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

M-A Kedda, F Lose, D Duffy, E Bell, P J Thompson, J Upham

CD14 is a multifunctional receptor which is constitutively expressed on the surface of monocytes, macrophages, and neutrophils and as a soluble form in serum. 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, and IL-12 is regarded as an obligatory signal for the maturation of naïve T cells into Th1 cells. IgE responses are regulated by inhibitory signals derived from Th1 type cells and by stimulatory signals from Th2 type cells. It is proposed that altered CD14 expression may affect the proportion of Th1 to Th2 cells, thereby influencing IgE responses 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, and subjects with allergic asthma have been reported to have increased expression of CD14 after acute allergen challenge and LPS inhalation. 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 and has been associated with altered CD14 and IgE levels in various ethnic populations.

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. 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. At final volume the PCR reaction contained 1 x PCR buffer (Qiagen), 1 x Q solution (Qiagen), 2 mM MgCl2, 200 μM each of dATP, dTTP, dCTP and dGTP (Promega, Madison, USA), 20 pmol of each primer (5'-GTGCCAACAGATGAGGTTCAC-3' and 5'-GCCTCTGACAA GTTTATGTAATC-3'), and 1 U of Taq DNA polymerase.

See end of article for authors' affiliations.
British (atopic asthma n = 125, controls n = 150); Dutch
94˚C for 30 seconds, 64 ˚C for 30 seconds, 72 ˚C for 1 minute,
and a final extension step at 72˚C for 10 minutes in a thermal
cycler (Eppendorf, Hamburg, Germany).

The restriction endonuclease AvaII recognise the restriction
sequence GG/TCC, present only in amplified DNA
fragments containing the T allele. The restriction mix
contained 1 x restriction buffer C (Promega, Madison, WI,
USA) and 1 U AvaII (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 χ² tests using the R
Statistics Program.13 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
multiple studies comparing healthy controls and asthma
phenotypes.27–35 Several polymorphisms
were characterised in different populations.5 9–12 20 25 26 We have
confirmed a weak association between the C-159T 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 27 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.24–26 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>
<thead>
<tr>
<th>Phenotype</th>
<th>No</th>
<th>Genotypes</th>
<th>Frequency of T allele</th>
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<tr>
<td>Asthma</td>
<td></td>
<td>CC CT TT</td>
<td></td>
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<tr>
<td>Non-asthma</td>
<td>443</td>
<td>0.28 0.51 0.21</td>
<td>0.460</td>
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<tr>
<td>All asthma</td>
<td>568</td>
<td>0.26 0.50 0.24</td>
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<td>Mild asthma</td>
<td>264</td>
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<td>Moderate asthma</td>
<td>225</td>
<td>0.27 0.52 0.21</td>
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<td>Severe asthma</td>
<td>79</td>
<td>0.27 0.46 0.27</td>
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<td>Atopy</td>
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<td>All non-atopic</td>
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<td>Non-atopic asthma</td>
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<td>Non-atopic non-asthma</td>
<td>226</td>
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</table>

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 vitro24 and in the serum of
children,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 asthma or asthma severity in a large well
phenotyped Australian white adult population.

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a region shown to be linked to Th2 prevalent phenotypes
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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
American white populations, and has also been found to be associated with non-atopic asthma and food allergy in various ethnic groups. 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 and the C allele has been associated with atopy, specifically to moulds, in a Czechoslovakian population.

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, with atopic asthma in an Icelandic population, or with atopy and/or asthma in two German white populations.

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. The -159T allele frequencies ranged between 0.60 in a Japanese population, 0.53 in a Chinese population, and 0.37 and 0.54 in all white populations: 0.37–0.41 in American; 0.38 in Polish; 0.42–0.46 in German; 0.45 in Icelandic; 0.47 in Australian; 0.52 in British; 0.53 in Dutch; 0.54 in Czechoslovakian. 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 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.

There is tight linkage disequilibrium across the CD14 promoter region 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. It is therefore highly likely that the CD14-159T allele is actually in linkage disequilibrium with the susceptibility variant on 5q.

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, 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. 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. 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. Thus, alleles which are associated with atopy might be expected to be over-represented in individuals with asthma relative to the general population. 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.

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 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 (FEV1). However, there was no association between genotype and asthma severity (also defined by mean FEV1) in a smaller predominantly white American population. We have used a more extensive set of criteria to define asthma severity and have confirmed that there is no relationship between asthma severity and the C-159T polymorphism in our population.

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