



## Genetic variants on 17q21 are associated with asthma in a Han Chinese population

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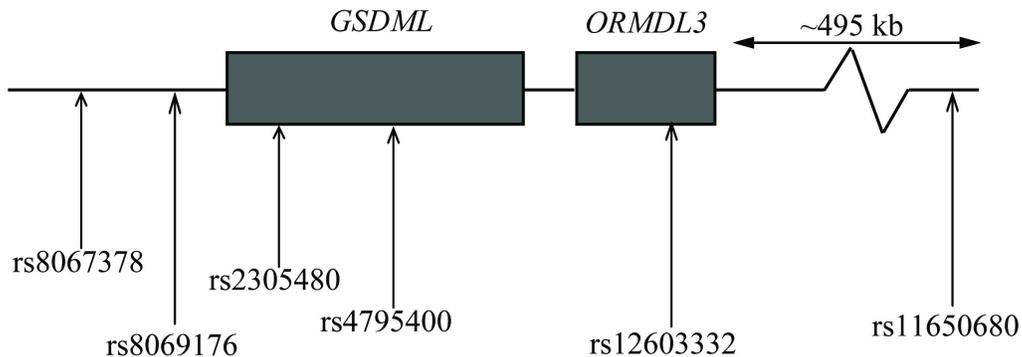
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**ABSTRACT.** A genome-wide study has shown an association between SNPs located on 17q21 and asthma. Such associations have been identified in several populations, but little is known about the Han Chinese population. We conducted a case-control study in a Han Chinese population to investigate the relationship between SNPs located on 17q21 and asthma; 241 asthmatic patients and 212 healthy controls were recruited from the outpatient clinics of the Nanfang Hospital, Guangdong Province, southern China. We genotyped six SNPs (rs8067378, rs8069176, rs2305480, rs4795400, rs12603332, and rs11650680) located on 17q21 with the Sequenom MassARRAY iPLEX platform. For two of these six loci (rs2305480 and rs8067378), there was evidence of association with asthma, and there was a weak association of asthma with rs8069176. We confirm that genetic variants on 17q21 are associated with asthma in the Han Chinese population.

**Key words:** Asthma; 17q21; Genetic variants; Susceptibility

## INTRODUCTION

Asthma is a disease of chronic airway inflammation. It is a serious public health problem throughout the world, affecting people of all ages. Its prevalence has increased during the second half of 20th century in most countries (Eder et al., 2006). Asthma is also a complex disease caused by multiple genetic and environmental factors. More than 100 genes have been reported to be associated with asthma, but few have been investigated in multiple populations (Ober and Hoffjan, 2006). In a whole-genome association study, Moffatt et al. (2007) identified a region on chromosome 17q21 that was strongly associated with asthma. Subsequently, a series of related studies were performed that highlighted this association in different ethnic groups (Sleiman et al., 2008; Madore et al., 2008; Galanter et al., 2008; Hirota et al., 2008; Tavendale et al., 2008; Leung et al., 2009). These findings suggested that orosomucoid 1 (ORM1)-like 3 (*ORMDL3*), gasdermin-like (*GSDML*), or adjacent genes located on chromosome 17q21 may be associated with asthma. However, these studies have mainly concentrated on Western populations. Our study aimed to investigate the associations between asthma and the identified SNPs on 17q21 in a Chinese population. In the present study, six SNPs in *GSDML*, *ORMDL3*, and the adjacent region were selected. Figure 1 shows the distribution of these six SNPs on the 17q21 region.



**Figure 1.** The distribution of six SNPs in our study along the chromosome 17q12-21 region.

## MATERIAL AND METHODS

### Subject recruitment

Patients and control subjects were obtained from individuals attending the outpatient clinics of Nanfang Hospital, Guangzhou, Guangdong Province, southern of China. All patients and control subjects were recruited between 2008 and 2010. The diagnosis of asthma was based on clinical symptoms, and clinical testing was performed by a physician on the basis of international asthma guidelines (GINA 2007). The control population consisted of outpatients without symptoms of asthma (wheezing, cough or dyspnea) and no diagnosis of asthma. The study protocol was approved by the Clinical Research Ethics Committee of Nanfang Hospital. Informed consent was obtained from all participants.

## Genotyping

Genomic DNA was extracted from 200  $\mu$ L peripheral blood using the Genomic DNA Purification kit (TIANGEN Biotech, Beijing, China) according to manufacturer instructions and stored at  $-70^{\circ}\text{C}$  until use. All SNPs were genotyped using the Sequenom MassARRAY matrix-assisted laser desorption/ionization time-of-flight mass spectrometry platform (Sequenom, San Diego, CA, USA). Primers were designed using a semi-automated method (Assay Design 3.1, Sequenom).

