Immunogenetics (2004) 56: 544–547
DOI 10.1007/s00251-004-0721-y

BRIEF COMMUNICATION

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Suggestive evidence of association of C-159T functional polymorphism of the *CD14* gene with atopic asthma in northern and northwestern Indian populations

Received: 17 June 2004 / Revised: 23 August 2004 / Published online: 18 September 2004

Abstract CD14 is a lipopolysaccharide receptor known to be an important modulator of Th1–Th2 response during early childhood. Genetic association studies of the *CD14* gene with asthma and atopic disorders have shown positive as well as negative results in different ethnic populations. The aim of this study was to test for association of C-159T functional promoter polymorphism with atopic asthma and serum IgE levels in northern and northwestern Indian populations. DNA was assayed for the *CD14* C-159T polymorphism in a case-control study involving atopic asthmatics (n=187) and healthy normal controls (n=227), and in a family-based association study of 106 trios. The case-control study showed an association at the genotypic (P=0.0146) as well as the allelic level (P=0.0048). Moreover, we observed a deviation of allelic transmission from random proportions (P=0.024) in the transmission disequilibrium test analysis. When we analyzed our results for serum total IgE levels, against this polymorphism, we observed a difference at the genotypic (P=0.0026) as well as at the allelic level (P=0.0016) in a case-control study, whereas no association in the quantitative transmission disequilibrium test analysis was obtained. These findings provide suggestive evidence of association of the *CD14* gene locus with atopic asthma in northern and northwestern Indian populations.

Keywords 5q31-33 · Asthma · *CD14* · Indian population · Quantitative transmission disequilibrium test

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Atopic asthma is a complex disease characterized by variable airway obstruction, bronchial hyperresponsiveness, elevated Th2-type cytokines, and high IgE levels (Cookson 1999). Both environmental and genetic factors play roles in the etiology of this disease (Holgate 1999; Cookson 1999). Various studies have revealed that exposure to bacterial infections or endotoxins during early childhood plays an important role in polarization of the immune response towards Th1 response, thus modulating the Th1–Th2 balance of the immune system (Strachan 1989; Holt et al. 1997). Bacterial endotoxin/lipopolysaccharide (LPS) binds to a glycoprotein receptor, i.e., CD14, which promotes Th1 responses. CD14 is expressed in myeloid cells, primarily in monocytes and in serum as soluble CD14 (sCD14). Binding of LPS to sCD14 causes activation of signaling via Toll-like receptor 4 (TLR4) in antigen-presenting cells (APC), resulting in secretion of IL-12 from these cells (Baldini et al. 2002). Reduced levels of CD14 were seen in the serum of atopic children from Sweden (Zdolsek et al. 2004).

The chromosomal location 5q31 has been shown to harbor many promising candidate genes for asthma/atopy (Marsh et al. 1994). *CD14* is present along with the Th2 cytokine gene cluster, viz. IL-3, -4, -5, -9, -13 and other susceptibility genes like *UGRP1*, β-2-adrenergic receptor gene (*ADRB2*), etc., in this region. A polymorphism at the −159 position in the *CD14* promoter has been identified and found to affect the binding affinity of Sp1, -2, and -3 transcription factors at the GC box in this region (LeVan et al. 2001) in vitro, thus changing the expression of CD14. Various studies correlating this polymorphism with the

Table 1 Allele and genotype frequencies and mean log-serum total IgE for C-159T polymorphism studied in the *CD14* gene. Numbers in *parentheses* indicate the frequency (percentage). *n* Number of individuals in each group

Allele and genotype	Asthma phenotype		log_{10}-total serum IgE, IU/ml (*n*=351)
	Patients *n*=187	Controls *n*=227	
C	178 (47.59)	172 (37.89)	2.71±0.54 (*n*=279)
T	196 (52.41)	282 (62.11)	2.57±0.63 (*n*=423)
CC	43 (22.99)	30 (13.22)	2.87±0.39 (*n*=50)
CT	92 (49.2)	112 (49.34)	2.62±0.59 (*n*=179)
TT	52 (27.81)	85 (37.44)	2.52±0.65 (*n*=122)

sCD14 level (Baldini et al. 1999; Kabesch et al. 2004) support its role in regulating CD14 levels. However, Heesen et al. (2001) found no such correlation in German populations.

