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ORIGINAL ARTICLE

Failure to Find an Association between CD14-159C/T Polymorphism and Asthma: A Family-based Association Test and Meta-analysis

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ABSTRACT

Background: CD14 is an essential component of the receptor for lipopolysaccharide (LPS) . LPS stimulates T-helper type 1 (Th1) cytokine expression, potentially suppressing Th2 immune responses involved in IgE-mediated allergic diseases. Previous studies have reported that -159C/T, a promoter polymorphism of *CD14*, is associated with total serum IgE levels and atopy, but other studies have shown conflicting results.

Methods: To examine possible associations of *CD14* polymorphisms with asthma susceptibility, we performed transmission disequilibrium tests (TDTs) of 137 Japanese families identified through children with atopic asthma.

Results: We found no association between -159C/T polymorphism and asthma (p= 0.37) . Quantitative TDT and ANOVA showed no association between the -159C/T genotype and total serum IgE levels. We also performed a meta-analysis of data from all available studies. Neither a fixed-effects model nor a random-effect model showed a significant odds ratio for the -159C/T polymorphism ($p > 0.1$).

Conclusions: Our data indicate that *CD14* does not contribute substantially to susceptibility to asthma. Further studies examining both genotypes and environmental factors will be necessary to elucidate the role of *CD14* in the development of allergic diseases.

KEY WORDS

CD14, genetics, haplotype, polymorphism, transmission disequilibrium test

INTRODUCTION

Allergic diseases such as asthma, atopic dermatitis, and allergic rhinitis, are growing major public health issues. Asthma affects nearly 155 million individuals worldwide.[1] Although environmental factors are important, there are strong genetic predispositions for the development of allergic diseases.

Several linkage studies have provided evidence for association of asthma and/or related traits with chromosome region 5q31.[2-4] There are many candidate genes clustered in this region including interleukin (*IL*)*4*,*IL5*,granulocyte-macrophage colony-stimulating factor (*GMCSF*), *IL9*, *IL13* and *CD14*.

The hygiene hypothesis, introduced by Strachan,[5] suggests that improved hygiene conditions in recent years have changed the type and level of stimulation from the microbial environment and that this may indirectly influence postnatal development of the immune system, leading to increased predisposition to allergic diseases in childhood. Natural immunity to some bacterial and viral infections induces a T-helper type 1 (Th1) pattern of cytokine release, potentially suppressing Th2 immune responses involved in IgE-mediated allergic diseases. Lipopolysaccharide (LPS) is a major component of the bacterial cell wall and is

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known to stimulate production of IL12, a Th1-type cytokine, by antigen-presenting cells.[6] CD14 is an essential component of the receptor for LPS,[7] and is expressed on the cell surface of monocytes, macrophages and neutrophils. CD14 has two protein forms: a glycosylphosphatidylinositol-anchored membrane protein form (mCD14) and a monocyte- or liver-derived soluble serum protein form (sCD14). Both mCD14 and sCD14 are critical for LPS-dependent signal transduction and inflammatory processes. Recently, several polymorphisms in *CD14* have been identified in both the coding and promoter regions. One of the polymorphisms, a C to T transition at position -159 (-159C/T), has been investigated as a candidate in asthma and allergic diseases. Gel-shift assay revealed that the T allele of -159C/T has a decreased affinity for DNA/protein interactions with SP1, SP2 and SP3 transcription factors.[8] While the -159C/T polymorphism is reported to be associated with elevated levels of sCD14 in serum and with total serum IgE levels[9] and atopy,[10] other reports have shown conflicting results.[11-13]

The aim of this study was to investigate whether the -159C/T polymorphism is associated with asthma in a Japanese population. To increase the sample size and power, we performed meta-analysis of all available studies and examined possible associations between asthma susceptibility and *CD14* polymorphisms.

METHODS

SUBJECTS

Probands of the families studied were asthmatic children treated at the Pediatric Allergy Clinic of the University Hospital of Tsukuba (Japan). A full verbal and written explanation of the study was given to all family members interviewed, and 137 families (466 members) gave informed consent and participated in this study. Informed consent for subjects younger than school age was provided by their parents. All the families had two parents and at least one proband. This study was approved by the Committee of Ethics of the University of Tsukuba.

