

# No Association of Polymorphisms in the 5' Region of the CD14 Gene and Food Allergy in a Japanese Population

Eduardo Campos<sup>1</sup>, Naoki Shimojo<sup>1</sup>, Yuzaburo Inoue<sup>1</sup>, Takayasu Arima<sup>1</sup>, Shuichi Suzuki<sup>1</sup>, Minako Tomiita<sup>1</sup>, Tomoko Matsuura<sup>1</sup>, Akira Hata<sup>2</sup>, Yoichi Suzuki<sup>2</sup>, Masahiko Aoyagi<sup>3</sup> and Yoichi Kohno<sup>1</sup>

## ABSTRACT

**Background:** The gene encoding CD14 is a positional candidate gene for allergic diseases as it is localized on chromosome 5q31, a region that is linked to atopy-related phenotypes.

Although it has been shown that a polymorphism in the 5' region of the CD14 gene is associated with food allergy in white subjects, it is not clear whether this association is also present in the Japanese population.

**Methods:** Eighty-eight children with food allergy were recruited along with 101 children controls without food allergy. DNA samples from these subjects were genotyped by PCR-based restriction fragment length polymorphism (RFLP) assay to investigate the relationship between two polymorphisms in the 5' region of the CD14 gene (C-159T and C-550T) and food allergy.

**Results:** There was no association among the CD14 alleles, dominant model or recessive model of either polymorphism with food allergy.

**Conclusions:** The CD14-159 and -550 polymorphisms might not play a major role in the pathogenesis of food allergy in Japanese children.

## KEY WORDS

5' region, CD14, food allergy, gene, polymorphism

## INTRODUCTION

Recent epidemiological studies show that the prevalence of allergic diseases in Japan is approximately 40% in children and 30% in adults,<sup>1</sup> with a prevalence rate of food allergy in infants of 8%,<sup>2</sup> which is similar to the rates in other developed countries such as France<sup>3</sup> and the United States.<sup>4</sup> Although changes in the environment may be one cause for the increase in allergic disorders in the last few decades; it has also been shown that there is a strong genetic predisposition for food allergies.<sup>5,6</sup>

The gene encoding CD14 is one of several genes related to the expression of allergic phenotypes, as it is located on chromosome 5q31, a region that is linked to atopy.<sup>7,8</sup> CD14 is a multifunctional receptor

that is expressed on the surface of monocytes, macrophages and neutrophils, and as a soluble form in serum.<sup>9</sup> It is the principal receptor for lipopolysaccharides (LPS), whose engagement results in the activation of antigen presenting cells, including macrophages and dendritic cells. The subsequent release of pro-inflammatory cytokines as IL-12<sup>10</sup> is regarded as an obligatory signal for the maturation of naive T cells into Th1 cells<sup>11,12</sup> and the suppression of Th2 type cell-dependent immunoglobulin E (Ig E) responses.<sup>13</sup>

In previous studies, the CD14 gene has been reported to contain several polymorphisms in both the coding and promoter regions.<sup>14</sup> The polymorphism at position -159, a C to T transition, has been investigated as a candidate gene for atopy,<sup>15</sup> although, so

<sup>1</sup>Department of Pediatrics, Graduate School of Medicine, <sup>2</sup>Department of Public Health, Graduate School of Medicine, Chiba University and <sup>3</sup>Department of Pediatrics, National Shimoshizu Hospital, Chiba, Japan.

Correspondence: Eduardo Campos, M.D., Department of Pediatrics, Graduate School of Medicine, Chiba University, 1-8-1 Ino-

hana, Chuo-ku, Chiba 260-8670, Japan.

Email: edcampos@graduate.chiba-u.jp

Received 24 January 2006. Accepted for publication 29 September 2006.

©2007 Japanese Society of Allergy

far, there have been conflicting results about the relationship between this polymorphism and atopic phenotypes.<sup>16-20</sup> Woo *et al.* recently reported an association between food allergy and the TT genotype of CD14 C-159T, particularly in white subjects in the United States of America.<sup>21</sup> Until now, no other studies on the association of CD14 polymorphisms with food allergy have been reported, especially in different ethnic groups.

The objective of this case control study was to investigate the association between the polymorphisms in the CD14-159 and -550 genes and food allergy in Japanese children.