Studies in different ethnic populations have shown the association of C-159T polymorphism with the skin-prick test (Ober et al. 2000; Koppelman et al. 2001; Buckov et al. 2003) and serum IgE levels (Gao et al. 1999; Baldini et al. 1999). However, other studies failed to replicate the association with atopic diseases (Cardaba et al. 2001; Sengler et al. 2003; Kabesch et al. 2004). In addition, no association was observed between *CD14* promoter polymorphisms and atopic asthma (Koppelman et al. 2001) or bronchial asthma (Heinzmann et al. 2003). Therefore, we have undertaken a case-control and a family-based study to investigate the association of C-159T polymorphism with atopic asthma and total serum IgE levels in northern and northwestern Indian populations.

For the case-control study, a total of 187 unrelated atopic asthmatic subjects (29.23±14.6 years) and 227 normal controls (27.2±5.6 years) were recruited from northern and northwestern India after clinical assessment based on the American Thoracic Society guidelines, as described earlier (Nagarkatti et al. 2002). Both populations are of Indo–Aryan origin. Individuals who had a prior history of smoking and/or parasitic infestation and the control samples with a history of asthma/allergies were excluded from the study. Similarly, for the family-based study, 106 families [proband mean age = 19.7±12.3 years, sex ratio (male:female) = 65.31:34.69] with a history of asthma/atopy comprising 294 individuals were recruited.

The presence of C-159T polymorphism was confirmed by sequencing the genomic DNA of 40 individuals (20 patients, 20 unrelated controls). PCR was done to amplify the −511 to −58 promoter region of the gene (Baldini et al. 1999). The sequencing results showed the presence of the previously reported C-159T functional polymorphism in our population. Additionally, no other polymorphisms were detected in the amplified region. Genotyping was done by PCR-restriction fragment length polymorphism (RFLP), using the *Ava*II restriction enzyme. The allele frequencies and genotype distributions were compared between the patients and controls using a chi square test of 2×2 tables. Hardy–Weinberg equilibrium for parents (family-based study) and for cases as well as for controls (case-control study) was calculated using the De Finetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The genotype and allele frequencies of the C-159T polymorphism in 187 unrelated atopic asthmatic patients and 227 ethnically matched controls are shown in Table 1. None of the observed genotype counts deviated significantly from those expected according to the Hardy–Weinberg equilibrium (P>0.40). The frequency of the −159 *C* allele was found to be higher in atopic asthmatic patients in comparison to the control subjects (0.476 vs 0.379, χ^2=7.91, *df*=1, P=0.0048). Individuals carrying the −159 *C* allele exhibited an increased risk of developing atopic asthma (OR=1.49, 95% CI=1.13−1.97). Similarly, a difference was seen at the genotype level when compared with the control group (χ^2=8.45, *df*=2, P=0.0146). The *CC* genotype was over-represented in the patient group (OR=1.96, 95% CI=1.17−3.28), whereas the *TT* genotype was found to be more frequent in controls (OR=1.55, 95% CI=1.02–2.36). According to the guidelines suggested by Colhoun and McKeigue (2003) for case-control studies, where a *P*-value in the range of 0.0005–0.00005 should be taken as the threshold for significance, our results indicate a suggestive evidence of association. Earlier case-control studies that showed positive associations of the *CD14* gene with serum IgE or SPT or CD14 levels had reported a *P*-value of more than 0.0005. Therefore, following the same criteria of Colhoun and McKeigue (2003), they should also be considered as suggestive evidence of association. In addition, the differences in the results between earlier studies (Koppelman et al. 2001; Heinzmann et al. 2003) and ours could be attributed to the differences in ethnicity or difference in the study design. It was also possible that we might have obtained a false-positive result due to population stratification. However, this possibility was ruled out by observing a similar genotypic distribution (P>0.05) for 39 microsatellite repeat markers, yet unlinked to asthma or atopy phenotype, among our case and control groups (data not shown).

