Association Between 17q12-21 Variants and Asthma in Japanese Women: rs11650680 Polymorphism as Potential Genetic Marker for Asthma

Yoshihiro Miyake,¹ Keiko Tanaka,¹ and Masashi Arakawa²

Epidemiological evidence on the relationship between single-nucleotide polymorphisms (SNPs) rs7216389 and rs11650680 on chromosome 17q12-21 and asthma is inconsistent. We examined this issue in young adult Japanese women. Case subjects were 202 women who had been diagnosed with asthma by a doctor, while 1290 women without doctor-diagnosed asthma served as control subjects. Adjustments were made for age and the presence of older siblings. There were no significant associations between SNP rs7216389 and asthma. Compared with the CC genotype of SNP rs11650680, the CT genotype, but not the TT genotype, was significantly inversely associated with asthma: the adjusted odds ratio for the CT genotype was 0.67 (95% confidence interval: 0.46–0.96). This inverse relationship was significant in women with late-onset asthma, but not in those with early-onset asthma. Under the dominant model, a significant inverse association was found between rs11650680 and asthma in women without older siblings, but not in those with older siblings; the interaction, however, was not significant. This is the first study to show that the CT genotype of SNP rs11650680 was significantly inversely associated with asthma, especially adult-onset asthma. We could not find evidence for interactions between rs11650680 and older siblings affecting asthma.

Introduction

IN 2007, A GENOME-WIDE association study of 994 cases and 1243 controls from the United Kingdom and Germany observed strong relationships between multiple single-nucleotide polymorphisms (SNPs) on chromosome 17q21 and childhood asthma in family and case-referent panels (Moffatt *et al.*, 2007). Among the identified SNPs, SNP rs7216389, which is located in an intron of the *GSDMB* gene in the 17q12 region, was most strongly associated with both childhood asthma and transcription levels of the nearby *ORMDL3* gene in the 17q12 region (Moffatt *et al.*, 2007). Significant positive relationships between the TT genotype or the T allele of SNP rs7216389 and asthma were shown in the above-mentioned genome-wide association study (Moffatt *et al.*, 2007) as well as in several genetic association studies among Japanese individuals (Hirota *et al.*, 2008), UK children (Tavendale *et al.*, 2008), Danish children (Bisgaard *et al.*, 2009), individuals from Iceland, Australia, The Netherlands, Germany, the United Kingdom, and Korea (Halapi *et al.*, 2010), French children (Smit *et al.*, 2010), Korean children (Yu *et al.*, 2011), UK adults (Binia *et al.*, 2011; Marinho *et al.*, 2012), Chinese adults in Hong Kong (Sy *et al.*, 2012),

Chinese children (Yang *et al.*, 2012), and US children (Çalışkan *et al.*, 2013). In contrast, in two studies on white US children (Sleiman *et al.*, 2008) and Chinese adults (Fang *et al.*, 2011), a significant positive relationship between the C allele of SNP rs7216389 and asthma was observed. A study of Chinese children in Hong Kong found that, compared with the TT genotype of rs7216389, the TC genotype, but not the CC genotype, was significantly inversely associated with asthma and that rs7216389 was not related to atopy or total plasma IgE levels (Leung *et al.*, 2009). In a study of Korean children, a significant inverse association was found between the combination of TC and CC genotypes of rs7216389 and asthma in comparison with the TT genotype (Kang *et al.*, 2012). No significant association was found between SNP rs7216389 and asthma in two studies of African Americans (Galanter *et al.*, 2008; Sleiman *et al.*, 2008).

SNP rs11650680 is located in the promoter region of *TOP2A* in the 17q21.2 region. The above-mentioned genome-wide association study also found that SNPs rs7216389 and rs11650680 were independently strongly associated with asthma (Moffatt *et al.*, 2007). The C allele of rs11650680 was significantly inversely related to asthma in a case-control study of African Americans (Galanter *et al.*, 2008). Significant

¹Department of Preventive Medicine and Public Health, Faculty of Medicine, Fukuoka University, Fukuoka, Japan.

²Health Tourism Research Center, Graduate School of Tourism Sciences, University of the Ryukyus, Okinawa, Japan.

inverse associations were found between the TT genotype of rs11650680 and asthma, atopy, and total plasma IgE levels in a study of Chinese children in Hong Kong (Leung *et al.*, 2009). In a cross-sectional study of Korean children, compared with the CC genotype of rs11650680, the CT and TT genotypes combined were significantly inversely related to asthma, although no significant association was observed between rs11650680 and asthma in an independent case-control study (Kang *et al.*, 2012). There was no relationship between SNP rs11650680 and asthma in three studies of UK adults (Marinho *et al.*, 2012), Chinese children (Yang *et al.*, 2012), and Chinese individuals (Li *et al.*, 2012).

