

A polymorphism controlling *ORMDL3* expression is associated with asthma that is poorly controlled by current medications

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Background: The specific genetic contributions to childhood asthma have been difficult to elucidate. A recent whole-genome association study suggested that single nucleotide polymorphisms at loci controlling the expression of the *ORMDL3* gene and others in the neighborhood of the *NRG1* and *ERO1LB* genes might be important.

Objective: We sought to replicate the associations of these genetic markers with asthma in a large population of asthmatic patients from Scotland and to assess the effect of these variants on asthma outcomes.

Methods: Using mouthwash-derived DNA and clinical interviews and measurements, we investigated the association of 3 single nucleotide polymorphisms in the candidate genes with susceptibility to asthma in a case-control study and also exacerbations in a group of 1054 patients aged 3 to 22 years.

Results: A common C/T polymorphism at a locus controlling *ORMDL3* gene expression (rs7216389) was significantly associated with the risk of childhood asthma ($P = 1.73 \times 10^{-12}$), with a single copy of the T allele conferring an odds ratio of 1.50 (95% CI, 1.24–1.81) and 2 copies of the T allele conferring an odds ratio of 2.11 (95% CI, 1.71–2.61), respectively. In asthmatic patients the T allele was associated with exacerbations of the condition ($P = .008$). Polymorphisms at the loci of nearby genes for *NRG1* (rs4512342) and *ERO1LB* (rs10924993) were associated with neither the occurrence of nor exacerbations of asthma.

Conclusion: A common genetic variation at a locus controlling the expression of the *ORMDL3* locus increases the susceptibility to asthma and is associated with poor control of the condition in children and young adults. (J Allergy Clin Immunol 2008;121:860–3.)

Key words: Asthma, *ORMDL3*, genetics, single nucleotide polymorphism, susceptibility, exacerbation

Asthma is a common chronic respiratory disorder in children and young adults and is particularly prevalent in Scotland.¹ Development of the condition is influenced by both genetic and environmental factors.² Although the investigation of genetic components of asthma has been facilitated by the introduction of chip-based high-throughput genotyping platforms and many putative single nucleotide polymorphism (SNP) markers have been identified in whole-genome association studies,^{3–6} it is crucial that replication studies are carried out in different populations to verify any discovered relationships between genotype and the presence of asthma.

Recently, in a whole-genome association study of 994 asthmatic children and 1243 control subjects, Moffatt et al⁷ identified 12 SNPs that passed a 1% false-discovery rate threshold. Seven of these SNPs mapped to a locus on 17q21, which determines expression of the *ORMDL3* gene. This is a member of a family of genes responsible for encoding transmembrane proteins of the endoplasmic reticulum. Two of the remaining SNPs mapped to loci relating to the *NRG1* and *ERO1LB* genes.

In a replication study, we used the BREATHE^{8,9} cohort of children and young adults with asthma and control subjects from the local population to validate SNPs in these 3 genes as asthma susceptibility markers. In addition, we used clinical data from the asthma cohort to investigate any association between these markers and the occurrence of exacerbations of the condition.

METHODS

Children and young adults (3–22 years) with physician-diagnosed asthma recruited into the BREATHE study^{8,9} formed the group of cases in this investigation. Participants attended primary or secondary clinics in 14 primary care practices and a secondary care asthma clinic in either Tayside or Dumfries. From 2004 through 2006, we collected mouthwash samples for DNA analysis from 1307 individuals; demographic, anthropometric, and clinical details were available for 1054 of these participants. A detailed history was obtained, including information on symptoms, treatment, and exacerbations. The asthma-prescribing level was determined in accordance with the British Thoracic Society (BTS) guidelines¹⁰ for physician-led management of asthma, as follows: step 0, no use of inhaled albuterol on demand within the past month; step 1, inhaled albuterol on demand; step 2, regular inhaled steroids plus inhaled albuterol on demand; step 3, regular inhaled salmeterol plus inhaled steroids with inhaled albuterol on demand; and step 4, regular inhaled salmeterol plus inhaled steroids plus oral montelukast with inhaled albuterol on demand. An asthma exacerbation was defined as the presence of 1 or more of the following 3 indicators during the period of study: (1) asthma-related hospital admission, (2) 1 or more days of asthma-related absence from school, and (3) prescribed course of oral steroids. All participants were of northern European origin. The study was approved by the Tayside Committee on Medical Research Ethics. Informed consent was provided by the patient and the