Further, to avoid the confounding effect of population admixture in case-control studies, the transmission disequilibrium test (TDT) was applied to analyze allelic association for *CD14* C-159T SNP with atopic asthma/atopy, using the TDT/STDT program, version 1.1 (http://genomics.med.upenn.edu/spielman/TDT.htm). We took 106 trios consisting of father, mother, and affected offspring, either atopic or atopic asthmatic, for our analysis. The TDT showed a preferential transmission of the −159 *C* allele to affected children, 54 transmitted versus 33 non-transmitted (χ^2 TDT=5.069, *df*=1, P<0.025, Table 2). Our results with the TDT analysis indicate a suggestive evidence of association with the disease. An earlier TDT study by Ober et al. (2000) also showed a suggestive linkage with the SPT phenotype in Hutterites,

Table 2 The transmission disequilibrium test (TDT). χ^2 TDT=5.069, *df*=1, *P*<0.025

Allele	Transmitted	Non-transmitted	Total
C	54	33	87
T	33	54	87
Total	87	87	174

following the guidelines of Lander and Kruglyak (1995). As C-159T polymorphism has been shown to affect Sp3 transcription-factor binding, it is possible that this polymorphism plays a direct role in regulating CD14 levels and thus modulating Th1–Th2 balance. Nevertheless, in a genetic association study, additional possibilities emerge as suggested by Weiss (2001). For example, the C-159T polymorphism might be in linkage disequilibrium with another allele at another nearby locus that could be the true causal allele. Therefore, it would be interesting to look for other polymorphisms in the *CD14* gene locus itself or in the flanking regions of the 5q31-33 chromosomal locus. Vercelli et al. (2001) studied five polymorphisms in the *CD14* promoter region and found only a few haplotypes because of high linkage disequilibrium. Notably, only haplotypes carrying the −159 *C* allele were found to be associated with high CD14 levels.

Next, we tested for association between genotypes or alleles and total IgE values, using one-way ANOVA for all individuals irrespective of their disease status (*n*=351). Total serum IgE levels were found to follow a log normal distribution in our study population. When the genetic effects of C-159T polymorphism were tested on log-transformed total serum IgE ($\log_{10}$ tsIgE) levels, a trend was observed at the allele level (*P*=0.0016, Fig. 1a; Table 1), as well as at the genotypic level (*F*=6.053, *df*=2, *P*=0.0026, Fig. 1b). The mean $\log_{10}$ tsIgE levels in individuals with *CC* genotype (2.87±0.39 IU/ml) were highest, followed by individuals with *CT* (2.62±0.59 IU/ml) and *TT* genotypes (2.52±0.65 IU/ml). The mechanism by which IgE levels are regulated is not clearly understood. Jabara and Vercelli (1994) suggested direct action of CD14 on monocytes, thereby inhibiting production of IgE from B lymphocytes. The *T* allele has been reported to reduce the binding affinity of Sp3 inhibitory transcription factor to the GC box in mono mac 6 cells and thus results in more *CD14* transcription (LeVan et al. 2001). The high levels of CD14 in serum can lead to modulation of immune response more towards Th1-type response and, hence, low IgE. Thus, the individuals with the *CC* genotype had the highest, and those with the *TT* genotype had the lowest, IgE levels. Further, quantitative transmission disequilibrium tests (QTDT) were used to simultaneously test for linkage and association between the *CD14* C-159T polymorphism and $\log_{10}$ tsIgE levels, using a QTDT program that can accommodate nuclear families of any size, with or without parental genotype data (Abecasis et al. 2000). The orthogonal model of Abecasis et al. (2000) was adopted in the QTDT analysis, where the genotype score is decomposed into orthogonal between-family (β_b) and within-family (β_w) components. Population stratification is tested according to whether $\beta_b=\beta_w$ as proposed by Fulker et al. (1999). Population stratification was not observed for the $\log_{10}$ tsIgE. Since false-positive results might be generated in multiple tests, to assess the reliability of the results, permutations (1,000 simulations) were performed. We have found no significant association between $\log_{10}$ tsIgE and C-159T CD14 polymorphism (overall empirical significance level = 0.6070). This points to the possibility that either the association observed in our case-control study could be a false positive, or our results with family-based analysis could be false negative due to the small number of trios in the family-based study. Because of missing IgE data or homozygous parental genotype, only 53 families were taken for QTDT analysis. It is a well-documented fact that exposure to endotoxins during early childhood plays an important role in modulating the Th1–Th2 balance (Strachan 1989). According to the hygiene hypothesis (Strachan 1989), the children who live in a cleaner environment have more chances of developing Th2 disorders, because they are not exposed to bacterial endotoxins during early childhood. CD14, being the receptor for LPS, becomes a prime candidate for genetic predisposition, and it becomes all the more important to study the effect of genetic variation of this gene in Th2 diseases in developing countries like India, where exposure to endotoxins in early childhood is very high. Moreover, the *CD14* gene is present in the major susceptibility region (5q31-33) for atopy and asthma (Marsh et al. 1994); earlier studies from our group on the *IL4* gene present in this region found evidence of an