In this study, we investigated the associations between SNPs rs7216389 and rs11650680 and asthma in young adult Japanese women using data from the Kyushu Okinawa Maternal and Child Health Study (KOMCHS). In addition, we performed a haplotype analysis and examined the possibility of an interaction between the SNPs and the presence of older siblings.

Materials and Methods

Study population

The KOMCHS is a prospective prebirth cohort study. Details of the baseline survey of the KOMCHS have been described elsewhere (Miyake *et al.*, 2013). Eligible women were those who became pregnant while living in one of the seven prefectures on Kyushu Island in southern Japan, with a total population of ~13.26 million, or in the 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 week of pregnancy provided their written informed consent to participate in the KOMCHS and completed the baseline survey. Around 4 months after delivery, 1492 women provided informed consent to genotyping. The ethics committee of the Faculty of Medicine, Fukuoka University approved the KOMCHS.

Selection of cases and control subjects

In the baseline survey, each participant filled out a self-administered questionnaire and mailed the completed questionnaire to the data management center. Research technicians completed missing or illogical data by telephone interview. The questionnaire included questions on age, presence of older siblings, and asthma.

Among the 1492 women whose DNA samples were available, 202 cases were those who had answered yes to the question: "Have you ever been diagnosed by a physician as having asthma?" The remaining 1290 women served as control subjects.

DNA extraction and genotyping

Genomic DNA from buccal specimens collected with BuccalAmp swabs (Epicenter BioTechnologies, Madison,

WI) was extracted using a QIAmp DNA mini kit (Qiagen, Inc., Valencia, CA). Genotyping of SNPs rs7216389 and rs11650680 was performed using TaqMan SNP Genotyping Assays on the StepOnePlus machine (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions.

Statistical analysis

SNPs under study were tested for deviation from a Hardy-Weinberg equilibrium using the chi-square test. Linkage disequilibrium was examined using Haploview software version 4.2 (Broad Institute, Cambridge, MA) (Barrett *et al.*, 2005). Logistic regression analysis was performed to estimate the crude odds ratios (ORs) and 95% confidence intervals (CIs) for asthma relative to the SNPs under investigation, with the reference category being the homozygote of the major allele. Multiple logistic regression analysis was used to adjust for age and the presence of older siblings. The statistical power calculation was performed using QUANTO version 1.2 (Gauderman, 2002). Haplotypes and their frequencies were inferred according to the expectation maximization algorithm. For differences in haplotype frequency between the cases and control groups, crude ORs and 95% CIs were estimated based on the frequency of each haplotype relative to all other haplotypes combined. The interaction was tested using a term of the product of two variables in a multiple logistic regression model. Excluding the calculation of linkage disequilibrium and statistical power calculation, all statistical analyses were carried out using STATA/SE software version 12.0 (StataCorp, College Station, TX).

Results

Women with asthma were more likely to be older than the control subjects (Table 1). There was no difference between case subjects and control subjects with regard to the presence of one or more older siblings.

The distributions of SNPs rs7216389 and rs11650680 were in the Hardy-Weinberg equilibrium in the cases ($p=0.85$ and 0.12 , respectively) and in the control subjects ($p=0.26$ and 0.14 , respectively). SNPs rs7216389 and rs11650680 were not in linkage disequilibrium.

Compared with a reference group of women with the CC genotype of SNP rs11650680, those with the CT genotype had a significantly reduced risk of asthma, while the TT genotype was not related to asthma according to our crude analysis (Table 2). Adjustment for age and the presence of older siblings did not appreciably alter the results: the adjusted OR for the CT genotype was 0.67 (95% CI: 0.46–0.96, $p=0.029$). This inverse association fell just short of the significance level under the dominant model ($p=0.059$).

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION

Variable	Cases (n = 202)	Controls (n = 1290)	p-Value ^a
Age, years, mean $\pm$ SD	31.5 $\pm$ 4.2	30.6 $\pm$ 4.2	0.008
Presence of one or more older siblings, n (%)	99 (49.0)	675 (52.3)	0.38

^aChi-square test or *t*-test.

