



IL3 SNP rs40401 variant is a risk factor for rhinoconjunctivitis in Japanese women: The Kyushu Okinawa Maternal and Child Health Study



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ABSTRACT

Background: No studies have investigated the relationship between *IL3* single nucleotide polymorphism (SNP) rs40401 and allergic rhinitis. We performed a case-control study to examine this issue and to assess interactions between the SNP and smoking or older siblings in young adult Japanese women.

Methods: Included were 393 women who met the criteria of the International Study of Asthma and Allergies in Childhood (ISAAC) for rhinoconjunctivitis. Controls were 767 women without rhinoconjunctivitis according to the ISAAC criteria who had not been diagnosed with allergic rhinitis by a doctor.

Results: Compared with women with the TT or TC genotype of *IL3* SNP rs40401, those with the CC genotype had a significantly increased risk of rhinoconjunctivitis: the adjusted OR was 1.52 (95% CI: 1.05–2.19). This positive relationship was significant under the additive model: the adjusted OR was 1.23 (95% CI: 1.02–1.47). The positive association fell just short of the significance level under the dominant or recessive model. There was no significant interaction between SNP rs40401 and smoking with respect to rhinoconjunctivitis. Compared with subjects with the TT or TC genotype of *IL3* SNP rs40401 who had one or more older siblings, those with the CC genotype who had no older siblings had a 2.33-fold increased risk of rhinoconjunctivitis; nevertheless, the interaction was not significant.

Conclusion: This is the first study to show a significant positive association between *IL3* SNP rs40401 variant and the risk of rhinoconjunctivitis. We could not find evidence for interactions between SNP rs40401 and smoking or older siblings affecting rhinoconjunctivitis.

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1. Introduction

Allergic rhinitis is the most common allergic disorder in Japan. In a nationwide study of 22,819 Japanese aged 20–79 years, the age-adjusted prevalence of rhinitis as defined according to the European Community Respiratory Health Survey questionnaire was 35.1% in men and 39.3% in women [1]. A complex interaction among genetic and environmental factors is likely to be involved in the development of allergic rhinitis.

Interleukin-3 (IL-3) is a cytokine that regulates the production and function of cells of the hematopoietic and immune systems and is expressed mainly by activated T lymphocytes and mast cells [2]. IL-3 also stimulates pro-inflammatory reactions from immune cells such as the release of histamine, IL-4, and IL-13 from basophils and eosinophil degranulation [2].

The *IL3* gene is located on chromosome 5q23–5q31. The *IL3* single nucleotide polymorphism (SNP) rs40401 variant in exon 1 arises due to a thymine-to-cytosine transversion at position +79, resulting in an amino acid change from serine to proline at residue

27 when including the leader sequence, or at residue 8 in the secreted form of human IL-3. To our knowledge, only four investigations have examined the relationship between *IL3* SNP rs40401 and asthma, and these have produced inconsistent results [3–6]. No studies have investigated the relationship between SNP rs40401 and allergic rhinitis or the gene-environment interactions associated with SNP rs40401 and allergic disorders.

Here, we conducted a case-control study of the relationship between *IL3* SNP rs40401 and rhinoconjunctivitis in a population of young adult Japanese women using data from the Kyushu Okinawa Maternal and Child Health Study (KOMCHS). We also investigated the possibility of an interaction between *IL3* SNP rs40401 and smoking or older siblings.

2. Subjects and methods

2.1. Study population

The KOMCHS is a prospective prebirth cohort study. Details of the baseline survey of the KOMCHS have been described elsewhere [7]. Eligible women were those who became pregnant while living in one of seven prefectures on Kyushu Island in southern Japan,

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with a total population of approximately 13.26 million, or in Okinawa Prefecture, an island chain in the southwest of Japan, with a total population of nearly 1.37 million. Between April 2007 and March 2008, we requested that 423 obstetric hospitals in the above-mentioned eight prefectures provide as many pregnant women as possible with a set of leaflets explaining the KOMCHS, an application form to participate in the study, and a self-addressed and stamped return envelope. Pregnant women who were willing to participate in the study returned the application form to the data management center. In total, 1757 pregnant women between the 5th and 39th weeks of pregnancy gave their full informed consent in writing to participate in the KOMCHS and completed the baseline survey. Around 4 months after delivery, 1492 women gave informed consent to genotyping. The ethics committee of the Faculty of Medicine, Fukuoka University approved the KOMCHS.

