggesting that results found in the genotyped subpopulation are likely to represent the total cohort. The high allele frequency of this polymorphism meant subject numbers remained adequate to detect significant associations.

The association between CD14 C-159T and childhood-onset atopy may result from a direct functional effect on sCD14 and IgE levels. Increased CD14 expression, as assessed by sCD14 levels and lower IgE levels, has been shown in individuals homozygous for the T allele (13). The CD14 C-159T polymorphism has also been shown to affect transcriptional activity (39), most likely through lowering the affinity of the CD14 regulatory region for Sp3 (40, 41). However, it is possible that the association could be due to linkage disequilibrium of CD14 C-159T with a polymorphism in another gene, although a more plausible candidate gene than CD14 in this region has not been identified

(42). The CD14 promoter was recently investigated in more detail and four novel single nucleotide polymorphisms identified in the region: A-1619G, G-1359T, A-1145G, and A-809C (42-44). The G-1359T, A-1145G, and C-159T polymorphisms were studied in a cohort of children and found to be in tight linkage disequilibrium (44). Homozygotes for the T/A/C haplotype were shown to have significantly higher IgE levels than homozygotes for both the G/G/T and G/A/C haplotype. Therefore, the possibility exists that one of these, rather than C-159T, may be the polymorphism responsible for the relationships we demonstrated. Ultimately, CD14 may therefore also need to be evaluated in a more comprehensive haplotype context.

A potential confounder specific to all longitudinal studies is the "era effect." This can be defined as an effect that occurs in populations in particular calendar years, not as a consequence of a particular year of life but rather as a consequence of temporally acting environmental factors (23). Prevalence of atopic disease was increasing in the 1980s but has continued to increase and has remained at high levels in recent years (45-47). Therefore, the effect shown in this study that disappeared with increasing age was more likely to be a result of a genetic rather than an

TABLE 5. CD14 C-159T GENOTYPE DISTRIBUTION IN SUBJECTS WITH AND WITHOUT AIRWAY HYPERRESPONSIVENESS FROM AGE 8 TO 25 YEARS

Age, yr	AHR			No AHR			χ^2 p Value
	n (%)			n (%)			
	CD14 –159CC	CD14 –159CT	CD14 –159TT	CD14 –159CC	CD14 –159CT	CD14 –159TT	
8	14 (44)	9 (28)	9 (28)	65 (24)	140 (51)	68 (25)	0.022
10	6 (30)	6 (30)	8 (40)	56 (26)	108 (51)	49 (23)	NS
12	7 (35)	5 (25)	8 (40)	54 (26)	101 (49)	52 (25)	NS
14	3 (33)	3 (33)	3 (33)	42 (24)	91 (51)	45 (25)	NS
16	4 (27)	6 (40)	5 (33)	52 (25)	100 (48)	55 (27)	NS
18	14 (36)	13 (33)	12 (31)	43 (23)	98 (52)	48 (25)	NS
25	10 (32)	12 (39)	9 (29)	64 (25)	125 (50)	63 (25)	NS

Definition of abbreviations: AHR = airway hyperresponsiveness; NS = not significant.

environmental, era-related cause. LPS level was not assessed in this study, therefore the possibility, although remote, exists that differences in CD14 genotypes over the course of the study might be related to a gene–environment interaction.

The use of longitudinal categories of disease was a novel approach to investigating the effect of genotype on asthma phenotype that allowed age relationships to be explored. The categorization of subjects into longitudinal groups was decided according to criteria that were based on available literature and expertise regarding the known influence of puberty on the course of asthma (48, 49). Cross-sectional analyses, a more established although not necessarily better or ideal method of analyzing genotype–phenotype associations, were done in this population in an attempt to validate the age cutoffs used for longitudinal analyses. Although data from an inconsistent number of subjects were analyzed using the two approaches, the value of doing both cross-sectional and longitudinal analyses was the ability to further support and interpret age-related findings. Nonetheless, age cutoffs were arbitrary and using different age and exclusion criteria may have resulted in different findings. Future analyses using definitions based on various age cutoffs may clarify this issue. Also, as with all studies, although defining longitudinal categories according to strict criteria increased the accuracy of definition and the validity of results, the number of subjects in each subgroup able to be analyzed became less. At times this resulted in small cell sizes that limited full investigation of persistent compared with remittent disease and may have restricted the ability to detect significant associations. Continuation of the use of this approach of longitudinal phenotype classification, including the use of different criteria, is necessary in more populations to explore these concepts further and expand on the findings of this work. Although data in this study were subject to several analyses, raising the possibility of type I errors, analyses were strictly prioritized and the main hypothesis tested using an *a priori* approach, therefore no adjustment for multiple comparisons was necessary. In assessing heterogeneous disorders, the delineation of homogenous subtypes increases the chances of finding significant genotype–phenotype correlations.

