

ORIGINAL ARTICLE

A CD14 gene polymorphism is associated with the IgE level for *Dermatophagoides pteronyssinus*

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Abstract

Conclusion. CD14/-159 plays a role in sensitization to *Dermatophagoides pteronyssinus* in Japanese patients with allergic rhinitis. **Objective.** An association between a polymorphism in the 5'-flanking region of the CD14 gene and atopic phenotypes has been identified in a pediatric American population and an adult Dutch population. The aim of this study was to determine whether the CD14/-159 polymorphism is associated with specific IgE levels in allergic rhinitis patients in a Japanese population. **Material and methods.** The CD14/-159 genotypes of 81 unrelated patients with allergic rhinitis and 78 healthy subjects were determined and compared. The total IgE level and the specific IgE levels for three aeroallergens were determined. The IgE levels for each genotype were compared. **Results.** The frequencies of CD14/-159 genotypes did not differ between the two groups. Among the allergic rhinitis patients, TT homozygotes had significantly fewer positive CAP-radioallergosorbent tests (CAP-RASTs) than CC homozygotes and CT heterozygotes, suggesting that the C allele is dominant. TT homozygotes also had significantly lower CAP-RAST scores for *Dermatophagoides pteronyssinus*, but not for *Cryptomeria japonica* or *Dactylis glomerata*, than CC homozygotes and CT heterozygotes. There was no significant association between total serum IgE levels and CD14/-159 genotypes in the allergic rhinitis patients.

Keywords: Allergic rhinitis, house dust mite, sensitization, specific IgE

Introduction

It has been consistently found in population studies that atopic diseases, such as asthma, allergic rhinitis and eczema, are associated with increased levels of total serum IgE [1]. Atopy is generally considered to be caused by an interaction between genetic and environmental factors. It has been suggested in recent studies [2] that bacterial infections in infancy may protect against the development of allergies. In 1997, Holt et al. [3] hypothesized that bacterial signals play a functional role in the maturation of the Th1-type immune response, thereby suppressing the Th2-type response, which may produce an atopic phenotype. Microbial components, such as lipopolysaccharides (LPSs), provide activation signals for the maturation of Th1 cells. Membrane-bound CD14 (mCD14) is a multifunctional receptor constitutively expressed primarily on the surface of monocytes, macrophages and neutrophils [4]. A

soluble form of CD14, sCD14, is abundant in serum and is apparently derived from secreted CD14 and enzymatically cleaved glycosyl-phosphatidylinositol-anchored mCD14 [4]. CD14 is a receptor that has specificity for LPS and other bacterial wall-derived components [4,5]. CD14 acts in concert with CD11b/CD18 and toll-like receptor 4 to elicit LPS-inducible gene expression [6]. CD14 engagement by these bacterial components is associated with strong IL-12 responses from antigen-presenting cells [5,7]. IL-12 is regarded as an obligatory signal for the maturation of naive T cells into Th1 cells [7]. IgE responses are regulated by inhibitory signals derived from Th1-type cells and by stimulatory signals provided by Th2-type cells [8].

A polymorphism in the 5'-flanking region of the CD14 gene was first reported by Baldini et al. [9]. They identified a C-to-T transition at base pair -159 from the major transcription start site (CD14/-159).

TT homozygotes had significantly higher sCD14 levels than individuals with the CC and CT genotypes. Among children who were skin test-positive to local aeroallergens, TT homozygotes had significantly lower levels of IgE than the other two genotypes. Koppelman et al. [10] found that, among skin prick test-positive individuals, homozygotes for the C allele had a higher number of positive skin tests, higher total serum IgE levels and more self-reported allergic symptoms, including rhinitis and hay fever, than subjects with the CT and TT alleles. They concluded that the -159 C-to-T promoter polymorphism in the CD14 gene may cause a more severe allergic phenotype. Arias et al. [11] reported that human B-cell function is regulated by interaction with sCD14. Interaction of sCD14 with B cells resulted in higher levels of IgG1 and marked inhibition of IgE production by activated tonsillar B cells and Ag-stimulated peripheral blood mononuclear cells. The gene encoding CD14 maps to band 5q31.1. It has been shown in genetic linkage studies [12] that 5q31.1 contains more than one locus that controls total serum IgE. This location may also be involved in the expression of asthma and bronchial hyperresponsiveness to non-specific stimuli [13].