## Statistical analysis

Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD) and the pairwise LD coefficient for the six SNPs in the controls were calculated by Haploview 4.2 (Daly Lab., Cambridge, MA, USA). The differences in the genotype distributions between the cases and control subjects were evaluated using the  $\chi^2$  test. The associations between polymorphisms and asthma risk were estimated using the odds ratio (OR) and 95% confidence intervals (95%CI) using binary logistic regression analyses, controlling for age and gender as covariates. The statistical tests, haplotypes, and their associations with asthma were analyzed using the web-based tool SNPstats (<http://bioinfo.iconcologia.net/SNPstats>). The results obtained with this calculator were consistent with the SPSS 13.0 software and have been summarized in another publication (Li et al., 2011). All comparisons were two-sided, and  $P < 0.05$  was considered to be significant.

## RESULTS

### Study population

In total, 241 unrelated patients with bronchial asthma and 212 control individuals were enrolled. The patient group consisted of 118 men and 123 women, with an average age of 41.48 years (SD = 16.03; range = 11-80 years). The control group consisted of 160 men and 52 women, with an average of 40.65 years (SD = 20.29; range = 18-89 years). The details of the patients and control subjects are listed in Table 1.

**Table 1.** Characteristics of asthma patients and healthy controls.

Variable	Case subjects (N = 241)	Control subjects (N = 212)
Mean age (years; means $\pm$ SD)	41.98 $\pm$ 16.17	40.71 $\pm$ 20.17
Male % (N)	48.96 (118)	75.47 (160)*
FEV1 (means $\pm$ SD)	59.88 $\pm$ 24.30	ND
FVC (means $\pm$ SD)	76.91 $\pm$ 84.29	ND
Smoking (%)	62.30	ND

ND = not done. \* $P < 0.05$  by the  $\chi^2$  test.

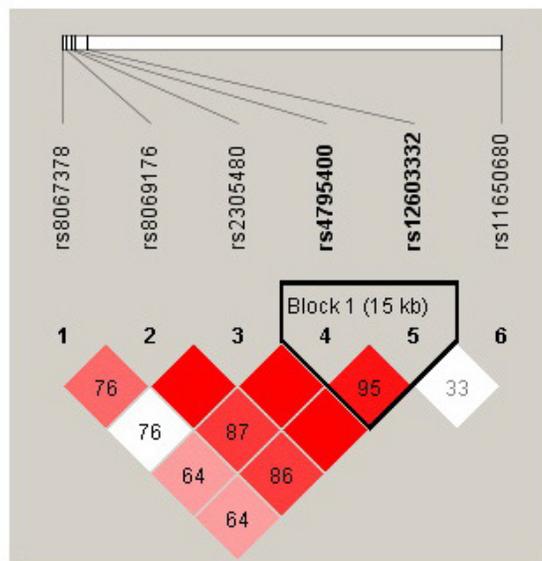
### Characterization of SNPs located on chromosome 17q21

The overall genotyping efficiency was 93.0%. Table 2 summarizes minor allele frequencies for the six SNPs. All SNPs followed HWE among the controls. The LD scores for

these SNPs among our control subjects were generally high for SNPs around the *GSDML* and *ORMDL3* genes (Figure 2). The SNP rs11650680, which is distant from the two genes, had a low LD compared to the other SNPs.

**Table 2.** Interethnic comparisons of minor allele frequencies for six SNPs at 17q21 in our subjects with HapMap data.

SNP	Minor allele	Present study		HapMap data			
		Control	Cases	Beijing	Japan	European	African
rs8067378	G	0.19	0.22	0.38	0.31	0.51	0.54
rs8069176	A	0.22	0.20	0.38	0.31	0.47	0.36
rs2305480	T	0.03	0.14	0.38	0.31	0.47	0.07
rs4795400	T	0.25	0.21	0.38	0.31	0.48	0.07
rs12603332	T	0.29	0.25	0.41	0.28	0.48	0.58
rs11650680	T	0.23	0.23	0.20	0.15	0.24	0.00



**Figure 2.** Linkage disequilibrium (LD) pattern among our 6 SNPs by the Haploview analysis. Numbers inside the boxes represent  $D'$  values for LD, and lacking numbers were 100.

### Asthma and individual polymorphisms

All genotype distributions for the six SNP markers in the case and control subjects are listed in Table 3. The relationship between asthma and these SNPs was analyzed by logistical regression in every chosen genetic model, and the co-dominant model is shown. Two of the six loci showed evidence of association (rs8067378 and rs2305480) with asthma. One locus, rs8069176, showed a weak trend toward an association with asthma. However, associations with asthma risk were not detected in the remaining three loci (rs4795400, rs12603332 and rs11650680).