Atopy is known to have an important association with asthma and AHR (50–53). The CD14 C–159T genotype was shown to be associated with AHR but not with wheeze or diagnosed asthma in this population. These findings may be due to the relatively low prevalence of diagnosed asthma in this unselected cohort, with few individuals with asthma taking regular preventive medication. Low numbers of subjects with AHR made conclusions difficult; however, despite this and the inherent variability of AHR, the use of longitudinal categories of disease detected an association between AHR and CD14 genotype. This emphasizes the advantage of longitudinal over cross-sectional studies in ascertaining the causes of genetic susceptibility and supports the need for these cohorts to be investigated into older ages. AHR could have been analyzed as both a dichotomous and continuous variable. However, as the main emphasis of this study was to assess age-related changes, and in view of the complexity of variable numbers, variable responses and multiple time points in this study, dichotomous classification of AHR was used to facilitate comparison of longitudinal and cross-sectional data and to maintain power to draw conclusions. Although in some studies phenotypes are studied in combination, such as wheeze with diagnosed asthma, in this study we specifically selected individual phenotypic parameters, in particular wheeze, because of the complexity of examining longitudinal data sets.

In this study, total serum IgE was measured on only two occasions, both of them in adulthood. This enabled us to comment on associations postpuberty but not on the change from childhood to adulthood. Analyses using populations with blood

taken from the same individuals in childhood and adulthood would be helpful in expanding our findings. Analyses of CD14 genotype within SPT-positive and SPT-negative subjects were performed to be consistent with previous work (13).

Longitudinal studies investigating the outcome of childhood asthma have shown atopy to be a major risk factor for the persistence of asthma into adult life (54–56). The potential therefore exists to identify, at a young age, those individuals with the CD14 –159C allele who are “at risk” of developing atopy in childhood and target them for early intervention strategies aimed at preventing sensitization and potentially “immunizing” in favor of the postnatal switch from Th2 to Th1 responses. In this population, only a small number of subjects had early-onset atopy that remitted after childhood, thus limiting some of the statistical conclusions. Longitudinal analysis of populations with larger numbers could confirm our findings and potentially provide insight into the differences between those subjects that develop early-onset atopy and go on to develop persistent disease from those subjects whose atopy resolves. In addition to its impact on atopic disorders, the CD14 C–159T genotype has implications in disorders of other systems. CD14 plays a crucial role as a mediator of the inflammatory response during gram-negative sepsis; therefore individuals at genetically decreased risk of allergic disease may be at increased risk of mortality associated with gram-negative sepsis (57, 58). Studies also report the CD14 C–159T genotype to be associated with altered risk of myocardial infarction (59, 60), atherosclerosis (61, 62), coronary artery lesions in Kawasaki disease (63), increased risk of alcoholic liver damage and cirrhosis (64), as well as renal disease (65).

In summary, this study showed that the CD14 –159CC genotype was associated with increased odds of childhood atopy and AHR, the association being present during midchildhood but not by early adulthood. These data are consistent with CD14 C–159T being responsible for an age-related susceptibility to the development of atopy.