TABLE 2. ORs AND 95% CIs FOR ASTHMA IN RELATION TO SNPs rs7216389 AND rs11650680 IN JAPANESE WOMEN

SNP	Model	Genotype	n (%)		Crude OR (95% CI)	Adjusted OR (95% CI) ^a
			Cases (n=202)	Controls (n=1290)		
rs7216389	Codominant	TT	115 (56.9)	675 (52.3)	1.00	1.00
		TC	76 (37.6)	528 (40.9)	0.84 (0.62–1.15)	0.84 (0.61–1.14)
		CC	11 (5.5)	87 (6.7)	0.74 (0.38–1.43)	0.71 (0.37–1.38)
	Additive				0.85 (0.67–1.09)	0.84 (0.66–1.08)
		Dominant			0.83 (0.62–1.12)	0.82 (0.61–1.10)
		Recessive			0.80 (0.42–1.52)	0.77 (0.40–1.47)
rs11650680	Codominant	CC	155 (76.7)	907 (70.3)	1.00	1.00
		CT	41 (20.3)	358 (27.8)	0.67 (0.47–0.97)	0.67 (0.46–0.96)
		TT	6 (3.0)	25 (1.9)	1.40 (0.57–3.48)	1.45 (0.58–3.60)
	Additive				0.80 (0.59–1.09)	0.80 (0.58–1.09)
		Dominant			0.72 (0.51–1.02)	0.71 (0.50–1.01)
		Recessive			1.55 (0.63–3.82)	1.60 (0.64–3.96)

^aAdjusted for age and presence of older siblings.

CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

No significant relationships were observed between SNP rs11650680 and asthma under the additive or recessive model. There were no significant associations between SNP rs7216389 and asthma in any genetic model. With regard to the relationship between SNP rs7216389 and asthma under the additive model (the adjusted OR: 0.84 [95% CI: 0.66–1.08, $p=0.17$]), the statistical power calculation revealed that, using our sample size, we could detect the gene-disease association for an OR of 0.703 with an accuracy of more than 80% at a significance level of 0.05 with a two-sided alternative hypothesis under the log-additive model.

Four haplotypes were constructed, but none of these were significantly associated with asthma (Table 3).

There was one case with missing data on age at onset of asthma. Under the codominant model, an inverse relationship between the CT genotype of SNP rs11650680 and asthma was statistically significant in women who had developed asthma at the age of 18 or older ($n=72$), but not in those who had developed asthma at the age of 17 or younger ($n=129$); the adjusted ORs were 0.53 (95% CI: 0.28–0.997, $p=0.049$) and 0.76 (95% CI: 0.49–1.17, $p=0.21$), respectively.

Under the dominant model, a significant inverse association was found between SNP rs11650680 and asthma in women without older siblings, but not in those with one or more older siblings; the interaction, however, was not sig-

nificant (Table 4). There was no interaction between SNP rs7216389 and the presence of older siblings.

Discussion

To our knowledge, the current study is the first to show that, compared with the CC genotype of SNP rs11650680, the CT genotype, but not the TT genotype, was significantly associated with a reduced risk of asthma, especially adult-onset asthma. On the other hand, the present study failed to detect a significant association between SNP rs7216389 and asthma. Our results concerning SNP rs11650680 are not consistent with those of three case-control studies of Chinese children (152 cases and 190 controls) (Yang *et al.*, 2012), Chinese individuals (241 cases [11–80 years] and 212 controls [18–89 years]) (Li *et al.*, 2012), and Korean children (931 cases and 480 controls) (Kang *et al.*, 2012) or with those of a cross-sectional study of 983 UK adults (Marinho *et al.*, 2012) showing the null relationship between this SNP and asthma, those of a study of Chinese children in Hong Kong that showed a significant inverse association between the TT genotype, but not the CT genotype, and asthma (315 cases and 192 controls) (Leung *et al.*, 2009), and those of a study of African Americans showing a significant inverse relationship between the C allele and asthma (261 cases [12–24 years] and 176 controls [8–40 years]) (Galanter *et al.*, 2008), but are partially consistent with those of a cross-sectional study of 1797 Korean children (including 162 children who had been diagnosed by a physician as having asthma) that showed a significant inverse association between the combination of the CT and TT genotypes and asthma (Kang *et al.*, 2012). The present results concerning SNP rs7216389 are in agreement with those of the above-mentioned study of African Americans (Galanter *et al.*, 2008) and those of another case-control study of African American children (Sleiman *et al.*, 2008), which found a lack of association between this SNP and asthma, but are not consistent with those of several studies showing significant associations. The inconsistency of our findings with those of other studies may be, at least partly, explained by the differences in the genetic backgrounds of the populations examined, definitions of asthma, and statistical power.