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Abbreviations used

BTS: British Thoracic Society

OR: Odds ratio

SNP: Single nucleotide polymorphism

parent/guardian, as relevant. The control population consisted of a random sample of 1593 specimens from a previous study of 2454 schoolchildren from the Tayside area, which investigated the role of genes in growth and development, as described previously,^{11,12} and clinical details were available for 1465 of the genotyped individuals. In addition, we determined rs7216389 allele frequencies in a random sample of 1494 adult nondiabetic specimens from a Tayside diabetes study population.¹³

DNA in the mouthwash specimens was extracted by using QIAamp 96 DNA blood kits (Qiagen, Hilden, Germany). Allelic discrimination assays for SNPs relating to *ORMDL3* expression (rs7216389), *NRG1* (rs4512342), and *ERO1LB* (rs10924993) were carried out by using the Applied Biosystems Custom Taqman SNP Genotyping assays (c/n 4332072) and a 7700 Sequence Detection System. All variants were in Hardy-Weinberg equilibrium ($P > .05$), and genotyping call rates ranged from 97% to 99%. Statistical analysis was carried out with SPSS for Windows version 14 (SPSS, Inc, Chicago, Ill). Differences in the genotype distributions between the groups of cases and control subjects were evaluated by using the χ^2 test. Logistic regression analysis controlling for age and exposure to cigarette smoke was used to determine odds ratios (ORs) for the occurrence of any exacerbation. The population attributable fraction was calculated by using the following equation: $\text{Population attributable fraction} = ((OR - 1)P) / (((OR - 1)P) + 1)$, where *OR* is the OR associated with the genotype and *P* is the allele frequency in the control population.

RESULTS

The characteristics of 1054 asthmatic patients and 1465 control subjects are listed in Tables I and II. The asthmatic group has good representation of mild-to-severe cases, as indicated by the numbers in each BTS treatment group. Although lung function test data show that in general asthma was well controlled in this group of patients, 37% had at least 1 asthma exacerbation event during the study period. The genotype distributions for the putative SNP markers in cases and population control subjects are listed in Table III. Although there was no difference in the genotype frequency for the SNPs rs10924993 and rs4512342, the T allele at rs7216389 was significantly enriched in asthmatic patients. In a local population of adults without type 2 diabetes, 1494 specimens were genotyped for rs7216389, producing a CC/CT/TT allele distribution of 422/740/332 and giving a T allele frequency of 0.470 (0.452-0.488), which is very similar to that observed in the local population of schoolchildren. In children, compared with the CC homozygous group, a single C/T substitution resulted in an OR for physician diagnosis of asthma of 1.50 (95% CI, 1.24-1.81; $P = 1.33 \times 10^{-5}$). There was an increase in risk of similar magnitude (OR, 1.41; 95% CI, 1.18-1.69; $P = 1.84 \times 10^{-4}$) on comparing the TT homozygous group with the CT heterozygotes. Thus the risk associated with the TT allele was double that of the CC allele (OR, 2.14; 95% CI, 1.71-2.61; $P = 1.81 \times 10^{-12}$; Table IV). By using binary logistic regression in a codominant (additive) model, the rs7216389 genotype conferred an allelic OR of 1.53 (95% CI, 1.34-1.75; $P = 2.54 \times 10^{-10}$), resulting in a population attributable fraction of 0.197.

There was no association between any of the SNPs studied and indices of lung function, possible allergic triggers of asthma, BTS treatment step, and family history (data not shown). In binary

TABLE I. Summary characteristics of 1054 asthmatic patients and 1465 population control subjects

	Asthmatic patients	Control subjects
Sex (male/female), N	624/430	741/724
Age (y)*	10.4 (7.0-13.1)	7.5 (6.6-8.5)
BMI (kg/m ²)*	19.2 (16.1-21.0)	17.2 (15.7-18.1)

*Mean (25th-75th percentile).

TABLE II. Clinical details of asthmatic patients

Lung function	Mean (25th-75th percentile)	n
Peak expiratory flow rate (% predicted)	87.9 (76.9-98.8)	833
FEV ₁ (% predicted)	95.8 (85.9-105.8)	836
Forced vital capacity (% predicted)	92.2 (83.2-101.3)	835
BTS step of treatment	N (%)	
0	31 (3.0)	
1	182 (17.5)	
2	588 (56.6)	
3	141 (13.6)	
4	96 (9.2)	
Asthma exacerbation in preceding 6 mo	N (%)	
Any hospital admission	118 (11.2)	
Any school absence	315 (29.9)	
One or more courses of oral steroids	214 (20.3)	
Any exacerbation	368 (34.9)	

