

Letter to the Editor

CD14* and *IL4R* gene polymorphisms modify the effect of day-care attendance on serum IgE levelsTo the Editor:*

The cause of atopy is generally traced to the interplay of genetic and environmental factors.¹ Day care appears to be one of the most frequently investigated environmental factors. Although the results of studies investigating the association between day-care attendance and atopy, as assessed by skin prick test responses, were inconsistent, all studies²⁻⁴ measuring serum IgE levels have thus far shown a constant decreasing effect on serum IgE levels.

Among the genes that show a gene-environment interaction for the development of atopy or allergic diseases, the most frequently investigated is the *CD14* gene.¹ However, there is no report that investigates interaction of this gene and day-care attendance. *CD14* is a pattern-recognition receptor involved in the clearance of bacterial endotoxin and is also known as a receptor of respiratory syncytial virus. We investigated *CD14*-159C/T (rs2569190) and *CD14*-550C/T (rs5744455) polymorphisms in Japanese patients with severe respiratory syncytial virus-induced bronchiolitis and found that *CD14*-550C/T but not *CD14*-159C/T was significantly associated with the condition.⁵

The IL-4 receptor α gene (*IL4R*) is also one of the most frequently investigated genes and has been shown to be associated with atopy and atopic diseases.⁶ The Ile50Val polymorphism (rs1805010) of the *IL4R* gene is a functional polymorphism and has been reported to be strongly associated with atopy and atopic asthma in the Japanese population. To date, only one study has reported the interaction of the *IL4R* Ile50Val polymorphism and day-care attendance in the first year of life.⁷ The result showed a significant gene-environment interaction for IFN- γ production at 1 year of age. However, it is not known whether this modified cytokine response affects the chance of having atopy or allergic diseases in the later period of life.

Here we report a relationship between serum total and specific IgE levels in Japanese elementary school children and day-care attendance during earlier life. Our results suggest that day-care attendance is associated with serum IgE levels, and this effect is modified by *CD14*-550C/T and *IL4R* Ile50Val polymorphisms. This is the first report that suggests an interaction between early-life day-care attendance and genetic variations on IgE levels in later life.

Children attending an elementary school located in the central area of Chiba city (population of approximately 930,000) were recruited for this study. We first asked all ($n = 843$) children for participate in the survey. We then sent a detailed questionnaire to those who had a positive response ($n = 582$). Children with congenital heart diseases and lung diseases caused by immature birth were excluded. A total of 473 school children aged 6 to 12 years were enrolled. Blood samples were collected from 411 children on 2 separate days (July 3 and 12, 2006) for serum and DNA preparation. A complete set of information on total and 8 specific IgE levels, genotypes, and environmental factors was obtained from 375 children. All parents provided written informed consent. The study protocol was approved by the Ethics Committee of Chiba University Graduate School of Medicine.

TABLE I. Characteristics of the subjects

Total no. of participants	473
Age (mo)	
Mean $\pm$ SD	111.1 $\pm$ 19.9
Range	76-147
Sex ratio (male:female)	1.00:1.01
Day-care attendance before age 2 y (%)	14.5
Total IgE (IU/mL), mean $\pm$ SD	
Male	254 $\pm$ 340
Female	241 $\pm$ 469
Prevalence of atopy (%)	
Male	76.9
Female	68.0
Prevalence of allergic disorders (%)	
Asthma	
Male	14.1
Female	6.6
Atopic dermatitis	
Male	11.5
Female	9.7
Allergic rhinitis	
Male	42.1
Female	31.2
Food allergy	
Male	3.0
Female	3.4

The status of allergic diseases was evaluated by using questions based on the International Study of Asthma and Allergies in Childhood. We asked whether the child regularly attends a day-care center where time is spent with other children at or before 2 years of age. For parents who responded yes to this question, the age of entry of their child to the day care center was obtained. The questionnaire also included the following items to assess possible confounding factors: number of siblings; number of older siblings; allergic diseases of parents and siblings (family history: scored as positive if parents, siblings, or both had any of 4 allergic diseases [asthma, allergic rhinitis, atopic eczema, and food allergy]); residential area (6 categories), type of house structure (5 categories), and floor type of bedroom (5 categories); yogurt/fermented food consumption; pet ownership; and smoking among family members.