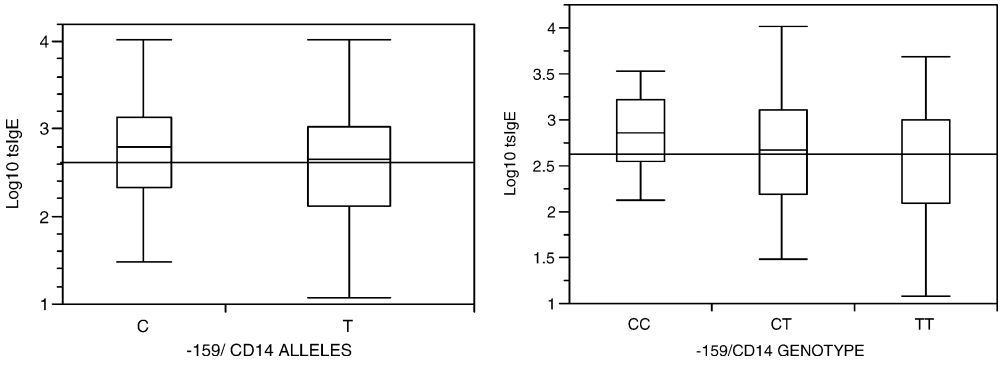

Fig. 1 **a** Boxplot of log transformed total serum IgE (*log$_{10}$ tsIgE*) levels in IU/ml by C-159T alleles (*P*=0.0016). **b** C-159T genotypes (*P*=0.0026). The boxplot illustrates medians and interquartile ranges

association with atopic asthma (Nagarkatti et al. 2004). Our results further emphasize the role of the 5q31 region in genetic predisposition to atopy/atopic asthma.

In conclusion, we found suggestive evidence of an association of the *CD14* C-159T polymorphism with atopic asthma and serum IgE in the Indian population that may intensify further research in the 5q31-33 chromosomal region.

Acknowledgements The authors would like to acknowledge CSIR for financial support. The contribution of clinicians Dr. Aditi Sinha, Dr. Virendra Kumar, Dr. S.K. Sharma, Dr. V.K. Vijayan, and Dr. P.V. Niphadkar is also acknowledged.

References

Abecasis GR, Cardon LR, Cookson WO (2000) A general test of association for quantitative traits in nuclear families. Am J Hum Genet 66:279–292

Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD (1999) 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 20:976–978

Baldini M, Vercelli D, Martinez FD (2002) CD14: an example of gene by environment interaction in allergic disease. Allergy 57:188–192

Buckova D, Holla LI, Schuller M, Znojil V, Vacha J (2003) Two *CD14* promoter polymorphisms and atopic phenotypes in Czech patients with IgE-mediated allergy. Allergy 58:1023–1026

Cardaba B, Moffatt MF, Fernandez E, Jurado A, Rojo M, Garcia M, Ansotegui IJ, Cortegano I, Arrieta I, Etxenagusia MA, del Pozo V, Urraca J, Aceituno E, Gallardo S, Palomino P, Cookson W, Lahoz C (2001) Allergy to dermatophagoides in a group of Spanish gypsies: genetic restrictions. Int Arch Allergy Immunol 125:297–306