**Table 3.** Six SNPs on 17q21 genotype distributions (%) and risk of asthma.

SNP	Genotype	Control (%)	Case (%)	OR* (95%CI)	P value
rs8067378	AA	117 (64.3)	119 (63.3)	1.00	0.041
	AG	61 (33.5)	55 (29.3)	0.95 (0.59-1.51)	
	GG	4 (2.2)	14 (7.5)	<b>3.88 (1.20-12.55)</b>	
rs8069176	GG	116 (58.0)	145 (64.4)	1.00	0.240
	AG	66 (33.0)	71 (31.6)	0.94 (0.61-1.44)	
	AA	18 (9.0)	9 (4.0)	0.48 (0.20-1.14)	
rs2305480	CC	193 (93.7)	181 (78.0)	1.00	<0.0001
	CT	13 (6.3)	39 (16.8)	<b>3.54 (1.79-7.01)</b>	
	TT	0 (0.0)	12 (5.2)	NA	
	CT+TT	13 (6.3)	51 (22.0)	<b>4.49 (2.32-8.71)</b>	
rs4795400	CC	115 (57.8)	139 (64.7)	1.00	0.620
	CT	67 (33.7)	61 (28.4)	0.81 (0.52-1.26)	
	TT	17 (8.5)	15 (7.0)	0.83 (0.38-1.80)	
rs12603332	CC	109 (52.7)	138 (59.2)	1.00	0.660
	CT	77 (37.2)	74 (31.8)	0.82 (0.54-1.26)	
	TT	21 (10.1)	21 (9.0)	0.86 (0.43-1.71)	
rs11650680	CC	127 (61.4)	137 (58.5)	1.00	0.480
	CT	65 (31.4)	86 (36.8)	1.18 (0.77-1.79)	
	TT	15 (7.2)	11 (4.7)	0.72 (0.31-1.67)	

OR = odds ratio; 95%CI = confidence interval at 95%. \*Adjusted for age and gender; P value calculated from chi-squared comparison of genotype frequencies in cases versus controls. The bold values indicate  $P < 0.05$ .

The most significant difference between the case and control groups in genotype frequency was identified for the SNP rs2305480 ( $P < 0.0001$ ). Compared to the CC genotype of rs2305480, the CT genotype conferred a covariate-adjusted 3.54-fold increased risk of asthma (95%CI = 1.79-7.01). Because the TT genotype was not detected in the control group, the OR for TT could not be calculated. The variant homozygous CT and TT genotypes of rs2305480 were significantly more frequent in asthmatic patients, and comparison of the CC genotype with the CT and TT genotypes revealed an increased risk of asthma (OR = 4.49; 95%CI = 2.32-8.71). The T allele of rs2305480 was also associated with an increased risk of asthma (OR = 4.13; 95%CI = 2.22-7.70) (data not shown). A significant difference in the genotype frequency for SNP rs8067378 was also seen between the case and control groups ( $P = 0.041$ ). The GG carriers of rs8067378 also showed an increased risk of asthma (OR = 3.38; 95%CI = 1.20-12.55) compared to the AA group. The best-fit model for rs8067378 was the recessive model, in which the GG genotype showed the higher risk of asthma (OR = 3.96; 95%CI = 1.24-12.65) compared to the AA and AG genotypes (data not shown).

Another SNP, rs8069176, showed a trend toward an association with asthma. The AA genotype of rs8069176 showed a decreased risk of asthma (OR = 0.48; 95%CI = 0.20-1.14) compared to the GG genotype. After stratification by gender, the AA genotype of rs8069176 showed a statistically significant decreased risk of asthma (OR = 0.29; 95%CI = 0.09-0.90) among male subjects (data not shown). The remaining three SNPs were not significantly associated with asthma (rs4795400, rs12603332 and rs11650680).

### Asthma and haplotypes located on chromosome 17q21

Six haplotype loci were assigned to the six SNPs in this study. Tables 4 and 5 summarize the analyses for individual haplotypes according to gender. The haplotype GACTTC and AACTTC were significantly protective against asthma, with the respective OR for the two

phenotypes being 0.29 and 0.11. The haplotype AACTTC was associated with a significant increase in the development of asthma, with an OR = 6.95. After stratification by gender, the statistically significant associations were only observed in male subjects for the haplotypes GACTTC and AACTTC.

**Table 4.** Relationship between asthma and haplotypes from six SNPs at 17q21 region (N = 453).

Haplotype	Frequencies			OR* (95%CI)	P value
	Total	Control	Case		
AGCCCC	0.504	0.497	0.512	1.00	
AGCCCT	0.156	0.167	0.144	0.84 (0.53-1.32)	0.440
GACTTC	0.071	0.116	0.036	<b>0.29 (0.12-0.72)</b>	0.008
AACTTC	0.032	0.045	0.013	<b>0.11 (0.02-0.72)</b>	0.022
GATTTC	0.031	NA	0.048	<b>6.95 (1.77-27.33)</b>	0.006

OR = odds ratio; 95%CI = confidence interval at 95%. Haplotypes were constructed from the following SNPs: rs8067378, rs8069176, rs2305480, rs4795400, rs12603332, and rs11650680. Only haplotypes with  $\geq 0.03$  frequencies were used. \*Adjusted for age and gender. The bold values indicate  $P < 0.05$ .