Genotyping of the *CD14*-550C/T polymorphism was performed as described previously,⁵ whereas that of the *IL4R* Ile50Val (rs1805010) polymorphism was carried out with the TaqMan allele-specific PCR method.⁸ Primer sequences were as shown in this article's Online Repository at www.jacionline.org.

Table I shows the characteristics of the investigated population. The percentage of children who had regularly attended day care before 2 years of age was 14.5%. Atopy was defined as the presence of positive (≥ 0.35 IU/mL) specific IgE level against at least 1 of the 8 allergens. Although the prevalences of asthma, atopic dermatitis, and food allergy were compatible with those in a recent large study,⁹ prevalences of allergic rhinitis and atopy were about 10 to 20 points higher, suggesting that children who had allergic rhinitis were more likely to attend this study.

TABLE II. Effects of day-care attendance on IgE levels when stratified by *CD14*–550C/T genotype

	C/C				C/T + T/T				Gene-environment interaction <i>P</i> value*
	Day-care attendance		Effect size or odds ratio (95% CI)	<i>P</i> value	Day-care attendance		Effect size or odds ratio (95% CI)	<i>P</i> value	
	No	Yes			No	Yes			
No. of subjects	169	22			157	28			
Log ₁₀ (total IgE)									
Mean	1.88	1.98	0.094 (−0.21 to 0.39)¶	0.54†	2.09	1.58	−0.50 (−0.26 to −0.76)¶	9.7 × 10 ^{−5} †	.0046**
SD	0.77	0.76			0.63	0.51			
Specific IgE (positive‡ rate)									
Mite	0.49	0.59	1.50 (0.61 to 3.69)#	.51§	0.61	0.32	0.30 (0.13 to 0.71)#	.0016§	.00047††
Cedar pollen	0.45	0.46	1.02 (0.42 to 2.45)#	.92§	0.57	0.32	0.35 (0.15 to 0.83)#	.032§	.116††
Atopy (rate)	0.77	0.68	1.60 (0.56 to 4.55)#	.38	0.81	0.50	0.24 (0.10 to 0.55)#	.00041	.0097‡‡

Boldface indicates statistically significant values.

*Adjusted for age, sex, number of siblings, and family history.

†Analysis of variance for log₁₀(total IgE [in international units per milliliter]).

‡Class ≥1 (≥0.35 IU/mL).

§Kruskal-Wallis test for IgE value (in international units per milliliter).

||χ² Test of independence.

¶Effect size.

#Odds ratio.

**General liner model.

††Generalized linear model (Poisson distribution, log link function).

‡‡Logistic regression.

Table II shows the association between day-care attendance and serum IgE levels or atopy after being stratified with the *CD14*–550C/T genotype. Day care significantly decreased total IgE levels ($P = 9.7 \times 10^{-5}$), mite-specific IgE levels ($P = .0016$), and rate of atopy ($P = .00041$) in individuals with the C/T or T/T genotype, whereas the effect of day care was not observed in those with the C/C genotype. Numbers of children with the C/T+T/T genotype and those with the C/C genotype were similar, suggesting that the difference is not likely due to the statistical power for detecting association. Multivariate analyses with confounding factors were performed to evaluate the significance of this gene-environment interaction. The interaction between the *CD14*–550C/T polymorphism and day care was significant for log₁₀(total IgE) ($P = .0046$), mite-specific IgE classes ($P = .00047$), and atopy ($P = .0097$) after adjusting for age, sex, family history, and number of siblings.

