ASTHMA

The CD14 C-159T polymorphism is not associated with asthma or asthma severity in an Australian adult population

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Background: CD14 functions as a multifunctional receptor for bacterial cell wall components including endotoxin and lipopolysaccharide and is likely to play a role in the polarisation of T lymphocytes into Th1 and Th2 subsets, thereby influencing the cytokine profile and subsequent IgE production in response to antigen/allergen contact in allergic phenotypes. A functional C-159T polymorphism has been described in the promoter region of the gene and has been associated with increased gene expression, atopy, and non-atopic asthma in different ethnic populations. A study was undertaken to examine the association between the C-159T polymorphism and asthma, asthma severity, and atopy in a large Australian white population.
Methods: PCR-RFLP analysis was used to characterise the C-159T polymorphism in mild (n = 264), moderate (n = 225) and severe (n = 79) asthmatic patients and non-asthmatic controls (n = 443), including atopic (n = 688) and non-atopic (n = 323) individuals. Association analyses were performed using χ^2 tests.
Results: There was no association between the polymorphism and asthma (p = 0.468) or asthma severity (p = 0.727), and only a very weak association with atopy (p = 0.084). A meta-analysis of all studies conducted to date revealed similar genotypic frequencies in white ethnic populations and confirmed that there was no overall association with atopy (p = 0.52) or asthma (p = 0.23), although there was significant between study heterogeneity (p = 0.01).
Conclusions: This study confirms that there is no association between the CD14 C-159T polymorphism and asthma or asthma severity and a weak association between this polymorphism and atopy in an adult population.

CD14 is a multifunctional receptor which is constitutively expressed on the surface of monocytes, macrophages, and neutrophils and as a soluble form in serum.[1] It is the principal receptor for lipopolysaccharide (LPS) or inhaled endotoxin, a potent inducer of lung inflammation which may activate innate immune pathways that promote Th1 differentiation and/or suppress Th2 dependent immunoglobulin E (IgE) responses. CD14 binding of LPS is associated with a strong IL-12 response by antigen presenting cells,[2 3] and IL-12 is regarded as an obligatory signal for the maturation of naïve T cells into Th1 cells.[3] IgE responses are regulated by inhibitory signals derived from Th1 type cells and by stimulatory signals from Th2 type cells.[4] It is proposed that altered CD14 expression may affect the proportion of Th1 to Th2 cells, thereby influencing IgE responses[5] and the associated inflammatory phenotype in allergic conditions such as asthma.

It has been suggested that asthmatic subjects are more sensitive to the effects of LPS than non-asthmatic subjects,[6] and subjects with allergic asthma have been reported to have increased expression of CD14 after acute allergen challenge[7] and LPS inhalation.[8] Thus, alterations in CD14 expression appear to be important, particularly in allergic asthma, and it is likely that its expression is regulated, at least partially, at the gene level. A functional single nucleotide polymorphism (C-159T) has been described in the promoter region of the *CD14* gene[5] and has been associated with altered CD14 and IgE levels in various ethnic populations.[5 9–12]

Previous studies have investigated the promoter polymorphism in populations of varying sizes and ethnicity, with limited definitions of asthma severity. We sought to investigate the association between the C-159T polymorphism and atopy and to determine if there was an association between this polymorphism and asthma and/or asthma severity in a large, carefully phenotyped adult Australian white ethnic population of non-asthmatic controls and patients with mild, moderate, and severe asthma.

METHODS

Subjects

Four hundred and forty three control individuals with no evidence of asthma, 264 individuals with mild asthma, 225 with moderate asthma, and 79 with severe asthma participated in this association study. The population, methods of recruitment, and disease severity categorisation have been described previously.[13] All subjects were unrelated and completed a detailed questionnaire and spirometric tests, and blood samples were obtained for DNA extraction. All individuals were tested for atopic status by skin prick testing, with a positive reaction resulting in a wheal of more than 3 mm diameter to at least one of the five aeroallergens tested (including cat, dog, house dust mite, mould mix and grass pollen mix).

The study protocol was approved by the Sir Charles Gairdner Hospital human research ethics committee and all subjects provided informed written consent to participate in this study.

Molecular methods

DNA was extracted from buffy coats using a commercially available DNA extraction kit (Qiagen, Hilden, Germany), following the manufacturers' instructions. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis was optimised following the protocol described by Baldini *et al*.[5] At final volume the PCR reaction contained 1× PCR buffer (Qiagen), 1× Q solution (Qiagen), 2 mM $MgCl_2$, 200 μM each of dATP, dTTP, dCTP and dGTP (Promega, Madison, USA), 20 pmol of each primer (5′-GTGCCAACAGATGAGGTTCAC-3′ and 5′-GCCTCTGACA GTTTTATGTAATC-3′), and 1 U of *Taq* DNA polymerase

(Qiagen). PCR amplification conditions were optimised with an initial denaturation step at 94℃ for 5 minutes, 35 cycles of 94℃ for 30 seconds, 64℃ for 30 seconds, 72℃ for 1 minute, and a final extension step at 72℃ for 10 minutes in a thermal cycler (Eppendorf, Hamburg, Germany).