2.2. Selection of cases and control subjects

In accordance with the questions in the International Study of Asthma and Allergies in Childhood (ISAAC) [8], the presence of rhinoconjunctivitis was defined as a positive response to the following two questions: 'In the last 12 months, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or flu?' and 'In the last 12 months, has this nose problem been accompanied by itchy-watery eyes?' Among the 1492 women whose DNA samples were available, 393 cases of rhinoconjunctivitis were identified. Among the 1099 remaining women who were eligible to serve as control subjects, 331 women were excluded who were not considered to have rhinoconjunctivitis as defined by the ISAAC criteria but who had answered 'yes' to the question: 'Have you ever been diagnosed by a physician as having allergic rhinitis?' One woman was also excluded due to incomplete data on smoking. Thus data from 393 cases and 767 control subjects were ultimately available for analysis.

2.3. DNA extraction and genotyping

Genomic DNA from buccal specimens collected with BuccalAmp swabs (Epicenter BioTechnologies, Madison, WI, USA) was extracted using a QIAmp DNA mini kit (Qiagen, Inc., Valencia, CA, USA). Genotyping of *IL3* SNP rs40401 was performed using a TaqMan SNP Genotyping Assay on the StepOnePlus machine (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

2.4. Statistical analysis

We tested agreement with Hardy–Weinberg equilibrium using the chi-square test. Logistic regression analysis was applied to estimate crude odds ratios (ORs) and 95% confidence intervals (CIs) of rhinoconjunctivitis in relation to *IL3* SNP rs40401, with the reference category being the homozygote of the ancestral allele based on the National Center for Biotechnology Information SNP database. 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 [9]. We examined multiplicative and additive interactions between *IL3* SNP rs40401 and smoking or older siblings with regard to the risk of rhinoconjunctivitis. Multiplicative interaction was estimated by introducing a multiplicative term into a multiple logistic regression model. Three measures for the additive interaction were calculated using the Excel sheet provided by Andersson et al. [10]: (1) relative excess risk due to interaction (RERI), (2) attributable proportion due to interaction (AP), and (3) synergy index (S). RERI is the excess risk due to an interaction

relative to the risk without exposure. AP refers to the attributable proportion of disease among individuals exposed to both factors that is due to the factors' interaction. S is the excess risk from both exposures when there is an additive interaction, relative to the risk from both exposures without an interaction. $RERI = 0$, $AP = 0$, or $S = 1$ means no interaction or strict additivity; $RERI > 0$, $AP > 0$, or $S > 1$ means a positive interaction or more than additivity; $RERI < 0$, $AP < 0$, or $S < 1$ means a negative interaction or less than additivity [11]. If any of the null values (0 in RERI and AP or 1 in S) falls outside the 95% CI of its respective measurement, then the additive interaction is considered statistically significant. Except for the statistical power calculation, all statistical analyses were performed using STATA/SE software version 12.0 (StataCorp, College Station, TX, USA).

3. Results

Compared with control subjects, cases were more likely to live on Kyushu Island but outside Fukuoka Prefecture and were less likely to live in Okinawa Prefecture and to have older siblings (Table 1). There were no differences between cases and control subjects with regard to age, smoking, or education.

There was no significant deviation from the Hardy–Weinberg equilibrium in the cases or in the control subjects ($P = 0.073$ and 0.206 , respectively).

Compared with a reference group of women with the TT genotype of *IL3* SNP rs40401, those with the CC genotype had a significantly increased risk of rhinoconjunctivitis, while no significant association was observed between the TC genotype and rhinoconjunctivitis (Table 2). The positive association was slightly strengthened after adjustment for the confounders under study: the adjusted OR for the CC genotype was 1.52 (95% CI: 1.05–2.19; $P = 0.025$). This positive relationship was significant under the additive model: the adjusted OR was 1.23 (95% CI: 1.02–1.47; $P = 0.027$). Under the dominant or recessive model, on the other hand, the positive association fell just short of the significance level ($P = 0.096$ and 0.051 , respectively). The statistical power calculation revealed that, using our sample size, we could detect the gene-disease association for an OR of 1.562 with an accuracy of more than 80% at a significance level of 0.05 with a two-sided alternative hypothesis under the recessive model.