**Table 5.** Relationship between asthma and haplotypes from six SNPs within gender.

Haplotype	Frequency	OR* (95%CI)	
		Female	Male
AGCCCC	0.504	1.00	1.00
AGCCCT	0.156	1.07 (0.51-2.28)	0.69 (0.39-1.23)
GACTTC	0.073	0.26 (0.06-1.06)	<b>0.24 (0.08-0.74)</b>
AACTTC	0.031	0.45 (0.04-5.33)	<b>0.10 (0.01-0.78)</b>
AGCTTC	0.030	1.37 (0.27-6.89)	1.50 (0.42-5.36)

OR = odds ratio; 95%CI = confidence interval at 95%. Haplotypes were constructed from the following SNPs: rs8067378, rs8069176, rs2305480, rs4795400, rs12603332, and rs11650680. \*Adjusted for age. The bold values indicate  $P < 0.05$ .

## DISCUSSION

The present study provides further data to support the association between asthma and variants located on chromosome 17q21. In this study, the most strongly associated SNPs were rs2305480 and rs8067378, which is consistent with recent studies (Flory et al., 2009; Bisgaard et al., 2009; Halapi et al., 2010). Additionally, Bouzigon et al. (2008) reported a significant association between rs2305480 and asthma in a French population. With regard to the SNP rs8067378, Moffatt et al. (2007) reported that rs8067378 was the most strongly associated marker for asthma in a family-based test. The association of rs8067378 with childhood asthma was also observed in a study investigating North Americans of European ancestry (Sleiman et al., 2008).

The two associated SNPs in this study, rs2305480 and rs8067378, are both located in *GSDML*, the first in an exon and the second upstream of the gene. The most prominent SNP, rs7216389, has been strongly associated with asthma in GWAS (Moffatt et al., 2007), also located in an intron of the *GSDML* gene. *GSDML* encodes a member of the gasdermin domain-containing protein family, whose function is currently unclear. Moffatt et al. (2007) found that the polymorphisms in *GSDML* and the transcript levels of *ORMDL3* were significantly associ-

ated with childhood asthma. It has been suggested that *GSDML* SNPs modify *ORMDL3* expression. Subsequently, Halapi et al. (2010) reported that several sequence variants of 17q21, including rs2305480 and rs8067378, are associated with asthma and affect the expression levels of both *GSDML* and *ORMDL3*. *ORMDL3* is a member of the gene family that encodes transmembrane proteins anchored in the endoplasmic reticulum, but their function is also unclear (Hjelmqvist et al., 2002). The variants in the *ORMDL3* gene associated with asthma have received little attention, and only two SNPs have been investigated: rs4378650 (Galanter et al., 2008; Wu et al., 2009) and rs12603332 (Galanter et al., 2008). Moffatt et al. (2007) initially described *ORMDL3* as an asthma susceptibility gene, but none of the associated SNPs in this study are directly located at *ORMDL3*. An increasing number of researchers have considered that the association between 17q21 and asthma may involve genes other than *ORMDL3* in this region (Wjst, 2008; Leung et al., 2009; Halapi et al., 2010). In the present study, high linkage was found between these SNPs in *ORMDL3* and *GSDML*. It is suggested that these variants may be co-regulated in gene expression of *GSDML* and *ORMDL3*, contributing to asthma susceptibility. Another SNP, rs8069176 upstream of the *GSDML* gene, has been found to be associated with asthma in a French population (Bouzigon et al., 2008). This association was not found among all subjects but in male subjects, along with the AA protective effect, similar to the study of Bouzigon et al. (2008).

A study by Galanter et al. (2008) in Mexicans and African Americans showed an association for rs12603332 and rs11650680. However, our data did not show such an association. The reason for this may be the difference in ethnic groups, or it may be that a larger sample needs to be investigated to provide sufficient statistical power to detect the association. This may also explain the discrepancy between our data and those from another study in children from Hong Kong (Leung et al., 2009), in which rs11650680 was shown to be associated with asthma. Asthma is a complex disease associated with numerous genetic and environmental factors. Our use of adult subjects may have weakened the power to detect significant genetic associations.

In conclusion, we confirmed the existence of genetic associations between asthma and the chromosome 17q21 in a Han Chinese population. Significant associations between two SNPs, rs2305480 and rs8067378 in the *GSDML* gene, and asthma were found in this study. Although the biological mechanism behind these associations remains unknown, the polymorphisms provide a predictor that may be useful for future targeted research into the prevention of asthma.

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