The restriction endonuclease *Ava*II recognises the restriction sequence GGTCC, present only in amplified DNA fragments containing the T allele. The restriction mix contained 1× restriction buffer C (Promega, Madison, WI, USA) and 1 U *Ava*II (Promega). Restricted products were electrophoresed on 1% agarose gels (Amresco, OH, USA). Expected fragment sizes were 497 bp for the C allele and 353+144 bp for the T allele.

Statistical methods

Genotype and allele frequencies were calculated for each phenotypic group. Comparisons of allele and genotype distribution were performed with χ^2 tests using the R Statistics Program.[14] The influence of the CD14 promoter polymorphism on specific asthma phenotypes was also examined using multivariate logistic regression analysis, and Hardy-Weinberg equilibrium analysis for each group was evaluated by the exact test implemented in the R Statistics Program. We performed a meta-analysis using the tabulated results from several populations: American white (atopic n = 161, non-atopic n = 122;[5] atopic asthma n = 99, non-atopic asthma n = 43, controls n = 49[15]); Polish (atopic asthma n = 50, controls n = 73[16]); Japanese (atopic asthma n = 100, non-atopic asthma n = 100, controls n = 100[17]); German (asthma n = 84, atopic n = 78, non-atopic n = 77, controls n = 119;[18] asthma n = 182, non-asthma n = 261[19]); British (atopic asthma n = 125, controls n = 150[17]); Dutch (atopic n = 172, non-atopic n = 143[12]); Chinese (asthma n = 258, non-asthma n = 92, atopic n = 261, non-atopic n = 89[20]); Czechoslovakian (atopic n = 37, non-atopic n = 101[11]); Icelandic (atopic asthma n = 94, controls n = 94[21]); and Australian (asthma n = 463[22]). For some studies, where genotypic frequencies were not available, we estimated genotypic counts based on the allele frequencies assuming Hardy-Weinberg equilibrium. The meta-analysis was performed using both fixed and random effects binomial regression, the latter using the approach of Whitehead and Whitehead.[23]

RESULTS

The CD14 C-159T promoter polymorphism was characterised in a large carefully phenotyped population of 1011 unrelated Australian white ethnic subjects described previously:[13] all subgroups had similar age and sex distributions and the asthma group as a whole had a significantly higher percentage of atopic subjects (83%) than the non-asthmatic control group (49%; $p<0.0001$).

Table 1 summarises the genotype analysis for all phenotypic groups. All groups studied were in Hardy-Weinberg equilibrium ($p\geq0.402$). There was no association between the C-159T polymorphism and asthma ($p = 0.468$) or disease severity ($p = 0.727$) in this population, although the T allele was slightly more common in patients with asthma, particularly those with mild and severe asthma. This effect was not influenced by age or sex. There was a very weak association between the C-159T polymorphism and atopy ($p = 0.084$), with the T allele slightly less common in non-atopic individuals.

We performed a meta-analysis using the tabulated results from several populations and, in a first analysis comparing healthy controls, all populations were shown to have similar genotypic frequencies with the exception of a Japanese population.[9] Both random effects and fixed effects logistic regression models concluded no overall association between asthma and CD14 genotype ($p = 0.23$, pooled allelic odds ratio 1.16, 95% CI 0.98 to 1.39). For atopy, again there was no overall association with the CD14 genotype ($p = 0.52$, pooled allelic odds ratio 0.83, 95% CI 0.58 to 1.20) but there was significant between-study heterogeneity ($p = 0.01$).

DISCUSSION

A functional C-159T polymorphism has been described in the promoter region of the CD14 gene and has been associated with increased CD14 expression in vitro[24] and in the serum of children,[5 20] and with altered serum IgE levels and skin test positivity in different populations.[5 9–12 20 25 26] We have confirmed a weak association between the C-159T polymorphism and atopy but found no association between this polymorphism and asthma or asthma severity in a large well phenotyped Australian white adult population.

The CD14 gene has been localised to chromosome 5q31, in a region shown to be linked to Th2 prevalent phenotypes including high total serum IgE levels and asthma, in a number of different populations.[27–35] Several polymorphisms have been described and investigated in the CD14 gene[5 11 19 26 36] and, of these, the C-159T promoter polymorphism has been the most extensively studied in atopic disease. In vitro studies using transient transfection assays in CD14 expressing monocytic cells showed that the C-159T polymorphism increases transcription by lowering the affinity of the CD14 regulatory region for Sp3,[24] a factor known to inhibit the activity of a number of promoters.[37–41] In clinical studies the C-159T TT genotype has been associated with higher circulating sCD14, a lower mean number of positive skin tests, and lower serum IgE levels in British and

Table 1 CD14 C-159T genotype and allele frequencies in an Australian adult population

