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ORIGINAL ARTICLE

Relationship Between Polymorphisms in *IL4* and Asthma in Japanese Women: The Kyushu Okinawa Maternal and Child Health Study

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Abstract

Background: Interleukin (IL) 4 plays a critical role in immune responses by acting as a growth factor for type 2 helper T cells and inducing immunoglobulin (Ig) class switching to IgE. Epidemiological evidence of the relationship between single-nucleotide polymorphisms (SNPs) in *IL4* and asthma is inconsistent.
Objectives: We examined the association between the *IL4* SNPs rs2243250, rs2070874, rs2227284, and rs2243290 and asthma in young adult Japanese women.
Methods: The study population comprised 89 women who met the criteria of the European Community Respiratory Health Survey (ECRHS) for asthma. The control group comprised 1281 nonasthmatic women (ECRHS criteria) who had not been diagnosed with asthma by a physician. Adjustment was made for age, region of residence, presence of older siblings, smoking, and education.
Results: Compared with the AA genotype of rs2243290, the AC genotype, but not the CC genotype, was significantly associated with a reduced risk of asthma: the adjusted odds ratio for the AC genotype was 0.62 (95%CI, 0.39-0.996). No evident relationships were found between rs2243250, rs2070874, or rs2227284 and asthma. None of the haplotypes were significantly associated with asthma. No significant interactions were found between the 4 SNPs under study and smoking with respect to the risk of asthma.
Conclusions: Ours is the first study in a non-Western population to show that the *IL4* SNP rs2243290 was significantly associated with the risk of asthma. Smoking did not significantly modify the gene-disease associations under study.

Key words: Asthma. *IL4*. Japanese women. Smoking. SNP.

Resumen

Antecedentes: La IL4 juega un papel crítico en la respuesta inmune, actuando como factor de crecimiento de las células TH2, induce a la producción de IgE. No hay evidencias epidemiológicas consistentes sobre la relación entre SNPs de IL4 y asma.
Objective: El objetivo de este estudio fue examinar la posible asociación entre SNPs rs2243250, rs2070874, rs2227284, y rs2243290 de IL4 y asma.
Métodos: La población de estudio incluyó 89 mujeres adultas jóvenes japonesas, que reunían los criterios de asma de la encuesta ECRHS. Como controles se incluyeron 1281 mujeres sin asma. Se ajustaron las muestras por edad, región de residencia, presencia de hermanos mayores, hábito tabáquico y educación.
Resultados: En cuanto a los resultados obtenidos, el genotiopo AC y no el CC, comparando con el genotipo AA del SNP rs2243290, estaba significativamente relacionado con un riesgo reducido a padecer asma. La OR ajustada para el genotipo AC era de 0,62 (95% CI: 0.39–0.996).No se encontró relación entre SNP rs2243250, rs2070874, o rs2227284 y asma.
Ningún haplotipo estaba significativamente realacionado con asma. No se encontraron interacciones significativas entre los 4 SNPs bajo estudio y el hábito de fumar con respecto al riesgo de padecer asma.
Conclusiones: En conclusión, este estudio es el primero realizado en una población no del oeste y muestra que el SNP rs2243290 de IL4 está relacionado significativamente con el riesgo de asma. El hábito tabáquico no modifica la asociación genética estudiada.

Palabras clave: Asma. IL4. Mujeres japonesas. Tabaco. SNP.

Introduction

Interleukin (IL) 4 plays a critical role in immune responses by acting as a growth factor for type 2 helper T cells (T_H2) and inducing immunoglobulin (Ig) class switching to IgE [1]. Previous genetic association studies have examined the relationships between the *IL4* single-nucleotide polymorphisms (SNPs) rs2243250 (–590C/T), located in the promoter region, and rs2070874 (–33C/T), located in the 5´ untranslated region, and asthma, but the results have been inconsistent [2-20]. Statistically significant associations were found between rs2243250 and rs2070874 and asthma in a cross-sectional study of German children [5], a case-control study of US white individuals [8], and a case-control study of Iranian individuals [15]. In a US case-control study, rs2243250 was significantly related to asthma in both African-American and Caucasian children [19]. A case-control study of Spanish adults showed a significant relationship between rs2070874 and persistent asthma [12]. On the other hand, investigations conducted in the US [14], the UK [2,6,16], Australia [2], Canada [7], Finland [11], Japan [3,4], Korea [9,10], China [13,18,20], and Taiwan [17] failed to detect a significant association between rs2243250 or rs2070874 and asthma. In the study by Basehore et al [8] in the US, significant associations were found between the SNP rs2227284 (3017G/T) in intron 2 and rs2243290 (8461C/A) in intron 3 and asthma.