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References

1. Corry DB, Kheradmand F. Induction and regulation of the IgE response. *Nature* 1999;402:B18–B23.
2. Feijen M, Gerritsen J, Postma DS. Genetics of allergic disease. *Br Med Bull* 2000;56:894–907.
3. Cookson WO, De Klerk NH, Ryan GR, James AL, Musk AW. Relative risks of bronchial hyper-responsiveness associated with skin-prick test responses to common antigens in young adults. *Clin Exp Allergy* 1991; 21:473–479.
4. Prescott SL, Holt PG. Abnormalities in cord blood mononuclear cytokine production as a predictor of later atopic disease in childhood. *Clin Exp Allergy* 1998;28:1313–1316.
5. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, Holt PG. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730–4737.
6. Holt PG, Macaubas C, Cooper D, Nelson DJ, McWilliam AS. Th-1/Th-2 switch regulation in immune responses to inhaled antigens: role of dendritic cells in the aetiology of allergic respiratory disease. *Adv Exp Med Biol* 1997;417:301–306.
7. Holt PG. Development of T-cell memory against inhalant allergens: risks for the future. *Clin Exp Allergy* 1999;29:8–13.
8. Holt PG. Potential role of environmental factors in the etiology and

- pathogenesis of atopy: a working model. *Environ Health Perspect* 1999; 107:485–487.
9. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;353:196–200.
 10. De Smedt T, Pajak B, Muraille E, Lespagnard L, Heinen E, De Baetselier P, Urbain J, Leo O, Moser M. Regulation of dendritic cell numbers and maturation by lipopolysaccharide *in vivo*. *J Exp Med* 1996; 184:1413–1424.
 11. Hilkens CM, Kalinski P, de Boer M, Kapsenberg ML. Human dendritic cells require exogenous interleukin-12-inducing factors to direct the development of naive T-helper cells toward the Th1 phenotype. *Blood* 1997;90:1920–1926.
 12. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995;13:437–457.
 13. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999;20:976–983.
 14. Koppelman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, Postma DS, Bleeker ER. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2001;163:965–969.
 15. Gao PS, Mao XQ, Baldini M, Roberts MH, Adra CN, Shirakawa T, Holt PG, Martinez FD, Hopkin JM. Serum total IgE levels and CD14 on chromosome 5q31. *Clin Genet* 1999;56:164–165.
 16. Buckova D, Holla LI, Schuller M, Znojil V, Vacha J. Two CD14 promoter polymorphisms and atopic phenotypes in Czech patients with IgE-mediated allergy. *Allergy* 2003;58:1023–1026.
 17. Leung TF, Tang NL, Sung YM, Li AM, Wong GW, Chan IH, Lam CW. The C-159T polymorphism in the CD14 promoter is associated with serum total IgE concentration in atopic Chinese children. *Pediatr Allergy Immunol* 2003;14:255–260.
 18. Segala C, Priol G, Soussan D, Liard R, Neukirch F, Touron D, Lepage T. Asthma in adults: comparison of adult-onset asthma with childhood-onset asthma relapsing in adulthood. *Allergy* 2000;55:634–640.
 19. Sherrill D, Stein R, Kurzius-Spencer M, Martinez F. On early sensitization to allergens and development of respiratory symptoms. *Clin Exp Allergy* 1999;29:905–911.
 20. Sengler C, Haider A, Sommerfeld C, Lau S, Baldini M, Martinez F, Wahn U, Nickel R. Evaluation of the CD14 C-159 T polymorphism in the German multicenter allergy study cohort. *Clin Exp Allergy* 2003; 33:166–169.
 21. Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GK. The -159 C→T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J Allergy Clin Immunol* 2003;112:438–444.
 22. Holt PG, Sly PD, Bjorksten B. Atopic versus infectious diseases in childhood: a question of balance? *Pediatr Allergy Immunol* 1997;8:53–58.
 23. Le Souef P. Use of cohorts with extensive longitudinal data in investigating the molecular genetics of asthma. *Clin Exp Allergy* 1998;28(Suppl 1):46–50.
 24. Peat JK. Asthma: a longitudinal perspective. *J Asthma* 1998;35:235–241.
 25. O'Donnell AR, Laing IA, Toelle B, Marks G, Peat JK, Goldblatt J, Le Souef PN. Association study of CC16 and CD14 polymorphisms in an unselected population assessed at age 8 and 25 years [abstract]. *Am J Respir Crit Care Med* 2000;161:A928.
 26. O'Donnell AR, Marks GB, Peat JK, Goldblatt J, LeSouef PN. Association study of CC16 and CD14 polymorphisms in an unselected population assessed at age 8 and 25 [abstract]. *Respirology* 2000;5:30.
 27. O'Donnell AR, Marks GB, Peat JK, Goldblatt J, LeSouef PN. CD14 C-159T polymorphism is associated with childhood onset atopy [abstract]. *Am J Respir Crit Care Med* 2002;165:A659.
 28. Peat JK, Britton WJ, Salome CM, Woolcock AJ. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. II: relative importance of associated factors. *Clin Allergy* 1987;17:283–290.