There was no significant multiplicative or additive interaction between *IL3* SNP rs40401 and smoking with respect to rhinoconjunctivitis (Table 3).

Women with one or more older siblings had a significantly lower prevalence of rhinoconjunctivitis than those without older siblings did in this population [12]. Compared with subjects with the TT or TC genotype of *IL3* SNP rs40401 who had one or more older siblings, those with the CC genotype who had no older siblings had a 2.33-fold increased risk of rhinoconjunctivitis; nevertheless, neither the multiplicative nor the additive interaction was significant (Table 4).

4. Discussion

To our knowledge, this is the first study to show a significant positive association between *IL3* SNP rs40401 variant and the risk of rhinoconjunctivitis. A study of Koreans aged 7–80 years including 477 cases and 158 control subjects demonstrated that, compared with the TT genotype of *IL3* SNP rs40401, the CC genotype was significantly associated with a reduced risk of asthma [3]. In a study in China, the CC genotype of SNP rs40401 was significantly inversely related to the risk of asthma under the recessive model in a initial sample consisting of 170 cases and 347 control subjects, but this inverse relationship was not validated in another

Table 1
Characteristics of the study population.

Variable	n (%)		<i>P</i> ^a
	Cases (N = 393)	Controls (N = 767)	
Age, years, mean ± SD	31.4 ± 4.2	31.3 ± 4.3	0.728
Region of residence			0.011
Fukuoka Prefecture	221 (56.2)	438 (57.1)	
Other than Fukuoka Prefecture in Kyushu	148 (37.7)	245 (31.9)	
Okinawa Prefecture	24 (6.1)	84 (11.0)	
Presence of one or more older siblings	181 (46.1)	419 (54.6)	0.006
Having ever smoked	125 (31.8)	224 (29.2)	0.360
Education, years			0.664
<13	93 (23.7)	168 (21.9)	
13–14	133 (33.8)	253 (33.0)	
≥15	167 (42.5)	346 (45.1)	

^a χ^2 test or *t* test.**Table 2**
Odds ratios (ORs) and 95% confidence intervals (CIs) for rhinoconjunctivitis associated with *IL3* SNP rs40401 in Japanese women.

Model	Genotype	n (%)		<i>P</i> ^a	Crude OR (95% CI)	<i>P</i>	Adjusted OR (95% CI) ^b	<i>P</i>
		Cases (N = 393)	Controls (N = 767)					
Co-dominant	TT	113 (28.8)	255 (33.3)	0.100	1.00		1.00	
	TC	200 (50.9)	390 (50.9)		1.16 (0.88–1.53)	0.306	1.18 (0.89–1.56)	0.260
	CC	80 (20.4)	122 (15.9)		1.48 (1.03–2.12)	0.032	1.52 (1.05–2.19)	0.025
Additive					1.21 (1.01–1.44)	0.036	1.23 (1.02–1.47)	0.027
Dominant					1.23 (0.95–1.61)	0.120	1.26 (0.96–1.64)	0.096
Recessive					1.35 (0.99–1.85)	0.059	1.37 (0.998–1.88)	0.051

^a χ^2 test.^b Adjusted for age, region of residence, presence of older siblings, smoking, and education.**Table 3**
Interaction between *IL3* SNP rs40401 and smoking history affecting rhinoconjunctivitis in Japanese women.