In order to further investigate associations between the *IL4* SNPs rs2243250, rs2070874, rs2227284, and rs2243290 and the risk of asthma based on newly gathered evidence, we conducted a case-control study in young adult Japanese women using data from the Kyushu Okinawa Maternal and Child Health Study (KOMCHS). We also carried out haplotype analyses and investigated the possibility of an interaction between these SNPs and smoking.

Methods

Study Population

KOMCHS is an ongoing prospective prebirth cohort study. From April 2007 to March 2008, the study organizers requested that 131 obstetric hospitals in Fukuoka Prefecture, the largest prefecture on Kyushu Island in southern Japan, with a total population of approximately 5.04 million, provide as many pregnant women as possible by handing out leaflets explaining the study, an application to participate, and a self-addressed stamped envelope in which to return the application. From May 2007 to March 2008, the study organizers also requested that 40 obstetric hospitals in Okinawa Prefecture, an island chain in the southwest of Japan, with a total population of almost 1.37 million, provide as many pregnant women as possible using the same set of documents. In addition, to increase sample size, pregnant women living in 6 prefectures on Kyushu Island other than Fukuoka Prefecture, with a total population of approximately 8.22 million, were provided with the same documents at 252 obstetric hospitals between August 2007 and March 2008. Pregnant women who intended to take part in the study returned the application form to the data management center. By the end of the baseline study, a total of 1757 pregnant women between weeks 5 and 39 of pregnancy had given their written informed consent to participate and had completed the baseline survey. Around 4 months after delivery, 1492 women gave their informed consent for genotyping to be performed. The ethics committee of the Faculty of Medicine, Fukuoka University approved the study.

Selection of Cases and Controls

In the baseline survey, each participant returned a self-administered questionnaire to the data management center. Research technicians completed missing data or resolved unclear data by telephone interview.

The questionnaire included questions on asthma based on the European Community Respiratory Health Survey (ECRHS) [21]. The presence of asthma was defined as the existence of either of 2 conditions: an asthma attack during the last 12 months or current use of asthma medication. The questionnaire also elicited information on age, region of residence, presence of older siblings, smoking habits, education, personal history of physician-diagnosed allergic disorders, and family history of allergic disorders.

Among the 1492 women whose DNA samples were available, 89 cases of asthma were identified based on the abovementioned definition. Among the 1403 remaining participants who were eligible to serve as controls, 121 women were excluded, as they were not considered to have asthma according to the ECRHS criteria but who had answered "yes" to the question: "Have you ever been diagnosed by a physician as having asthma?" We further excluded 1 control with incomplete data on smoking. Thus, the final sample consisted of 89 cases and 1281 controls.

DNA Extraction and Genotyping

Research technicians or participants themselves collected buccal specimens with BuccalAmp swabs (Epicenter BioTechnologies). Genomic DNA was extracted using a QIAmp DNA mini kit (Qiagen, Inc). *IL4* SNPs were genotyped using TaqMan SNP Genotyping Assays on the StepOnePlus system, according to the manufacturer's instructions (Applied Biosystems).