## METHODS

### SUBJECTS

Eighty-eight childhood patients with food allergy, with a mean 7.1+/-5.0 years of age were included from the Pediatrics Allergy Clinic at the Chiba University Hospital. All patients experienced immediate adverse reactions upon ingestion of offending foods. CAP RAST score to the offending food allergens was 2 or more in all patients. The diagnosis of food allergy was confirmed by open or blind challenge tests unless the patients experienced anaphylactic reactions to offending foods. Sixty-seven patients had positive RAST results to more than one food and the foods most frequently related to allergy were egg and milk. At the time of blood sampling, 44 patients had active reactions to food and 44 had outgrown food allergy. One hundred and one healthy control children volunteers (mean = 9.45+/-1.5 years of age) were recruited from the Attached Elementary School of Education Department of Chiba University. Healthy controls had no history of food allergy upon careful history taking. Total serum IgE was less than 60 IU/ml and they had no specific IgE antibodies to major airborne and food allergens.

Approval from the Ethics Committee of Chiba University was received. All parents of the children participating in this study gave their informed consent.

### IgE ANTIBODY ANALYSIS

Total serum IgE levels (IU/ml) were analyzed with laser nephelometry and allergen-specific IgE antibodies were measured by fluorescent enzyme immunoassay (AutoCAP system, Pharmacia Diagnostics AB, Uppsala, Sweden).

### MOLECULAR METHODS

Genomic DNA was isolated from EDTA-anticoagulated whole blood according to the instructions for the QIA.amp DNA Blood Kit from QIAGEN (Japan).

### DETECTION OF THE BI-ALLELIC POLYMORPHISM C-159T IN THE 5' REGION OF THE CD14 GENE

The presence of -159C or -159T alleles was analyzed by means of PCR using the method reported previously.<sup>14</sup> The forward primer for CD14-159 was 5'-GTGCCAACAGATGAGGTTTCAC-3' and the reverse primer was 5'-GCCTCTGACAGTTTATGTAATC-3'. Commercially available *Ava*II endonuclease (New England Biolabs, Tokyo, Japan) is specific for the sequence GGTC, which is present only among carriers of the CD14 -159T allele. The digested fragments were separated by electrophoresis in 2% agarose gels and visualized by ethidium bromide staining. The homozygous C allele of the CD14 gene appears as a single 497 bp (base pair) band and the homozygous T allele as 144 and 353 bp fragments. Heterozygotes exhibited all bands: 144, 353 and 497 bp.

### DETECTION OF THE BI-ALLELIC POLYMORPHISM C-550T IN THE 5' REGION OF THE CD14 GENE

The C-550T polymorphism was detected using a modification of the technique reported previously.<sup>22</sup> The forward primer for CD14-550 was 5'-GGAAGGGGAATTTTCTTTAGGC-3' and the reverse primer was 5'-GGCAGTGTCTGATGACTCA-3'. PCR was performed in a final volume of 15 µl, containing 25 ng of genomic DNA, 200 µM dNTP, 2.4 mM MgCl<sub>2</sub>, 10× buffer (TAKARA Bio Inc, Japan) containing 50 mM KCl, 10 mM Tris-HCl buffer (pH 8.4), 0.4 U *Taq* polymerase (TAKARA Bio Inc) and 0.1 µM each of the forward and reverse primers. Cycling conditions were 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 60°C annealing for 30 seconds, 72°C for 40 seconds and a final extension at 72°C for 10 minutes. An aliquot (8.5 µl) of the PCR product was digested with 5 U of the restriction enzyme *Hae*III (New England Biolabs) at 37°C for 16 hours. The final products were separated in 2% agarose gels. The digestion revealed fragments of 236 bp length for the C allele and 258 bp for the T allele.

### STATISTICAL ANALYSIS

All statistical analyses were performed using the program packages SNPalyze ver. 4.1 (Dynacom Co. USA) and GraphPad Prism ver. 4.0 (GraphPad Software Inc. USA). The chi-square ( $\chi^2$ ) test and Fischer's exact test were used to compare differences in genotype or allele frequency among groups. Odds ratio (OR), confidence intervals (CI) and *P* values were calculated, and *P* values <0.05 were considered to indicate significant differences. Serum IgE levels (IU/ml) were transformed to log<sub>10</sub> values and the difference in the mean log (IgE) values among the three different genotype groups were assessed by analysis of variance (ANOVA). Power of this study was calculated with the aid of Sample Power program (SPSS,