 29. Peat JK, Britton WJ, Salome CM, Woolcock AJ. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. III: effect of exposure to environmental allergens. *Clin Allergy* 1987;17:291–300.
 30. Salome CM, Peat JK, Britton WJ, Woolcock AJ. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. I: relation to respiratory symptoms and diagnosed asthma. *Clin Allergy* 1987;17: 271–281.
 31. Peat JK, Toelle BG, Dermand J, van den Berg R, Britton WJ, Woolcock AJ. Serum IgE levels, atopy, and asthma in young adults: results from a longitudinal cohort study. *Allergy* 1996;51:804–810.
 32. Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;38:760–765.
 33. Peat JK, Woolcock AJ. Sensitivity to common allergens: relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas of Australia. *Clin Exp Allergy* 1991; 21:573–581.
 34. Gergen PJ, Turkeltaub PC, Kovar MG. The prevalence of allergic skin test reactivity to eight common aeroallergens in the US population: results from the second National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 1987;80:669–679.
 35. Barbee RA, Lebowitz MD, Thompson HC, Burrows B. Immediate skin-test reactivity in a general population sample. *Ann Intern Med* 1976; 84:129–133.
 36. Hamilton RG AN. Measurement of total serum IgE and allergen specific IgE antibody. Washington, DC: American Society for Microbiology; 1992.
 37. Dawson-Saunders B, Trapp RG. Basic and clinical biostatistics. East Norwalk, CT: Appleton and Lange; 1994.
 38. Srivastava P, Helms PJ, Stewart D, Main M, Russell G. Association of CCR5Delta32 with reduced risk of childhood but not adult asthma. *Thorax* 2003;58:222–226.
 39. LeVan TD, Bloom JW, Bailey TJ, Karp CL, Halonen M, Martinez FD, Vercelli D. A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. *J Immunol* 2001;167:5838–5844.
 40. Hata Y, Duh E, Zhang K, Robinson GS, Aiello LP. Transcription factors Sp1 and Sp3 alter vascular endothelial growth factor receptor expression through a novel recognition sequence. *J Biol Chem* 1998;273: 19294–19303.
 41. Majello B, De Luca P, Lania L. Sp3 is a bifunctional transcription regulator with modular independent activation and repression domains. *J Biol Chem* 1997;272:4021–4026.
 42. Vercelli D, Baldini M, Martinez F. The monocyte/IgE connection: may polymorphisms in the CD14 gene teach us about IgE regulation? *Int Arch Allergy Immunol* 2001;124:20–24.
 43. Vercelli D, Baldini M, Stern D, Lohman IC, Halonen M, Martinez F. CD14: a bridge between innate immunity and adaptive IgE responses. *J Endotoxin Res* 2001;7:45–48.
 44. Baldini M, Kabesh M, Graves P, Erikson R, Vercell D, Martinez F. Detection of four novel polymorphisms in the CD14 promoter and association of their haplotypes with total serum IgE levels [abstract]. *Am J Respir Crit Care Med* 2000;161:A928.
 45. Peat JK. The rising trend in allergic illness: which environmental factors are important? *Clin Exp Allergy* 1994;24:797–800.
 46. Peat JK, van den Berg RH, Green WF, Mellis CM, Leeder SR, Woolcock AJ. Changing prevalence of asthma in Australian children. *BMJ* 1994; 308:1591–1596.
 47. Robertson CF, Heycock E, Bishop J, Nolan T, Olinsky A, Phelan PD. Prevalence of asthma in Melbourne schoolchildren: changes over 26 years. *BMJ* 1991;302:1116–1118.
 48. Martin AJ, McLennan LA, Landau LI, Phelan PD. The natural history of childhood asthma to adult life. *BMJ* 1980;280:1397–1400.
 49. Kjellman B, Hesselmar B. Prognosis of asthma in children: a cohort study into adulthood. *Acta Paediatr* 1994;83:854–861.
 50. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;320:271–277.
 51. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* 1991;325:1067–1071.
 52. Clifford RD, Radford M, Howell JB, Holgate ST. Prevalence of atopy and range of bronchial response to methacholine in 7 and 11 year old schoolchildren. *Arch Dis Child* 1989;64:1126–1132.
 53. Zimmerman B, Feanny S, Reisman J, Hak H, Rashed N, McLaughlin FJ, Levison H. Allergy in asthma. I: the dose relationship of allergy to severity of childhood asthma. *J Allergy Clin Immunol* 1988;81:63–70.
 54. Strachan D, Gerritsen J. Long-term outcome of early childhood wheezing: population data. *Eur Respir J Suppl* 1996;21:42s–47s.
 55. Kokkonen J, Linna O. The state of childhood asthma in young adulthood. *Eur Respir J* 1993;6:657–661.
 56. Godden DJ, Ross S, Abdalla M, McMurray D, Douglas A, Oldman D, Friend JA, Legge JS, Douglas JG. Outcome of wheeze in childhood: symptoms and pulmonary function 25 years later. *Am J Respir Crit Care Med* 1994;149:106–112.
 57. Landmann R, Calandra T, Zimmerli W. Soluble CD14 in septic shock. *Prog Clin Biol Res* 1995;392:375–380.
 58. Gibot S, Cariou A, Drouet L, Rossignol M, Ripoll L. Association between