Each family member was questioned regarding allergic symptoms and underwent a physical examination by a pediatrician. Asthma was diagnosed according to the criteria established by the National Institutes of Health (USA) with minor modifications (National Heart Blood and Lung Institute, 1995). Criteria included the following: two or more episodes of wheezing and shortness of breath during the past year and reversibility of the wheezing and dyspnea, either spontaneously or by bronchodilator treatment. Patients treated with systemic steroids were excluded from this study. Because wheezing is often associated with viral respiratory infection in young children, only subjects older than 3 years of age were evaluated for the asthma phenotype.

GENOTYPING AND TRANSMISSION DISEQUILIBRIUM TESTS (TDT)

Genomic DNA was extracted from peripheral blood leukocytes. The regions of interest were amplified by PCR with primers 5'-GGTTGGATAGTGCAGAGT-3' and 5'-CCCTGATCACCTCCCAC-3'. The expected PCR product size was 263 bp. Amplified PCR products were subjected to *Ava*II digestion. Expected product sizes for the T allele were 79 bp and 184 bp, and expected product size for the C allele was 263 bp. Digested products were run on 2% agarose gels.

Family-based association tests were performed with a TDT as implemented by the ASPEX Program (http：//aspex.sourceforge.net/). Quantitative TDT tests of total serum IgE levels were performed with the QTDT program.[14]

META-ANALYSIS

We performed computer-based searches (National Library of Medicine, MEDLINE) and hand searches of relevant journals to identify studies published prior to March 2005 that were related to asthma and *CD14*. For the electronic searches, we used a combination of key words related to *CD14* and asthma. We included articles published in any languages. To be eligible for inclusion in the present study, studies had to meet all of the following criteria: design, case-controlled trials; subjects, patients with asthma; method, genotype of *CD14* -159C/T polymorphism; and results, genotype and allele data. We included our data in the meta-analysis. Control alleles in the present study referred to an artificially constructed control population consisting of non-transmitted parental alleles as described by Kirov *et al.*[15]

Meta-analysis was performed with the Mantel-Haenszel method as a fixed-effects model test and the DerSimonian-Laird method as a random-effects model test. Heterogeneity among studies was tested by the χ^2 statistic obtained by adding the weighted squares of the deviation of each estimate from the pooled estimate. Publication bias was examined by funnel plot of the reported effect assessed with the odds ratio against the standard error of the natural logarithm of the odds ratio ($p > 0.10$). We estimated odds ratios by comparing asthmatic cases with controls in the same study and calculated odds ratios under the hypothesis that individuals possessing the -159C allele were more susceptible to asthma.

RESULTS

Frequencies of the -159 CC, CT, and TT genotypes in parents were 21, 55 and 24%, respectively. Allele frequency for T was estimated at 52%, and the distribution did not deviate from the expected Hardy-Weinberg equilibrium ($p = 0.09$).