**Table 1** Demographic characteristics of patients and controls and concomitant allergic diseases

Characteristic	Patients	Controls	P value
No. of subjects	88	101	
Age ± SD (y)	7.1 ± 5.0	9.4 ± 1.5	<0.0001
Sex (No.)			
Male	53	58	
Female	35	43	
Log IgE ± SD	2.9 ± 0.5	1.1 ± 0.3	<0.0001
Concomitant allergic diseases (No.):			
Atopic dermatitis	55	—	
Bronchial asthma	29	—	

SD: standard deviation  
y: years

**Table 2** CD14 C-159T genotype frequencies in food allergy patients

Population	Genotypes (%)		
	-159CC	-159CT	-159TT
Food Allergy (n = 88)	20 (22.7)	39 (44.3)	29 (32.9)
Control (n = 101)	22 (21.7)	48 (47.5)	31 (30.7)

No significant association was found

Inc. Chicago, IL).

## RESULTS

Demographic characteristics and concomitant allergic diseases of patients and healthy controls are shown in Table 1.

The genotype distributions for both polymorphisms were consistent with Hardy-Weinberg equilibrium in both allergic and control groups. The allele frequencies for CD14-159 were: -159C: 46.6% patients *vs* 45.5% controls; -159T: 53.4% patients *vs* 54.5% controls; the C versus T allele ratio showed no significant difference ( $p = 0.8$ ). Table 2 summarizes the codominance model of the C-159T polymorphism, comparing the frequencies of CC, CT and TT genotypes between patients and control subjects.

To explore the dominant-recessive character of the C-159T polymorphism, we evaluated the relevant homozygote with the heterozygote and alternate homozygote combined by using the  $\chi^2$  test with 1 degree of freedom (*df*). The test of the CC genotype (e.g. CC *vs* CT/TT) revealed no difference between food allergy patients and healthy controls ( $p = 0.8$ ), and for the TT genotype (TT *vs* CT/CC) as well, no relationship was found ( $p = 0.7$ ).

Concerning the C-550T polymorphism, the frequencies of the alleles were: -550C: 75% patients *vs* 75.7% controls; -550T: 25% patients *vs* 24.3% controls;

**Table 3** CD14 C-550T genotype frequencies in food allergy patients

Population	Genotypes (%)		
	-550CC	-550CT	-550TT
Food Allergy (n = 88)	49 (55.7)	34 (38.6)	5 (5.7)
Control (n = 101)	57 (56.4)	39 (38.6)	5 (4.9)

No significant association was found

**Table 4** CD14 (C-159T, C-550T) Haplotype analysis

Haplotype	Control freq.	Food Allergy freq.	Chi-square	P value
C-C	0.455	0.449	0.0161	0.899
C-T	0.302	0.301	0.0003	0.985
T-T	0.243	0.250	0.0278	0.868

no significant difference in the C versus T allele ratio was found ( $p = 0.8$ ). Table 3 shows the frequency of genotypes CC, CT and TT. An evaluation of the CC genotype *vs* others showed no difference between patients and controls ( $p = 0.9$ ), and no significant difference was found for the TT genotype ( $p = 0.8$ ).

Comparing the frequencies of haplotypes between patients and controls, we could not find any significant difference in both groups (Table 4).

There was also no association between total serum IgE levels and CD14C-159T genotypes (Table 5) or CD14C-550T (Table 6) for either food allergy patients or healthy control children.

The frequency of CD14-159 TT genotype in controls was 0.307 (Table 2). Assuming that the odds ratio (OR) of a risk genotype were 2.31, the present study (a sample size of 88 cases and 101 controls) had a statistical power of 80% to detect an association with the disease at  $P = 0.05$ . This power is enough to replicate the results of Woo JG *et al.*<sup>21</sup> In their study they found that the frequency of -159TT genotype increased from 0.10 (control) to 0.30 (food allergy) OR = 3.9.

## DISCUSSION

Food allergy clearly has a genetic component.<sup>23</sup> Even though some candidate genes have been investigated,<sup>24</sup> Woo *et al.* found a strong association with CD14 in a white population in the United States of America. They suggested that one possible explanation for the association between CD14-159T and food allergy might be that lipopolysaccharide (LPS) in foods acts as an adjuvant and increases the specific IgE response to foods in susceptible individuals.<sup>21</sup> There may also be an increased pro-inflammatory response in the gut caused by the presence of the CD14-159T allele, which would cause an elevation in

**Table 5** Log total IgE levels (IU/ml) geometric mean  $\pm$  SD by CD14 C-159T genotypes in food allergy patients and healthy control children