logistic regression, although there were no significant associations of the rs10924993 and rs4512342 SNPs with the occurrence of asthma exacerbations, the genotype of the rs7216389 SNP did have a significant association with asthma exacerbations (Table V-VII). Both school absence (OR, 1.25; 95% CI, 1.02-1.53; $P = .03$) and oral steroid use (OR, 1.32; 95% CI, 1.04-1.66; $P = .02$) over the previous 6 months were individually associated with the rs7216389 genotype, and the risk of any exacerbation was 1.30 (95% CI, 1.07-1.59; $P = .008$). Although the best-fitting model was the codominant model, a single C/T substitution did not significantly increase the risk of the occurrence of any asthma exacerbation (OR, 1.11; 95% CI, 0.765-1.618; $P = .578$); however, the TT homozygote was associated with increased risk over both the CT (OR, 1.469; 95% CI, 1.076-2.006; $P = .0156$) and CC genotypes (OR, 1.634; 95% CI, 1.098-2.431; $P = .0154$).

DISCUSSION

In this study of possible genetic markers of susceptibility to childhood asthma, we confirm that the rs7216389 C/T polymorphism at locus 17q21 controlling *ORMDL3* gene expression is strongly associated with the occurrence of the condition. The effect is dose dependent with each C to T substitution at the SNP site, increasing the odds of the occurrence of asthma by about 50%, with the homozygous T allele being associated with double the odds of the homozygous C allele. The strength of the association is underlined by considering that our asthmatic population represented the full continuum of the severity of the condition, with mild-to-severe cases equally represented.

TABLE III. Genotype distribution in asthmatic patients and population control subjects

Locus/group	Genotype/n						
rs7216389(C/T)*	CC	CT	TT	n	HWE†	AAF‡	P value
Asthmatic patients	251	627	401	1279	0.834	0.559	1.73×10^{-12}
Control subjects	450	751	340	1541	0.425	0.464	
rs10924993(T/G)*	TT	TG	GG	n	HW	AAF	P value
Asthmatic patients	411	609	274	1294	0.084	0.553	.901
Control subjects	488	733	329	1550	0.082	0.551	
rs4512342(T/G)*	TT	TG	GG	n	HW	AAF	P value
Asthmatic patients	1007	251	24	1282	0.075	0.117	.847
Control subjects	1220	316	21	1557	0.916	0.115	

*Ancestral alleles are bold and underlined, as defined from dbSNP build 128.

†Hardy-Weinberg equilibrium χ^2 P value.

‡AAF ancestral allele frequency.

TABLE IV. *ORMDL3* genotype and risk of asthma

Risk	Reference	OR	95% CI	P value
CT	CC	1.497	1.241–1.805	1.33×10^{-5}
TT	CT	1.413	1.181–1.690	1.84×10^{-4}
TT	CC	2.114	1.711–2.612	1.81×10^{-12}

Asthma is a complex condition with several contributing pathologic processes, including airway remodeling, allergic response, atopy, and inflammation. Genetic variation in all of these processes and individual variation in response to pharmacologic therapies combine to form the range of phenotypes seen in the condition. Candidate asthma susceptibility genes,¹⁴ such as *ADAM33*,¹⁵ *IL13*,¹⁶ filaggrin null mutations,^{12,17,18} and others, typically confer increased risk with ORs in the range of 1.2 to 2.0. That each variation conveys a modest increase in risk confirms the multifactorial nature of the condition. To explain more of the genetic contribution, whole-genome association studies are useful in identifying novel candidates. The strength of these studies lies in the discovery of new putative markers in previously unexplored areas of the genome. However, the scale of these analyses is such that the number of false-positive associations is a major problem.¹⁹ This is reinforced by our finding that 2 loci reported with P values of association of less than 10^{-6} did not replicate in a large well-powered study. It is important that initial discoveries in whole-genome analyses are verified in substantial replication studies, regardless of the estimated false-discovery rate.