- a genomic polymorphism within the CD14 locus and septic shock susceptibility and mortality rate. *Crit Care Med* 2002;30:969–973.
59. Hubacek JA, Rothe G, Pit'ha J, Skodova Z, Stanek V, Poledne R, Schmitz G. C(-260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999;99:3218–3220.
60. Unkelbach K, Gardemann A, Kostrzewa M, Philipp M, Tillmanns H, Haberbosch W. A new promoter polymorphism in the gene of lipopolysaccharide receptor CD14 is associated with expired myocardial infarction in patients with low atherosclerotic risk profile. *Arterioscler Thromb Vasc Biol* 1999;19:932–938.
61. Risley P, Jerrard-Dunne P, Sitzler M, Buehler A, von Kegler S, Markus HS. Promoter polymorphism in the endotoxin receptor (CD14) is associated with increased carotid atherosclerosis only in smokers: the Carotid Atherosclerosis Progression Study (CAPS). *Stroke* 2003;34:600–604.
62. Eilertsen KE, Olsen JO, Brox J, Østerud B. Association of the -159 C → T polymorphism in the CD14 promoter with variations in serum lipoproteins in healthy subjects. *Blood Coagul Fibrinolysis* 2003;14:663–670.
63. Nishimura S, Zaitzu M, Hara M, Yokota G, Watanabe M, Ueda Y, Imayoshi M, Ishii E, Tasaki H, Hamasaki Y. A polymorphism in the promoter of the CD14 gene (CD14/-159) is associated with the development of coronary artery lesions in patients with Kawasaki disease. *J Pediatr* 2003;143:357–362.
64. Jarvelainen HA, Orpana A, Perola M, Savolainen VT, Karhunen PJ, Lindros KO. Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. *Hepatology* 2001;33:1148–1153.
65. Yoon HJ, Shin JH, Yang SH, Chae DW, Kim H, Lee DS, Kim HL, Kim S, Lee JS, Kim YS. Association of the CD14 gene -159C polymorphism with progression of IgA nephropathy. *J Med Genet* 2003;40:104–108.