Statistical Analysis

Hardy-Weinberg equilibrium was tested among the controls using the χ^2 test. Linkage disequilibrium was examined using Haploview software version 4.2 (Broad Institute) [22]. Estimations of crude odds ratios (ORs) and 95% confidence intervals (CIs) for asthma in relation to the SNPs under study were made by means of logistic regression analysis, with the reference category being the homozygote of the major allele. Multiple logistic regression analysis was used to adjust for age, region of residence, presence of older siblings, smoking, and education. The statistical power calculation was performed using QUANTO version 1.2 [23]. Haplotypes and their frequencies were inferred by means of the expectation maximization algorithm. For differences in haplotype frequency between the cases and controls, crude ORs and 95%

CIs were estimated based on the frequency of each haplotype relative to all other haplotypes combined. The multiplicative interaction was estimated by introducing a multiplicative term into a multiple logistic regression model. Excluding the calculation of linkage disequilibrium and statistical power, all computations were performed using STATA/SE software version 12.0 (StataCorp).

Results

Compared with controls, women with asthma were more likely to be younger, with a lower educational level, a personal history of physician-diagnosed atopic eczema and allergic rhinitis, and a family history of asthma and allergic rhinitis (Table 1).

Among the controls, the genetic distributions of rs2243250, rs2070874, rs2227284, and rs2243290 did not deviate from Hardy-Weinberg equilibrium (*P*=.47, .47, .28, and .51, respectively). All SNP pairs were in strong linkage disequilibrium (D'=0.96 to 0.99, r^2=0.69 to 0.98) (Table 2).

Compared with a reference group of women with the AA genotype of rs2243290, those with the AC genotype had a significantly reduced risk of asthma; the CC genotype, however, was not significantly associated with asthma in our crude analysis (Table 3). Adjustment for confounders under investigation did not materially alter the results: the adjusted OR for the AC genotype was 0.62 (95%CI, 0.39-0.996). No evident relationships were found between rs2243250, rs2070874, or rs2227284 and asthma. As for rs2070874, compared with the TT genotype, the combination of the TC and CC genotypes was nonsignificantly associated with a reduced risk of asthma (adjusted OR, 0.71; 95%CI, 0.46-1.10): the statistical power calculation revealed that, using our sample size, we could detect a gene-disease association for an OR of 0.539 with an accuracy of more than 80% under the dominant model.

When haplotypes with a frequency of less than 1% in either cases or controls were excluded, 3 constructed haplotypes remained, but none of these were significantly associated with the risk of asthma (Table 4).

Table 1. Characteristics of the Study Population[a]

Variable	Cases (n=89)	Controls (n=1281)	*P*[b]
Age, years, mean (SD)	30.4 (4.2)	31.5 (4.2)	.02
Region of residence			.79
Fukuoka Prefecture	47 (52.8)	723 (56.4)	
Other than Fukuoka Prefecture in Kyushu	32 (36.0)	431 (33.7)	
Okinawa Prefecture	10 (11.2)	127 (9.9)	
Presence of 1 or more older siblings	52 (58.4)	669 (52.2)	.26
Having ever smoked	34 (38.2)	390 (30.4)	.13
Education, years			<.0001
<13	34 (38.2)	268 (20.9)	
13-14	32 (36.0)	440 (34.4)	
≥15	23 (25.8)	573 (44.7)	
History of physician-diagnosed atopic eczema	35 (39.3)	207 (16.2)	<.0001
History of physician-diagnosed allergic rhinitis	63 (70.8)	494 (38.6)	<.0001
Family history of asthma[c]	30 (33.7)	217 (16.9)	<.0001
Family history of atopic eczema[c]	20 (22.5)	219 (17.1)	.20
Family history of allergic rhinitis[c]	50 (56.2)	549 (42.9)	.01

[a]All values are expressed as No. (%) unless otherwise indicated.
[b]χ^2 test or t test.
[c]A family history of asthma, atopic eczema, or allergic rhinitis (including Japanese cedar pollinosis) was considered to be present if 1 or more parents or siblings of the study participants had been diagnosed by a physician as having any of these allergic disorders.

Table 2. Pairwise Linkage Disequilibrium of *IL4* Polymorphisms[a]

	rs2243250	rs2070874	rs2227284	rs2243290
rs2243250		0.99	0.96	0.99
rs2070874	0.98		0.96	0.99
rs2227284	0.69	0.69		0.97
rs2243290	0.97	0.98	0.70	

[a]r^2 below and D'above the diagonal.