Table III shows the association between day-care attendance and serum IgE levels or atopy after being stratified with the *IL4R* Val50Ile genotype. The effects of day care on total and some specific IgE levels were significant in Val/Ile heterozygotes but not in Val/Val or Ile/Ile homozygotes. In Val/Ile individuals day care significantly decreased total IgE levels ($P = .0012$), mite-specific ($P = .011$) and cedar pollen-specific ($P = .034$) IgE levels, and rate of atopy ($P = .018$). No such trend was observed in Val/Val or Ile/Ile individuals. The numbers of Val/Val and Val/Ile individuals were similar. It is therefore unlikely that the lack of significant association in Val/Val individuals was due to smaller statistical power for detecting association. When the significance of gene-environment interaction was assessed with the confounding factors, the interaction term between *IL4R* and day-care attendance was significant for log₁₀(total IgE) ($P = .019$) and mite-specific ($P = .0025$) and cedar pollen-specific ($P = .040$) IgE classes but not for atopy.

Total IgE levels in 4 genotype groups (group 1: *CD14* C/C, *IL4R* Ile/Ile+Val/Val; group 2: *CD14* C/C, *IL4R* Val/Ile; group 3: *CD14* C/T+T/T, *IL4R* Ile/Ile+Val/Val; and group 4: *CD14*

C/T+T/T, *IL4R* Val/Ile) were compared to evaluate the combined effect of 2 polymorphisms on total IgE levels. Fig 1 shows the box plot of log₁₀(total IgE) in 4 genotype groups. Among children who attended day care compared with group 1, the mean log₁₀ (total IgE) values of groups 2, 3, and 4 decreased by 0.41, 0.35, and 0.69, respectively. This magnitude of change suggests that the effects of *CD14* and *IL4R* were additive. The children in group 4 showed significantly ($P = .0046$) lower total IgE levels than those in group 1. On the other hand, among children who did not attend day care, the log₁₀(total IgE) levels of children in groups 3 ($P = .031$) and 4 ($P = .036$) were significantly higher than those of children in group 1. The *CD14* C/T and T/T genotypes appeared to show the opposite effect on the serum total IgE level in children who did not attend day care compared with those who did attend day care. When we examined the effect of day care in each genotype group, the effect was not sufficiently large to show a significant change in IgE level in groups 2 and 3, in which individuals had only 1 IgE level-decreasing genotype. However, in group 4, in which individuals had 2 IgE level-decreasing genotypes, the effect was sufficiently large to show a significant difference ($P = .00018$). Significance of interaction between the *CD14* and *IL4R* genotypes was also evaluated by using general linear models in which age, sex, family history, number of siblings, and day care were included as variables. The interaction term of the 2 genes was not significant, suggesting an independent effect of the *CD14* and *IL4R* genes.

The interaction of the *CD14* gene with day-care attendance suggests that the mechanism of the effect of day care involves at least in part a response to infection, environmental endotoxin exposure, or both. The interaction of the *IL4R* gene with day-care attendance suggests that the mechanism also involves those related to T_H2 cell proliferation and IgE production. These results suggest that the complex nature of mechanisms underlies the effect of day-care attendance on serum IgE levels.

Environmental factors investigated in the present study were determined based on a questionnaire on past day-care attendance,

TABLE III. Effects of day-care attendance on IgE levels when stratified by *IL4R* Val50Ile genotype