The aim of this study was to determine whether the CD14/-159 polymorphism was associated with allergic rhinitis in a Japanese population. We compared the CD14/-159 genotype frequencies in allergic rhinitis patients and normal subjects. Moreover, we examined total and specific IgE levels to determine whether IgE levels were associated with the genetic polymorphism.

Material and methods

Subjects

The genotype frequency of CD14/-159 was determined in 81 unrelated patients (41 males, 40 females; mean age 32.6 ± 12.0 years; age range 20–74 years) with allergic rhinitis and 78 healthy subjects. The patients visited the outpatient clinic at Mie University Hospital. The diagnosis of allergic rhinitis was based on the following criteria: (i) symptoms of sneezing, watery rhinorrhea and nasal obstruction; (ii) compatible findings in an anterior rhinoscopic examination; (iii) eosinophilia in nasal smears; and (iv) a CAP-radioallergosorbent (CAP-RAST) score of ≥ 2 for *Dermatophagoides pteronyssinus*, *Cryptomeria japonica* or *Dactylis glomerata*.

The 78 normal control subjects (38 males, 40 females) were unrelated. They resided in the same district as the patient group. The control subjects met the following criteria: (i) absence of symptoms

of sneezing, watery rhinorrhea and nasal obstruction; (ii) normal findings in an anterior rhinoscopic examination; (iii) no eosinophilia in nasal smears; and (iv) CAP-RAST scores of < 2 for *Dermatophagoides pteronyssinus*, *Cryptomeria japonica* or *Dactylis glomerata*. They had no nasal diseases and no family history of allergic diseases. The mean age of the control group was 29.0 ± 9.8 years (range 18–60 years), which was not significantly different from that of the allergic rhinitis group.

Written informed consent for genetic studies and all other procedures was obtained from all subjects. The study was approved by the Ethics Committee of Mie University (30 January, 2002; approval No. 178). The procedures followed were in accordance with the ethical standards of the Ethics Committee and with the Helsinki Declaration of 1975, as revised in 1983.

Serum sCD14 levels

Serum sCD14 levels were determined using a commercially available ELISA kit supplied by R&D Systems (Minneapolis, MN). A total of 12 CC homozygotes, 30 CT heterozygotes and 14 TT homozygotes were randomly recruited from the allergic rhinitis patients for this assay.

Total serum IgE

Total serum IgE levels were measured using paper radioimmunosorbent test kits (Pharmacia Diagnostic, Piscataway, NJ). The assay threshold was 0.1 IU/ml, as described elsewhere [14].

CAP-RAST

Serum IgE antibodies to three local aeroallergens (*Dermatophagoides pteronyssinus*, *Cryptomeria japonica* and *Dactylis glomerata*) were determined using the CAP system (Pharmacia), according to the manufacturer's instructions.

Molecular genetic methods

A Sepagene (Sankojunyaku, Japan) kit was used to extract genomic DNA from 100 μ l of peripheral blood. Genotyping of the CD14/-159 polymorphism was performed according to the protocol described by Baldini et al. [9] with minor modifications. Polymerase chain reaction (PCR) was performed in a volume of 50 μ l containing 80 ng of DNA, 200 mmol/l dNTPs, 1.5 mM $MgCl_2$, $10 \times$ buffer (Takara Biochemicals, Ohtsu, Japan), 0.5 U Taq polymerase (Takara Biochemicals), and 0.5 mM primer (5'-GTGCCAACAGATGAGGTTTCAC-3' and 5'-GCCTCTGACAGTTTATGTAATC-3').

The following reaction conditions were used: 35 cycles at 94°C for 45 s, 54°C for 45 s, 72°C for 1 min and 72°C for 10 min. After phenol–chloroform extraction, PCR-amplified DNA was digested with 5 U of *Ava* II in 2 ml of the manufacturer's buffer (Promega, Tokyo, Japan) at 37°C for 1 h. Reaction products were separated on a 2% agarose gel and stained with ethidium bromide. *Ava* II digests the PCR products only when the T allele is present. The length of the uncut product is 497 bp, whereas those of the digested products are 144 and 353 bp.