Preferential transmission of either the -159C or

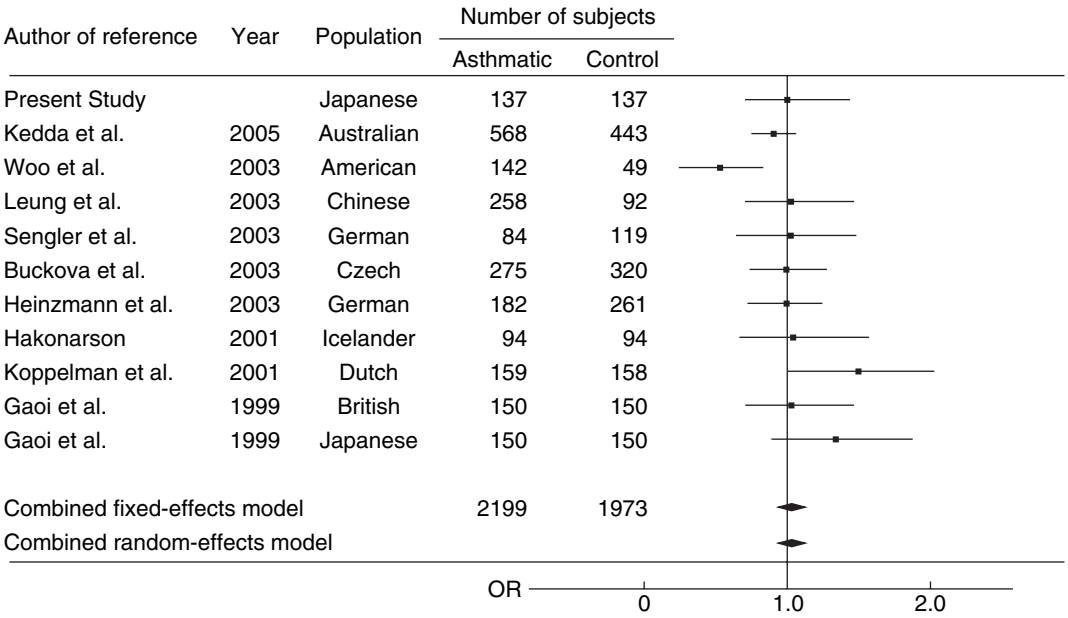

Fig. 1 Meta-analysis of 10 published studies of CD14 polymorphism and asthma

-159T allele was not observed by TDT analysis (transmitted 121 versus not transmitted 108 for -159C, p = 0.37). Mean log [total serum IgE] levels of parents with the CC, CT and TT genotypes were 1.99, 1.99 and 2.05, respectively; no significant association was observed between groups (p = 0.83 for ANOVA). By quantitative TDT analysis, no associations were observed between log [total serum IgE] levels and either allele of the -159C/T polymorphism (p = 0.19).

The total number of subjects included in the meta-analysis was 2199 patients with asthma and 1973 control individuals (Fig. 1).[10-13, 16-20] No publication bias was observed by funnel plot of the reported effects of -159C/T (p = 0.67). Overall, the combined odds ratio of the fixed-effects model for asthma with -159C was 1.0053 (95% confidence interval (CI), 0.92 to 1.096; p = 0.92; test of homogeneity, Q = 15.61, df = 10, p = 0.11). The odds ratio of the random effects model for -159C was 1.017 (95% CI 0.91 to 1.14; p = 0.77).

DISCUSSION

The TDT analysis in the present study showed no association between *CD14* -159C/T polymorphism and asthma or total serum IgE levels. Conflicting results are often observed in studies of the genetics of complex traits. Some studies show associations between a polymorphism and a disease, whereas others fail to replicate the results. Discrepancies may be due to differences in the populations studied, difficulty in defining the phenotypes, or lack of statistical power. We performed a meta-analysis to increase the power of the analysis of asthma and the *CD14*–159C/T polymorphism. However, we found no association of asthma and the -159C/T polymorphism.

There are several potential limitations of our meta-analysis. First, meta-analyses are limited by the numbers of studies included. Traditionally, meta-analyses are most powerful when large numbers of studies are included. It is possible that the influence of the *CD14*-159C/T polymorphism for susceptibility to asthma is quite small. The meta-analysis performed in the present study had the power (β= 0.8) to detect significant odds ratios as low as 1.19. However, this may not be enough to identify modest genetic effects in common diseases such as asthma.

Regulation of CD14 expression appears to be important in diseases such as gram-negative septic shock,[21] HIV infection[22] and malaria.[23] The functional -159C/T polymorphism of *CD14* has been studied extensively in relation to various diseases and has been shown to be associated with coronary artery complications in Kawasaki disease,[24] and in IgA nephropathy.[25] The -159C/T polymorphism is also associated with serum sCD14 levels, with subjects with the TT genotype showing the highest levels of sCD14.[13,26] Both TDT and meta-analysis in the present study failed to show involvement of the -159C/T polymorphism in the development of asthma. Because CD14 expression in blood cells is influenced by exposure levels,[27] differences in endotoxin load may contribute to individual disease susceptibility. In developed countries, endotoxin levels in the environment are very low, so there is the possibility of failing to show involvement of the -159C/T polymorphism in the development of asthma. However, the endotoxin switch hypothesis proposed by Vercelli[28] suggests that exposure to certain antigen concentrations may result in opposite responses, depending on the environmental context. According to this hypothesis, the

Th cytokine profile would not differ in response to very low or very high endotoxin exposure, regardless of genotype, but the -159C/T polymorphism may effect different Th cytokine responses in response to intermediate levels of exposure. Further studies examining both environmental endotoxin exposure and CD14 genotypes will be necessary to elucidate the role of CD14 in allergic diseases.

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