Table 3. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) For Asthma According to *IL4* Polymorphisms

SNP			No. (%) Cases (n=89)	No. (%) Controls (n=1281)	*P*[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[a,b]
rs2243250	Genotype	TT	43 (48.3)	520 (40.6)	.19	1.00	1.00
		TC	33 (37.1)	602 (47.0)		0.66 (0.41–1.06)	0.67 (0.42–1.08)
		CC	13 (14.6)	159 (12.4)		0.99 (0.52–1.89)	1.04 (0.54–2.01)
	Allele	T	119 (66.9)	1642 (64.1)	.46	1.00	
		C	59 (33.1)	920 (35.9)		0.88 (0.63–1.23)	
rs2070874	Genotype	TT	44 (49.4)	517 (40.4)	.17	1.00	1.00
		TC	33 (37.1)	604 (47.2)		0.64 (0.40–1.02)	0.65 (0.41–1.05)
		CC	12 (13.5)	160 (12.5)		0.88 (0.45–1.71)	0.94 (0.48–1.85)
	Allele	T	121 (68.0)	1638 (63.9)	.28	1.00	
		C	57 (32.0)	924 (36.1)		0.84 (0.59–1.17)	
rs2227284	Genotype	TT	49 (55.1)	647 (50.5)	.70	1.00	1.00
		TG	32 (36.0)	515 (40.2)		0.82 (0.52–1.30)	0.84 (0.53–1.35)
		GG	8 (9.0)	119 (9.3)		0.89 (0.41–1.92)	0.95 (0.43–2.07)
	Allele	T	130 (73.0)	1809 (70.6)	.49	1.00	
		G	48 (27.0)	753 (29.4)		0.89 (0.62–1.26)	
rs2243290	Genotype	AA	45 (50.6)	517 (40.4)	.11	1.00	1.00
		AC	32 (36.0)	603 (47.1)		0.61 (0.38–0.97)	0.62 (0.39–0.996)
		CC	12 (13.5)	161 (12.6)		0.86 (0.44–1.66)	0.90 (0.46–1.76)
	Allele	A	122 (68.5)	1637 (63.9)	.21	1.00	
		C	56 (31.5)	925 (36.1)		0.81 (0.58–1.14)	

Abbreviation: OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]χ^2 test.
[b]Adjusted for age, region of residence, presence of older siblings, smoking, and education.

Table 4. Haplotype Analysis of 3 *IL4* Polymorphisms in Relation to Asthma[a]

Haplotype[b]	Frequency, No. (%) Cases (2N=178)	Frequency, No. (%) Controls (2N=2562)	Crude OR (95% CI)[c]
TTTA	118 (66.3)	1619 (63.2)	1.15 (0.82–1.61)
CCTC	10 (5.6)	180 (7.0)	0.79 (0.36–1.52)
CCGC	46 (25.8)	736 (28.7)	0.86 (0.60–1.23)

Abbreviation: OR, odds ratio.
[a]Rare haplotypes (frequency less than 1% in either cases or controls) were deleted.
[b]Haplotype order is rs2243250, rs2070874, rs2227284, and rs2243290.
[c]Crude OR for each haplotype is relative to all other haplotypes combined.

Table 5. Association Between *IL4* Polymorphisms and Asthma, Stratified by Smoking History

SNP	Genotype	Never Smoked: No. of Cases/Controls	Never Smoked: Adjusted OR (95% CI)[a]	Smoked: No. of Cases/Controls	Smoked: Adjusted OR (95%CI)[a]	*P* For Interaction
rs2243250	TT	25/356	1.00	18/164	1.00	.85
	TC + CC	30/535	0.78 (0.45–1.36)	16/226	0.75 (0.36–1.55)	
rs2070874	TT	26/353	1.00	18/164	1.00	.98
	TC + CC	29/538	0.72 (0.42–1.25)	16/226	0.77 (0.37–1.59)	
rs2227284	TT	28/442	1.00	21/205	1.00	.62
	TG + GG	27/449	0.94 (0.54–1.63)	13/185	0.78 (0.37–1.64)	
rs2243290	AA	27/352	1.00	18/165	1.00	.83
	AC + CC	28/539	0.66 (0.38–1.15)	16/225	0.77 (0.37–1.59)	

Abbreviations: OR, odds ratio; SNP, single-nucleotide polymorphism.
[a]Adjusted for age, region of residence, presence of older siblings, and education.