In our study we found that the frequency of the T allele in the group of asthmatic patients was 0.559, which is lower than the value (0.62) reported by Moffat et al.⁷ A possible explanation is that although the severity of asthma in our group of cases ranged from mild to severe, that in the group reported by Moffat et al was step 3 asthma or worse. In addition, the T allele frequency in our control schoolchildren (0.464) was less than that observed in the unaffected individuals in the initial study (0.52). This might be due to the family-based structure of this study, in which families enriched for asthma phenotypes would be enriched for this risk allele, regardless of affected status. Our estimate of the T allele frequency in the control children was validated when we found a comparable value (0.470) in a local population of adult control subjects. A similar frequency (0.48) has been reported in the National Center for Biotechnology Information rs database, and

TABLE V. *ORMDL3* genotype and risk of specific asthma exacerbations

Clinical outcome	OR	95% CI	P value
Hospital admission	1.283	0.955–1.724	.098
School absence	1.251	1.022–1.530	.030
Oral steroid use	1.320	1.048–1.662	.018

TABLE VI. *ORMDL3* genotype distribution and any asthma exacerbation

	Genotype/n (%)			OR (CI)*	P value
	CC	CT	TT		
No exacerbations	140 (70.7)	311 (65.9)	172 (57.0)		
Any exacerbation	58 (29.3)	161 (34.1)	130 (43.0)	1.304 (1.071–1.587)	.008

*From binary logistic regression adjusted for age and exposure to cigarette smoke.

TABLE VII. *ORMDL3* T allele and risk of any asthma exacerbation

Risk	Reference	OR	95% CI	P value
CT	CC	1.112	0.765–1.618	.578
TT	CT	1.469	1.076–2.006	.0156
TT	CC	1.634	1.098–2.431	.0154

in addition, there is corroborating evidence in the National Institutes of Health Cancer Genetic Markers of Susceptibility whole-genome association study, where the T allele frequency was found to be 0.487 in 2000 cancer-free individuals from the United States.^{20,21} In any study of genetic susceptibility, population stratification is a consideration. One advantage of our study is that we have a relatively genetically homogeneous population, thus minimizing the chance that our results occur because of stratification. In general, population stratification has proved not to be a problem in United Kingdom-wide studies of asthma and other common diseases,²² and the results reported here reflect those observed in the German population.⁷

In addition to its effects on asthma susceptibility, our study shows that the rs7216389 polymorphism is associated with the risk of asthma exacerbations. The biologic mechanism behind these associations remains to be explained. Although the SNP is located within the first intron of *GSDML*, the focus has been on the fact that the SNP is strongly associated with the expression of the neighboring gene *ORMDL3* but is not associated with the expression of the *GSDML* gene.⁷ *GSDML*, however, encodes the protein gasdermin B. Gasdermins are a family of proteins implicated in epithelial barrier function and skin differentiation, and therefore it will be important to determine whether the asthma association is due to changes in the function of the *GSDML* gene.²³ Little is known about the *ORMDL* family of genes other than that they are responsible for encoding transmembrane proteins of the endoplasmic reticulum. Given that the gene is highly expressed in lymphocytes, it is possible there might be a role for this gene on the immunologic aspects of asthma. The possibility that another SNP in linkage disequilibrium with rs7216389 might underlie the relationship reported here cannot be entirely ruled out. Polymorphic variation in a number of different genes, like that for peroxisome proliferator activated receptor γ ,²⁴ have been shown recently to regulate exacerbation risk in the same population. Asthma exacerbation risk is also regulated by

β -adrenoceptor genotype in those receiving regular treatment with inhaled β_2 -agonists.^{25,26} Functional variation within a number of proteins, occurring as a result of population variation in polymorphisms, could thus act as a significant determinant for asthma exacerbation risk in children and young adults with asthma. Because asthma exacerbations are responsible for a significant component of costs within the health service and arguably constitute the most important clinical outcome asthma physicians seek to control,²⁵ the further understanding of the complex interplay between the above mechanisms constitutes a major research goal. Interestingly, unlike our results with polymorphic variation in the filaggrin gene, there was no evidence of association of *ORMDL3* genotype and parent-reported eczema, suggesting that the mechanisms underlying exacerbation risk might not be driven through the atopic pathway.

Although our findings regarding the replication of the association provide a high degree of certainty as to their robust nature, it is clear that our findings with regard to exacerbations are less robust. Indeed, these analyses would not be significant after correction for multiple testing when including the tests on the nonreplicating SNPs, but the strong evidence of the association of rs7216389 with asthma provides plausible *a priori* evidence for involvement in these secondary measures of the condition. The fact remains, however, that the role of these variants in asthma control will have to be confirmed by replication in other studies.

In summary, we confirm that the rs7216389 SNP controlling expression of the *ORMDL3* gene is robustly associated with asthma susceptibility in children and young adults and, given its association with increased exacerbations on current medications, might in the future provide an alternative pathway for the targeting of therapeutics for the management of asthma.

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Key Messages

- Polymorphism at a locus controlling the expression *ORMDL3* is a robust predictor for physician-diagnosed asthma in children and young adults.
- In asthmatic patients, the T allele of the polymorphism is associated with exacerbations of the condition.

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