Statistical analysis

As TT homozygous patients had fewer positive CAP-RASTs and lower CAP-RAST scores for *Dermatophagoides pteronyssinus* than the CC homozygous and CT heterozygous patients, we chose a recessive model for the T allele. To study this recessive model, TT homozygotes were compared to individuals with the CT or CC genotypes. The number of positive CAP-RASTs, CAP-RAST scores, serum IgE levels and serum sCD14 levels were not normally distributed. Phenotypic differences between carriers of different genotypes were assessed using non-parametric statistics (Mann–Whitney U-test).

Results

Genotypes were obtained for all 159 subjects. The frequencies of CC, CT and TT genotypes for the entire study population were 20.8%, 48.4% and 30.8%, respectively. The allele frequency for C was estimated to be 45.0%, and the distribution was very similar to the expected Hardy–Weinberg equilibrium.

CD14/-159 genotype frequencies in the allergic rhinitis group and control group

The frequencies of the CD14/-159 genotypes in the two groups are shown in Table I. The genotype distribution did not differ significantly between the two groups.

Table I. CD14/-159 genotype frequencies.

Genotype	CD14/-159 genotype frequency; n (%)	
	Allergic rhinitis	Control subjects
CC	19 (23.4)	14 (18.0)
CT	40 (49.4)	37 (47.4)
TT	22 (27.2)	27 (34.6)
Total	81 (100)	78 (100)

CD14/-159 genotype, total serum IgE levels and sensitization to allergens

There was no significant association between total serum IgE levels and CD14/-159 genotypes among the allergic rhinitis patients. The allergic rhinitis patients with the TT genotype had a smaller number of positive CAP-RASTs than the CC homozygous and CT heterozygous patients ($p=0.015$) (Figure 1). When we examined specific IgE levels for three common allergens, we found that allergic rhinitis patients with C alleles (CC and CT) had significantly higher CAP-RAST scores for *Dermatophagoides pteronyssinus* than TT homozygous patients ($p=0.0013$ in a recessive model for the T allele) (Figure 2). No associations were found between the specific IgE levels for the other two aeroallergens (*Cryptomeria japonica* and *Dactylis glomerata*) and CD14/-159 genotypes (Figures 3 and 4).

Association between CD14/-159 and sCD14

The serum sCD14 levels of the CC homozygous patients were significantly lower than those of the CT heterozygous ($p=0.024$) and TT homozygous patients ($p=0.045$) (Figure 5).

Discussion

The ethnicity of study subjects is a key factor in genetic polymorphism studies. Baldini et al. [9] found that the association between total serum IgE levels and CD14/-159 genotypes reached statistical significance among non-Hispanic white children, but not among Hispanic children. Koppelman et al. [10] examined an adult Dutch population and found that

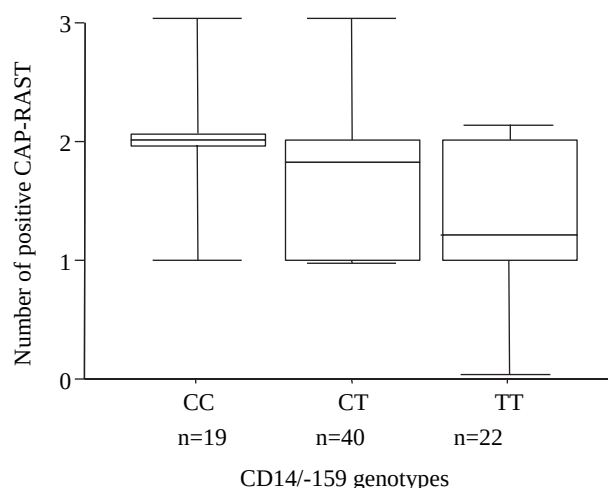


Figure 1. Box-and-whisker plots showing the number of positive CAP-RASTs for three allergens: *Dermatophagoides pteronyssinus*, *Cryptomeria japonica* and *Dactylis glomerata*. The boxplot shows the means and interquartile ranges; the whiskers represent the 10th and 90th percentiles.