We did not find that smoking significantly modified the gene-disease associations under study (Table 5).

Discussion

To our knowledge, this is the first study in a non-Western population to show that the *IL4* SNP rs2243290 was significantly associated with the risk of asthma. Our results for this SNP are consistent with those of a case-control study of US Caucasians that found a significant association between rs2243290 and asthma [8] but not with those of a case-control study of African-Americans showing no relationship between rs2243290 and asthma [14]. In the present study, no significant associations were found between the SNPs rs2243250, rs2070874, or rs2227284 and asthma. Our results are consistent with those of other studies that found no relationship between rs2243250 or rs2070874 and asthma [2-4,6,7,9-11,13,14,16-18,20] but not with those of other studies showing significant associations between any of the 3 SNPs and asthma [5,8,12,15,19]. The inconsistency between our findings and those of several previous studies may be at least partly explained by differences in the genetic backgrounds of the populations examined, definitions of asthma, and statistical power.

The mechanisms for the significant association between rs2243290 and asthma are unknown. Rosenwasser et al [24] showed that the T allele of rs2243250 was associated in vitro with enhanced *IL4* transcription and higher binding affinity to nuclear transcription factors.

We found no significant interactions between the 4 SNPs under study and smoking with respect to the risk of asthma. A UK longitudinal birth cohort study showed no interaction between rs2070874 and smoking affecting lung function [16].

The current study has 2 main methodological strengths. The study participants were homogeneous in that they were all pregnant women and adjustment was made for several confounders.

Our study is also subject to a series of limitations. First, the participation rate cannot be calculated because the exact number of eligible pregnant women who were provided with the abovementioned KOMCHS documents is not available. In addition, we were not able to assess differences between participants and nonparticipants, because information on personal characteristics such as age, socioeconomic status, and history of allergic disorders was not available for nonparticipants. Our participants were probably not representative of Japanese women in the general population: for example, the distribution of educational status differed considerably from that of the general population. According to the 2000 population census of Japan, the percentages of women aged 30 to 34 years in Fukuoka Prefecture with years of education of <13, 13-14, ≥15, and unknown were 52.0%, 31.5%, 11.8%, and 4.8%, respectively [25]. The corresponding figures for the present study in the control group were 20.9%, 34.4%, 44.7%, and 0.0%, respectively. Therefore, the present population might have been more aware of health-related matters than the general population. Nevertheless, the distribution of all 4 SNPs under study was consistent with Hardy-Weinberg equilibrium, and any selection bias associated with genotype distribution would be negligible.

Second, the definition of asthma was based on the ECRHS questions [21]. No attempt was made to ascertain outcome status through reviews of medical records. The possibility of nondifferentially misclassifying outcome would have given rise to an underestimation of our results. Among the 89 patients who met the ECRHS criteria, however, 83 (93.3%) had been diagnosed with asthma by a physician. In addition, we were not able to distinguish atopic from nonatopic asthma, as data on serum IgE levels or skin prick test results were not available in this study. We do know, however, that about 71% of the cases had a personal history of physician-diagnosed allergic rhinitis and 39% had a personal history of physician-diagnosed atopic eczema.

Third, the number of cases was rather small for a valid genetic association study; however, a significant association between rs2243290 and asthma was detected. The lack of significant relationships between the other SNPs and asthma might be ascribed to insufficient statistical power.

Fourth, correction for multiple testing, an appropriate element in initial exploratory analyses, was not performed in this study. As this is a hypothesis testing study and part of the current findings is a replication of previously published results, we think that correction for multiple testing would cause us to underestimate our results.

Fifth, although adjustment was made for some confounders, residual confounding effects could not be ruled out.

Our findings show that the *IL4* SNP rs2243290, but not rs2243250, rs2070874, or rs2227284, is significantly associated with the risk of asthma. However, we could not provide evidence that smoking interacts with any of the 4 SNPs in the etiology of asthma. Future evaluations should involve larger population-based case-control studies and functional studies.

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