	Val/Val				Val/Ile				Ile/Ile				Gene-environment interaction <i>P</i> value*
	Day-care attendance		Effect size or odds ratio (95% CI)	<i>P</i> value	Day-care attendance		Effect size or odds ratio (95% CI)	<i>P</i> value	Day-care attendance		Effect size of odds ratio (95% CI)	<i>P</i> value	
	No	Yes			No	Yes			No	Yes			
No. of subjects	125	18			152	27			49	5			
Log ₁₀ (total IgE)													
Mean	1.94	1.91	−0.058 (−0.38 to 0.27)¶	.72†	1.88	1.55	−0.44 (−0.71 to −0.18)¶	.0012†	1.99	2.32	0.33 (−0.31 to 0.97)¶	.12†	.019**
SD	0.64	0.72			0.57	0.56			0.69	0.52			
Specific IgE (positive‡ rate)													
Mite	0.57	0.56	0.95 (0.35 to 2.57)#	.51§	0.52	0.30	0.39 (0.16 to 0.94)#	.011§	0.59	0.80	2.76 (0.29 to 26.5)#	.36§	.0025††
Cedar pollen	0.50	0.50	1.01 (0.38 to 2.73)#	.93§	0.51	0.30	0.41 (0.17 to 0.99)#	.034§	0.55	0.40	0.54 (0.083 to 3.54)#	.91§	.040††
Atopy (rate)	0.74	0.72	0.93 (0.31 to 2.82)#	.90	0.74	0.52	0.37 (0.16 to 0.86)#	.018 	0.76	0.80	1.30 (0.13 to 12.8)#	.82	.118‡‡

Boldface indicates statistically significant values.

*Adjusted for age, sex, number of siblings, and family history.

†Analysis of variance for log₁₀(total IgE [in international units per milliliter]).

‡Class ≥1 (≥0.35 IU/mL).

§Kruskal-Wallis test for IgE value (in international units per milliliter).

||χ² Test of independence.

¶Effect size.

#Odds ratio.

**General liner model.

††Generalized linear model (Poisson distribution, log link function).

‡‡Logistic regression.

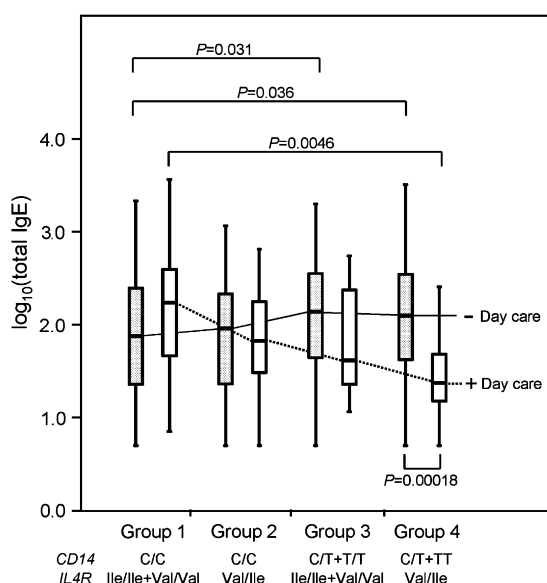


FIG 1. Total IgE levels in 4 groups of children classified based on a combination of *IL4R* and *CD14* genotypes. Box plot of log₁₀(total IgE) values is shown for children who attended day care (+ Day care) and for those who did not (− Day care). Results are presented as medians and interquartile ranges. Only significant *P* values (<.05) are shown.

and therefore recall bias can be a potential problem. The number of subjects investigated in this study was not so large and might be the acceptable minimum for investigating gene-environment interactions. The subjects evaluated were children who attended a single school and lived in a medium-populated city, thus representing those living in rather small regional environments in

Japan. Nevertheless, these characteristics of the present sample might have contributed to minimizing the variances of background and outcome parameters and might have resulted in the positive findings obtained from a relatively small number of subjects. It is necessary to perform a cohort study to follow children with or without day-care attendance until they reach school age to validate the current observations.

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PRIMERS FOR *IL4R* GENOTYPING (5' TO 3')

TaqMan probe (FAM-TACAGGTGACCAGCCTAACCCAGC CCCTGT-TAMRA); common primer (TGGAGGCATGTCCCG GACAC); Ile (A) allele primer (CGCCTCCGTTGTTCTCAG GGGT); and Val (G) allele primer (CGCCTCCGTTGTTCTC AGGGGC).