Colhoun HM, McKeigue PM, Davey Smith G (2003) Problems of reporting genetic associations with complex outcomes. Lancet 361:865–872

Cookson W (1999) The alliance of genes and environment in asthma and allergy. Nature 402:B5–B11

Fulker DW, Cherny SS, Sham PC, Hewitt JK (1999) Combined linkage and association sib-pair analysis for quantitative traits. Am J Hum Genet 64:259–267

Gao PS, Mao XQ, Baldini M, Roberts MH, Adra CN, Shirakawa T, Holt PG, Martinez FD, Hopkin JM (1999) Serum total IgE levels and *CD14* on chromosome 5q31. Clin Genet 56:164–165

Heesen M, Blomeke B, Schluter B, Heussen N, Rossaint R, Kunz D (2001) Lack of association between the −260 C→T promoter polymorphism of the endotoxin receptor *CD14* gene and the CD14 density of unstimulated human monocytes and soluble CD14 plasma levels. Intensive Care Med 27:1770–1775

Heinzmann A, Dietrich H, Jerkic S P, Kurz T, Deichmann KA (2003) Promoter polymorphisms of the *CD14* gene are not associated with bronchial asthma in Caucasian children. Eur J Immunogenet 30:345–348

Holgate ST (1999) Genetic and environmental interaction in allergy and asthma. J Allergy Clin Immunol 104:1139–1146

Holt PG, Sly PD, Bjorksten B (1997) Atopic versus infectious diseases in childhood: a question of balance? Pediatr Allergy Immunol 8:53–58

Jabara HH, Vercelli D (1994) Engagement of CD14 on monocytes inhibits the synthesis of human Igs, including IgE. J Immunol 153:972–978

Kabesch M, Hasemann K, Schickinger V, Tzotcheva I, Bohnert A, Carr D, Baldini M, Hackstein H, Leupold W, Weiland SK, Martinez FD, Mutius E, Bein G (2004) A promoter polymorphism in the *CD14* gene is associated with elevated levels of soluble CD14 but not with IgE or atopic diseases. Allergy 59:520–525

Koppelman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, Postma DS, Bleecker ER (2001) Association of a promoter polymorphism of the *CD14* gene and atopy. Am J Respir Crit Care Med 163:965–969

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

LeVan TD, Bloom JW, Bailey TJ, Karp CL, Halonen M, Martinez FD, Vercelli D (2001) A common single nucleotide polymorphism in the *CD14* promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. J Immunol 167:5838–5844

Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, and Beaty TH (1994) Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 264:1152–1156

Nagarkatti R, Rao CB, Rishi JP, Chetiwal R, Shandilya V, Vijayan V, Kumar R, Pemde HK, Sharma SK, Sharma S, Singh AB, Gangal SV, and Ghosh B (2002) Association of IFNG gene polymorphism with asthma in the Indian population. J Allergy Clin Immunol 110:410–412

Nagarkatti R, Kumar R, Sharma SK, Ghosh B (2004) Association of *IL4* Gene Polymorphisms with Asthma in North Indians. Int Arch Allergy Immunol 134:206–212

Ober C, Tsalenko A, Parry R, and Cox NJ (2000) A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. Am J Hum Genet 67:1154–1162

Sengler C, Haider A, Sommerfeld C, Lau S, Baldini M, Martinez F, Wahn U, Nickel R (2003) Evaluation of the CD14 C-159 T polymorphism in the German Multicenter Allergy Study cohort. Clin Exp Allergy 33:166–169

Strachan DP (1989) Hay fever, hygiene, and household size. Br Med J 299:1259–1260

Vercelli D, Baldini M, Martinez F (2001) The monocyte/IgE connection: may polymorphisms in the *CD14* gene teach us about IgE regulation? Int Arch Allergy Immunol 124:20–24

Weiss ST (2001) Association studies in asthma genetics. Am J Respir Crit Cell Mol Biol 164:2014–2015

Zdolsek HA, Jenmalm MC, LeVan TD, Bloom JW, Bailey TJ, Karp CL, Halonen M, Martinez FD, Vercelli D (2004) Reduced levels of soluble CD14 in atopic children. Clin Exp Allergy 34:532–539