CD14C-159T Genotypes	<i>n</i>	Log Total IgE among food allergy patients	<i>n</i>	Log Total IgE among healthy control children
CC	20	3.05 $\pm$ 0.48	22	1.00 $\pm$ 0.40
CT	39	2.93 $\pm$ 0.51	48	1.12 $\pm$ 0.38
TT	29	2.72 $\pm$ 0.61	31	1.05 $\pm$ 0.33
		<i>P</i> = 0.13		<i>P</i> = 0.45

**Table 6** Log total IgE levels (IU/ml) geometric mean  $\pm$  SD by CD14 C-550T genotypes in food allergy patients and healthy control children

CD14C-550T Genotypes	<i>n</i>	Log Total IgE among food allergy patients	<i>n</i>	Log Total IgE among healthy control children
CC	49	2.81 $\pm$ 0.55	57	1.06 $\pm$ 0.36
CT	34	2.98 $\pm$ 0.51	39	1.09 $\pm$ 0.37
TT	5	3.12 $\pm$ 0.64	5	1.05 $\pm$ 0.50
		<i>P</i> = 0.26		<i>P</i> = 0.84

the production of CD14 by monocytes and macrophages and, therefore, an increase in inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12 and TNF- $\alpha$ ;<sup>12,25</sup> this response, then, might be related to enhanced sensitization to food allergens, principally in early childhood.<sup>21</sup>

In contrast to Woo's results, we found no association between this polymorphism and food allergy in a Japanese population. The mean age of the patients in our study (7.1 years) was similar with that in Woo's study (5.2 years) and patients with food allergy in the present investigation and theirs developed their symptoms in childhood. The food allergy phenotype was also the same and IgE-mediated food allergy was studied in both investigations.

The power of this study was estimated to be enough (80%) if OR of the risk genotype of CD14-159 TT genotype were 2.31. This OR value seemed to be lower than the estimated OR value observed in Woo's study (3.9). However, if we consider the lower limit of 95% CI of their OR (1.5), the power would be estimated to be 26%, which is underpowered.

The frequency of TT genotype of C-159T in controls in our study was 30.7%, which is substantially different from Woo's result in the U.S. white population and similar to the results of Nishimura *et al.*<sup>26</sup> and Gao *et al.*<sup>27</sup> in the Japanese population. Thus, one explanation for the discrepancy between our results and those of Woo may be the ethnic difference. Another possibility is a difference in environment such as amounts of LPS. In studies of the association between CD14 C-159T polymorphism and atopy-related traits, different results have been reported for the same population, such as Caucasians<sup>15,19</sup> and this

may be explained by the differences in environments. The difference in gene frequencies and environment may lead to different results; for example, two studies in a Japanese population could not confirm the association between CD14 C-159T polymorphism and total serum IgE level,<sup>26,27</sup> whereas Baldini *et al.* reported that C-159T in the CD14 gene was associated with serum total IgE concentration in a white population.<sup>14</sup>

An association between the CD14-550T allele and high sCD14 in milk but low sCD14 in plasma has been demonstrated,<sup>22</sup> but the relationship with atopic diseases was not studied. In our study, no association between the CD14C-550T polymorphism and food allergy was found. The relationship between this polymorphism and other atopic diseases awaits clarification.

In conclusion, we could not confirm an association between food allergy and CD14-159 and -550 polymorphisms in a Japanese population. A standardized assessment of environmental conditions and the combination of polymorphisms in the promoter and coding regions of the CD14 gene may be necessary to evaluate the existence of an association between gene polymorphisms and food allergy.

## ACKNOWLEDGEMENTS

We are very grateful to all the children/families and control volunteers who participated in our study, and want to express our gratitude to the staff of Chiba University Hospital and Shimoshizu Hospital for their assistance in the recruitment of patients and control volunteers. We also thank Dr. Ohto for reviewing the paper.