Phenotype	No	Genotypes CC	CT	TT	Frequency of T allele
Asthma					
Non-asthma	443	0.28	0.51	0.21	0.460
All asthma	568	0.26	0.50	0.24	0.487
Mild asthma	264	0.25	0.50	0.25	0.496
Moderate asthma	225	0.27	0.52	0.21	0.469
Severe asthma	79	0.27	0.46	0.27	0.506
Atopy					
All atopic	689	0.25	0.52	0.23	0.490
All non-atopic	322	0.32	0.48	0.20	0.443
Atopic asthma	472	0.25	0.50	0.25	0.490
Non-atopic asthma	96	0.28	0.53	0.19	0.450
Atopic non-asthma	217	0.24	0.56	0.20	0.480
Non-atopic non-asthma	226	0.33	0.46	0.21	0.440

American white populations,[5 9 26] and has also been found to be associated with non-atopic asthma and food allergy in various ethnic groups.[15] Conversely, the CC genotype has been associated with higher levels of total serum IgE and a higher number of positive skin tests in a population from the Netherlands[12 20 26] and the C allele has been associated with atopy, specifically to moulds, in a Czechoslovakian population.[11]

However, the associations between the C-159T polymorphism and atopic phenotypes have not always been consistent and, in contrast to these other findings, the CD14-159T allele was over-transmitted to atopic individuals in an inbred Hutterite population. Similarly, we have shown that the -159T allele was slightly more common in atopic adults. There was no association of this polymorphism with atopy in an Hispanic population,[5] with atopic asthma in an Icelandic population,[21] or with atopy and/or asthma in two German white populations.[18 19]

Although some populations have been relatively small, the allele frequencies of the C-159T polymorphism have not varied significantly between all populations investigated to date, with the exception of a Japanese population.[9] The -159T allele frequencies ranged between 0.60 in a Japanese population,[9] 0.53 in a Chinese population,[20] and 0.37 and 0.54 in all white populations: 0.37–0.41 in American;[5 15] 0.38 in Polish;[16] 0.42–0.46 in German;[18 19] 0.45 in Icelandic;[21] 0.47 in Australian; 0.52 in British;[9] 0.53 in Dutch;[12] 0.54 in Czechoslovakian.[11] A meta-analysis of all populations studied to date revealed that there was no overall association between the C-159T polymorphism and asthma ($p = 0.23$) or atopy ($p = 0.52$); however, it did reveal significant between-study heterogeneity ($p = 0.01$).

It is difficult to explain the inconsistency of association studies for the C-159T polymorphism, even between populations of similar ethnicity. Environmental factors may differ between the study populations[18] but, as has been highlighted by a number of authors, association studies are commonly difficult to replicate between different populations, especially when different phenotypic markers are analysed.[18 19]

There is tight linkage disequilibrium across the CD14 promoter region[42] which would strongly favour linkage between C-159T and polymorphisms in CD14 or another gene on 5q, and may account for some of the associations seen to date. In support of this, the CD14-159T allele has been shown to be over-transmitted to atopic individuals in an inbred Hutterite population, only when on a haplotype with marker D5S642 previously shown to be linked to atopy in this population.[43] It is therefore highly likely that the CD14-159T allele is actually in linkage disequilibrium with the susceptibility variant on 5q.[10]

It is also possible that the C-159T polymorphism has an age related influence on the development of atopy. The polymorphism has been associated with increased CD14 expression only in the serum of children,[5 20] and there was no difference in serum CD14 levels or the expression of membrane bound levels of CD14 in a sample of adult blood donors with different CD14 genotypes.[44] Additionally, a longitudinal study in Australian white subjects aged 8–25 years found that individuals with the CC genotype were more likely to have early onset atopy and early onset airway hyperresponsiveness, suggesting that the influence of -159C on the atopic genotype may be age specific.[25] There is strong evidence to suggest that atopy and asthma are closely related entities, with a positive association between total serum IgE and number of positive skin tests, bronchial hyperresponsiveness, and development of asthma and doctor diagnosed asthma.[45 46] Thus, alleles which are associated with atopy might be expected to be over-represented in individuals with asthma relative to the general population.[12] However, to date, no association has been found between the C-159T polymorphism and atopic asthma. We have confirmed this in our population and in a meta-analysis of all populations studied, suggesting that separate genes on chromosome 5q may regulate susceptibility to high total serum IgE levels and bronchial responsiveness.[5]

Similarly, there are no publications showing an association between the CD14 C-159T polymorphism and asthma severity, although it has been suggested that it might modify the severity of airflow obstruction in asthmatics. A recent conference abstract reported a study conducted on 418 Australian adult asthmatics[22] in which neither the CT nor TT genotypes were associated with life threatening asthma, although the TT genotype was found to be weakly associated with lower forced expiratory volume in 1 second (FEV_1). However, there was no association between genotype and asthma severity (also defined by mean FEV_1) in a smaller predominantly white American population.[15] We have used a more extensive set of criteria to define asthma severity[13] and have confirmed that there is no relationship between asthma severity and the C-159T polymorphism in our population.

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