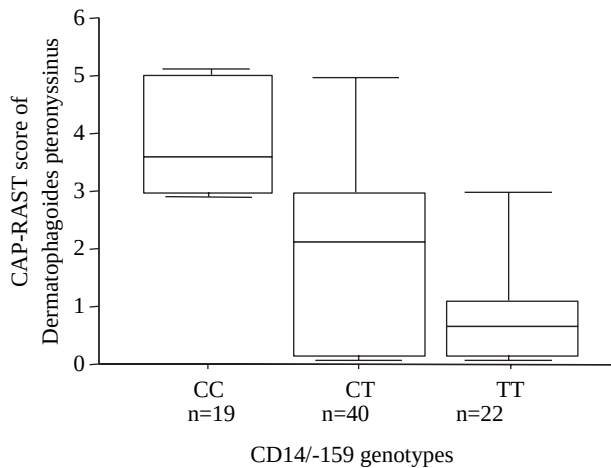


Figure 2. Association between the CD14/-159 polymorphism and the CAP-RAST score for *Dermatophagoides pteronyssinus*. For other details, see Figure 1.

CC homozygous individuals had a higher number of positive skin tests and a higher total IgE level only among skin prick test-positive individuals. The participants in our study were all Japanese residents of the Mie district, which means that they were a very homogenous and restricted group.

The frequencies of CD14/-159 genotypes in the allergic rhinitis group and the control group were not significantly different. This finding is in agreement with the results of a previous study [15], in which no significant association was found between CD14/-159 genotype and atopy or asthma in a Japanese population. Recently [16], it was reported that the influence of CD14/-159 on the atopic phenotype may be age-specific, exerting an effect during the middle of childhood which is no longer apparent by early adulthood. The lack of association between CD14/-159 genotypes and allergic phenotypes found

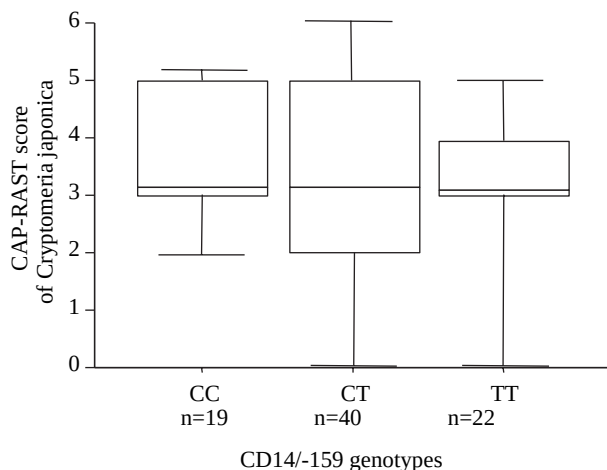


Figure 3. Lack of association between the CD14/-159 polymorphism and the CAP-RAST score for *Cryptomeria japonica*. For other details, see Figure 1.

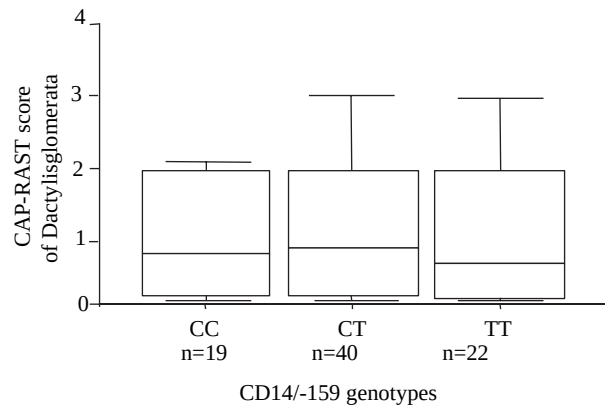


Figure 4. Lack of association between the CD14/-159 polymorphism and the CAP-RAST score for *Dactylis glomerata*. For other details, see Figure 1.

in the present study may be explained by this age-related factor.

We do not perform skin prick tests on a regular basis in our outpatient practice. However, we use the CAP-RAST system to examine specific IgE levels and patients' sensitization to aeroallergens. Thus, we compared the number of positive CAP-RAST scores (≥ 2) with CD14/-159 genotypes. In the allergic rhinitis group, patients with the TT genotype had a smaller number of positive CAP-RASTs than the CC homozygotes and CT heterozygotes. This result suggests that the C allele is dominant. In a study of white American children [9], a highly significant difference was found in the number of positive skin tests between the combined group (CC plus CT) and the TT subjects, which also suggests that the C allele is dominant. However, in an adult Dutch population, the C allele was associated with a higher number of positive skin tests in a codominant model [10].