TABLE 3. HAPLOTYPE ANALYSIS OF SNPs rs7216389 AND rs11650680 IN RELATION TO ASTHMA IN JAPANESE WOMEN

Haplotype ^a	n (%)		Crude OR (95% CI) ^b
	Cases (2n=404)	Controls (2n=2580)	
TC	265 (65.6)	1584 (61.4)	1.20 (0.96–1.51)
TT	41 (10.1)	294 (11.4)	0.88 (0.61–1.25)
CC	86 (21.3)	588 (22.8)	0.92 (0.70–1.19)
CT	12 (3.0)	114 (4.4)	0.66 (0.33–1.22)

^aHaplotype order is rs7216389 and rs11650680.

^bCrude OR for each haplotype is relative to all other haplotypes combined.

TABLE 4. ASSOCIATION BETWEEN SNPs rs7216389 AND rs11650680 AND ASTHMA, STRATIFIED BY PRESENCE OF OLDER SIBLINGS IN JAPANESE WOMEN

		Presence of older siblings				
		No		Yes		
SNP	Genotype	No. of cases/controls	Adjusted OR (95% CI) ^a	No. of cases/controls	Adjusted OR (95% CI) ^a	p-Value for interaction
rs7216389	TT	59/302	1.00	56/373	1.00	0.35
	TC+CC	44/313	0.71 (0.46–1.08)	43/302	0.95 (0.62–1.45)	
rs11650680	CC	81/419	1.00	74/488	1.00	0.27
	CT+TT	22/196	0.59 (0.36–0.97)	25/187	0.87 (0.54–1.42)	

^aAdjusted for age.

We are unable to explain why we observed a significant inverse association between the CT genotype of SNP rs11650680 and asthma. Kang *et al.* (2012) demonstrated that atopic asthmatic Korean children with the CT or TT genotype of rs11650680 showed lower eosinophil cationic protein levels than did those with the CC genotype, and that nonatopic asthmatic children with the CT or TT genotype of rs11650680 showed higher methacholine provocation test values than did those with the CC genotype. SNP rs11650680 might be related to eosinophilic inflammation and bronchial hyperresponsiveness. In the present study, an inverse relationship between the CT genotype of SNP rs11650680 and asthma was stronger in women with late-onset asthma than in those with early-onset asthma. This SNP might have more of an impact on nonatopic asthma than on atopic asthma; however, we were not able to distinguish atopic from nonatopic asthma because neither data on serum IgE levels nor skin prick test results were available in the current study.

SNP rs7216389 is associated with the expression levels of *GSDMB* and *ORMDL3* genes in white blood cells (Halapi *et al.*, 2010). There are also significant positive associations between the number of T alleles of rs7216389 and the expression levels of *GSDMB* and *ORMDL3* in human rhinovirus-stimulated peripheral blood mononuclear cells from adults (Çalışkan *et al.*, 2013). In cord blood mononuclear cells, compared with the CC genotype of SNP rs7216389, the TT genotype was significantly associated with increased Der p 1-activated *ORMDL3* gene expression and with increased Der p 1-induced and phytohemagglutinin-induced *GSDMA* gene expression; moreover, the TT genotype showed significantly increased IL-17 secretion in unstimulated and phytohemagglutinin-stimulated cord blood (Lluis *et al.*, 2011). Given these findings, as well as those of previous epidemiological studies showing significant relationships between SNP rs7216389 and asthma, the lack of significant inverse association between this SNP and asthma in the present study might be due to insufficient statistical power.

Although the interaction between SNP rs11650680 and the presence of older siblings affecting asthma was not significant, the inverse association between the combination of the CT and TT genotypes and asthma was significant only in women without older siblings. Given that the presence of older siblings is a factor in the hygiene hypothesis, there might be an interaction between this SNP and some types of

environmental factors related to the hygiene hypothesis with respect to asthma.

Certain methodological weaknesses of the current study should be borne in mind. In the baseline survey, the participation rate must have been fairly low, and our subjects were probably not representative of Japanese women in the general population. Nevertheless, the distribution of the two SNPs under investigation was consistent with the Hardy–Weinberg equilibrium, and any selection bias based on genotype distribution is expected to be negligible. The outcome in the present study was self-reported, doctor-diagnosed asthma. The possibility of nondifferential outcome misclassification would introduce a bias toward the null.

Conclusion

The current study showed that the CT genotype of *TOP2A* SNP rs11650680 was significantly inversely associated with asthma in comparison with the CC genotype, while the *GSDMB* SNP rs7216389 was not related to asthma in young adult Japanese women. There were no significant interactions between these SNPs and the presence of older siblings affecting asthma. We acknowledge that the present results need to be confirmed by both larger case–control studies with smaller likelihoods of selection bias and functional studies.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Yoshihiro Miyake, MD, PhD

Department of Preventive Medicine and Public Health

Faculty of Medicine

Fukuoka University

Fukuoka 814-0180

Japan

E-mail: miyake-y@fukuoka-u.ac.jp

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