## REFERENCES

1. Furuta Y, Yoshioka M, Sugiyama T, Miyasaki K. [Rapidly increasing allergic diseases.] *Hokkaido Igaku Zasshi* 2005; **80**:3-7 (in Japanese).
2. Hikino S, Nakayama H, Yamamoto J, Kinukawa N, Sakamoto M. Food allergy and atopic dermatitis in low birthweight infants during early childhood. *Acta Paediatr.* 2001;**90**:850-855.
3. Fremont S. Biological exploration of food allergy. *Ann. Biol. Clin.* 2002;**60**:405-410.
4. Sampson HA. Food allergy. Part 1. immunopathogenesis and clinical disorders. *J. Allergy Clin. Immunol.* 1999;**103**:717-728.
5. Harris JR, Magnus P, Samuelson SO, Tambs K. No evidence for effects of family environment on asthma. A retrospective study of Norwegian twins. *Am. J. Respir. Crit. Care Med.* 1997;**156**:43-49.
6. Laitinen T, Rasanen M, Kaprio J, Koskenvuo M, Laitinen L. Importance of genetic factors in asthma: a population based twin-family study. *Am. J. Respir. Crit. Care Med.* 1998;**157**:1073-1078.
7. Meyers DA, Postma DS, Panhuysen CI *et al.* Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994;**23**:464-470.
8. Marsh DG, Neely JD, Breazeale DR *et al.* Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentration. *Science* 1994; **264**:1152-1156.
9. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol.* 1995;**13**:437-457.
10. Akira S, Takeda K, Kaisho T. Toll like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol.* 2001;**2**:675-680.
11. Macatonia SE, Hosken NA, Litton M *et al.* Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+T cells. *J. Immunol.* 1995;**154**:5071-5079.
12. Verhasselt V, Buelens C, Willems F, De Groot D, Haefliger-Cavaillon N, Goldman M. Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells; evidence for a soluble CD14-dependent pathway. *J. Immunol.* 1997;**158**:2919-2925.
13. Romagnani S. Induction of Th1 and Th2 responses: a key role for the "natural" immune response? *Immunol. Today* 1992;**13**:379-381.
14. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez F. 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.* 1999;**20**:976-983.
15. Koppelman GH, Reijmerink NE, Colin Stine O *et al.* Association of a promoter polymorphism of the CD14 gene and atopy. *Am. J. Respir. Crit. Care Med.* 2001;**163**:965-969.
16. Kedda MA, Lose F, Duffy D, Bell E, Thompson PJ, Upham J. The CD14 C-159T polymorphism is not associated with asthma or asthma severity in an Australian adult population. *Thorax* 2005;**60**:211-214.
17. Heinzmann A, Dietrich H, Jerkic SP, Kurz T, Deichmann KA. Promoter polymorphisms of the CD14 gene are not associated with bronchial asthma in Caucasian children. *Eur. J. Immunogenet.* 2003;**30**:166-169.
18. Hakonarson H, Bjornsdottir US, Ostermann E *et al.* Allelic frequencies and patterns of single-nucleotide polymorphisms in candidate genes for asthma and atopy in Iceland. *Am. J. Respir. Crit. Care Med.* 2001;**164**:2036-2044.
19. Sengler C, Haider A, Sommerfeld C *et al.* Evaluation of the CD14 C-159T polymorphism in the German Multicenter Allergy Study cohort. *Clin. Exp. Allergy* 2003;**33**:166-169.
20. Buckova D, Holla LI, Schuller M, Znojil V, Vacha J. Two CD14 promoter polymorphisms and atopic phenotypes in Czech patients with IgE-mediated allergy. *Allergy* 2003; **58**:1023-1026.
21. Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GK. The -159 C→T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J. Allergy Clin. Immunol.* 2003;**112**:438-444.
22. Guerra S, Lohman I, Le Van T, Wright A, Martinez F, Halonen M. The differential effect of genetic variation on soluble CD14 levels in human plasma and milk. *AJRI* 2004; **52**:204-211.
23. Sicherer SH, Furlong TJ, Maes HH, Desnick RJ, Sampson HA, Gelb BD. Genetics of peanut allergy: a twin study. *J. Allergy Clin. Immunol.* 2000;**106**:53-56.
24. Gawronska-Szklarz B, Pawlik A, Czaja-Bulsa G, Gornik W, Luszczewska-Kutrzeba T, Wrzesniewska J. Genotype of N-acetyl transferase (NAT2) polymorphism in children with immunoglobulin E-mediated food allergy. *Clin. Pharmacol. Ther.* 2001;**69**:372-378.
25. Morrison DC, Ryan JL. Endotoxins and disease mechanisms. *Annu. Rev. Med.* 1987;**38**:417-432.
26. Nishimura F, Shibasaki M, Ichikawa K, Tadao A, Noguchi E. Failure to Find an Association between CD14-159C/T Polymorphism and Asthma: A Family-based Association Test and Meta-analysis. *Allergol. Int.* 2006;**55**:55-58.
27. Gao PS, Mao XQ, Baldini M *et al.* Serum total IgE levels and CD14 on chromosome 5q31. *Clin. Genet.* 1999;**56**:164-165.