To analyze the effect of CD14 polymorphism on specific IgE production, we compared CD14 polymorphism with specific IgE levels (CAP-RAST

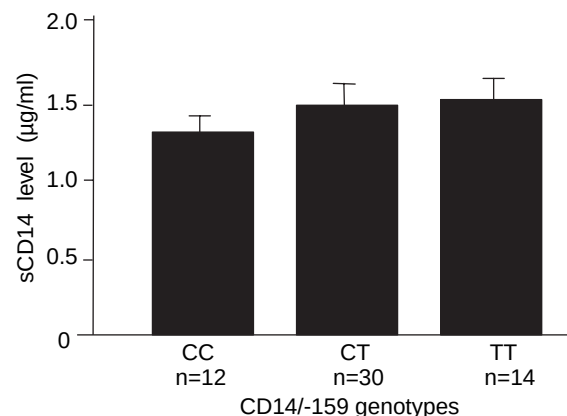


Figure 5. Serum levels of sCD14 as a function of CD14/-159 genotype.

scores) for three allergens. Among allergic rhinitis patients, only specific IgE levels for *Dermatophagoides pteronyssinus*, and not those for *Cryptomeria japonica* and *Dactylis glomerata*, had a strong association with the CD14 polymorphism.

In Czech patients with IgE-mediated allergy, the frequency of the C allele was higher in patients with a positive skin prick test for molds than in patients without reactivity to this antigen [17]. This was the first report of an association between CD14 polymorphism and IgE levels specific for house dust mite and pollens. It is generally thought that the genes controlling pollen antigen-specific IgE production are associated with human leukocyte antigens (HLAs). For example, Japanese cedar pollinosis is associated with HLA-DP5 [18], and short ragweed pollinosis is associated with DR2/Dw2 [19]. In general, significant associations are found only with highly purified simple allergens and not with more complex common allergens [20]. The complex allergens of house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) have not been associated with a specific type of HLA [21,22]. Although the exact mechanism of the association between CD14 polymorphism and *Dermatophagoides pteronyssinus* is unknown, it is assumed that specific IgE production against complex common allergens may be influenced by CD14 polymorphism.

We did not find a significant association between total serum IgE levels and CD14/-159 genotypes in allergic rhinitis patients. This result may be explained by the observation that patients with allergic rhinitis often do not have increased serum IgE levels. While some authors [9,10] have reported an association between this genotype and total serum IgE levels, this association is not always observed. In a recent large German population study [23], no association was found between this genotype and total IgE levels, although this polymorphism was associated with levels of serum sCD14.

The functional role of CD14/-159 was indicated by the finding that CC homozygotes have lower levels of sCD14 and a lower density of CD14 on monocytes than TT homozygotes [11,24]. In the present study, the serum sCD14 levels of the CC homozygous patients were significantly lower than those of CT heterozygous and TT homozygous patients. This result supports the functional role of CD14/-159 polymorphism in their correlation with serum sCD14 levels.

Recently, LeVan et al. [25] reported that this polymorphism (T allele) affects Sp protein binding and increases human CD14 promoter activity. They developed a reporter system to assess the transcriptional activity of the two alleles in a monocytic cell

line. They found greater transcriptional activity for the T allele than the C allele. This functional difference was associated with a decreased affinity of Sp proteins (Sp1, -2 and -3) for the GC box that encompasses the polymorphic site. Because the Sp3 transcription factor often inhibits promoter activity, LeVan et al. [25] hypothesized that the increased transcriptional activity of the CD14/-159T variant may be caused by impaired Sp3-DNA interactions at the polymorphic GC box.

Conclusions

In summary, we found that TT homozygotes had a significantly smaller number of positive CAP-RASTs than both CC homozygotes and CT heterozygotes among Japanese patients with allergic rhinitis. The CD14/-159 polymorphism in allergic rhinitis patients was associated with the level of specific IgE for *Dermatophagoides pteronyssinus*, but not those for *Cryptomeria japonica* or *Dactylis glomerata*. Thus, the CD14/-159 polymorphism plays a role in sensitization to house dust mites in a Japanese population.

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