Genotype	Smoking history					
	No			Yes		
	No. cases/controls	Adjusted OR (95% CI) ^a	<i>P</i>	No. cases/controls	Adjusted OR (95% CI) ^a	<i>P</i>
TT + TC	209/459	1.00	0.024	104/186	1.22 (0.90–1.64)	0.199
CC	59/84	1.54 (1.06–2.24)		21/38	1.26 (0.71–2.23)	0.431
<i>P</i> for multiplicative interaction = 0.26						
Measures of additive interaction ^b						
Relative excess risk due to interaction (RERI) = −0.50 (95% CI: −1.43-0.44)						
Attributable proportion due to interaction (AP) = −0.40 (95% CI: −1.30-0.50)						
Synergy index (S) = 0.34 (95% CI: 0.02-5.45)						

^a Adjusted for age, region of residence, presence of older siblings, and education.^b Statistically significant when the 95% CI of RERI > 0, the 95% CI of AP > 0, or the 95% CI of S > 1, indicating additive interaction.**Table 4**
Interaction between *IL3* SNP rs40401 and older siblings affecting rhinoconjunctivitis in Japanese women.

Genotype	Older siblings					
	Yes			No		
	No. cases/controls	Adjusted OR (95% CI) ^a	<i>P</i>	No. cases/controls	Adjusted OR (95% CI) ^a	<i>P</i>
TT + TC	141/339	1.00	0.466	172/306	1.40 (1.07–1.85)	0.016
CC	40/80	1.17 (0.76–1.80)		40/42	2.33 (1.44–3.77)	0.001
<i>P</i> for multiplicative interaction = 0.29						
Measures of additive interaction ^b						
Relative excess risk due to interaction (RERI) = 0.76 (95% CI: −0.39-1.90)						
Attributable proportion due to interaction (AP) = 0.32 (95% CI: −0.06-0.70)						
Synergy index (S) = 2.31 (95% CI: 0.63-8.46)						

^a Adjusted for age, region of residence, smoking, and education.^b Statistically significant when the 95% CI of RERI > 0, the 95% CI of AP > 0, or the 95% CI of S > 1, indicating additive interaction.

independent sample of 202 cases and 332 control subjects [6]. No significant association was found between SNP rs40401 and asthma in a study in China (423 cases and 114 control subjects) [4]. The current findings are at variance with these results.

IL3 SNP rs40401 leads to a change in amino acid at residue 8 from serine to proline; these different forms are available as recombinant proteins. Schweiger et al. [13] have shown that recombinant human IL-3/proline 8 induced enhanced proliferation of the

erythropoietic cell line TF-1 compared with recombinant human IL-3/serine 8, while there was a greater release of leukotrienes and IL-4 after blood leukocytes were primed with recombinant human IL-3/serine 8 rather than recombinant human IL-3/proline 8, and TNF- α production was also greater in response to recombinant human IL-3/serine 8. These results regarding mediator release are likely to support the previously cited findings showing a significant inverse relationship between the CC genotype of SNP rs40401 and asthma [3,6], but are not likely to be in agreement with the observed significant positive relationship between the CC genotype of SNP rs40401 and rhinoconjunctivitis.

This is the first study to assess gene-environment interactions in relation to *IL3* SNP rs40401 and allergic disorders; however, no significant interactions were observed between SNP rs40401 and smoking or older siblings affecting rhinoconjunctivitis although subjects with the CC genotype who had no older siblings had a 2.33-fold increased risk of rhinoconjunctivitis. A genome-wide association meta-analysis of allergic rhinitis found no consistent evidence that any SNPs modify the protective effect of increasing birth order [14].

The current study had methodological advantages in that study subjects were homogeneous in terms of all being pregnant and in that adjustment was made for several confounders.

There were several limitations. The participation rate cannot be calculated. Our subjects were probably not representative of Japanese women in the general population: for example, the distribution of educational status among participants differed considerably from that among the general population. According to the 2000 population census of Japan, the proportions of women aged 30–34 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 [15]. The corresponding figures for the control group in the current study were 21.9%, 33.0%, 45.1%, and 0.0%, respectively. Nevertheless, the distribution of SNP rs40401 was consistent with Hardy–Weinberg equilibrium and the selection bias associated with genotype distribution would be negligible.

The definition of rhinoconjunctivitis was based on the questions in the ISAAC questionnaire. The possibility of non-differential outcome misclassification might have biased the magnitude of the observed associations toward the null. The current study size was rather small for a valid genetic association study, although significant associations were detected. The lack of a significant additive interaction between SNP rs40401 and older siblings might be attributable to insufficient statistical power. Although adjustment was made for some confounders, residual confounding effects could not be ruled out.

The results of the present Japanese case-control study reveal that the CC genotype of *IL3* SNP rs40401 is significantly associated with an increased risk of rhinoconjunctivitis. Nevertheless, we could not find evidence for interactions between *IL3* SNP rs40401 and smoking or older siblings that affect rhinoconjunctivitis. The current findings must be confirmed by further epidemiological studies with larger numbers of subjects in addition to functional studies.

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References

- [1] Konno S, Hizawa N, Fukutomi Y, Taniguchi M, Kawagishi Y, Okada C, et al. The prevalence of rhinitis and its association with smoking and obesity in a nationwide survey of Japanese adults. *Allergy* 2012;67:653–60.
- [2] Broughton SE, Dhagat U, Hercus TR, Nero TL, Grimbaldston MA, Bonder CS, et al. The GM-CSF/IL-3/IL-5 cytokine receptor family: from ligand recognition to initiation of signaling. *Immunol Rev* 2012;250:277–302.
- [3] Park BL, Kim LH, Choi YH, Lee JH, Rhim T, Lee YM, et al. Interleukin 3 (*IL3*) polymorphisms associated with decreased risk of asthma and atopy. *J Hum Genet* 2004;49:517–27.
- [4] Zhang H, Zhang Q, Wang L, Chen H, Li Y, Cui T, et al. Association of *IL4R* gene polymorphisms with asthma in Chinese populations. *Hum Mutat* 2007;28:1046.
- [5] Kabisch M, Depner M, Dahmen I, Weiland SK, Vogelberg C, Niggemann B, et al. Polymorphisms in eosinophil pathway genes, asthma and atopy. *Allergy* 2007;62:423–8.
- [6] Hong X, Zhou H, Tsai HJ, Wang X, Liu X, Wang B, et al. Cysteinyl leukotriene receptor 1 gene variation and risk of asthma. *Eur Respir J* 2009;33:42–8.
- [7] Miyake Y, Tanaka K, Okubo H, Sasaki S, Arakawa M. Fish and fat intake and prevalence of depressive symptoms during pregnancy in Japan: baseline data from the Kyushu Okinawa Maternal and Child Health Study. *J Psychiatr Res* 2013;47:572–8.
- [8] Strachan D, Sibbald B, Weiland S, Ait-Khaled N, Anabwani G, Anderson HR, et al. Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International Study of Asthma and Allergies in Childhood (ISAAC). *Pediatr Allergy Immunol* 1997;8:161–76.
- [9] Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. *Stat Med* 2002;21:35–50.
- [10] Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. *Eur J Epidemiol* 2005;20:575–9.
- [11] Knol MJ, VanderWeele TJ, Groenwold RH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. *Eur J Epidemiol* 2011;26:433–8.
- [12] Miyake Y, Tanaka K, Arakawa M. Sibling number and prevalence of allergic disorders in pregnant Japanese women: baseline data from the Kyushu Okinawa Maternal and Child Health Study. *BMC Public Health* 2011;11:561.
- [13] Schweiger A, Stern D, Lohman IC, Baldini M, Martinez FD, Halonen M. Differences in proliferation of the hematopoietic cell line TF-1 and cytokine production by peripheral blood leukocytes induced by 2 naturally occurring forms of human IL-3. *J Allergy Clin Immunol* 2001;107:505–10.
- [14] Ramasamy A, Curjuric I, Coin LJ, Kumar A, McArdle WL, Imboden M, et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol* 2011;128:996–1005.
- [15] Statistics Bureau, Ministry of Public Management, Home Affairs, Posts and Telecommunications. 2000 Population Census of Japan, vol. 3–2–40, Labour Force Status of Population, Industry (Major Groups) of Employed Persons, and Education: Fukuoka-ken. Tokyo: Statistics Bureau, Ministry of Public Management, Home Affairs, Posts and, Telecommunications; 2002 [in Japanese].