

Letter to the Editor

Genetic polymorphism regulating ORM1-like 3 (*Saccharomyces cerevisiae*) expression is associated with childhood atopic asthma in a Japanese population

To the Editor:

Bronchial asthma is a complex disease caused by a combination of genetic and environmental factors.¹ A recent study using genome-wide association analysis has shown that single nucleotide polymorphisms (SNPs) on the chromosome 17q21.1 locus contribute to the risk of childhood asthma.² Genetic variants of the region were strongly associated in *cis* with transcript levels of *ORM1-like 3* (*S. cerevisiae*; *ORMDL3*) in EBV-transformed lymphoblastoid cell lines from children with asthma.² The *ORMDL3* transcripts were strongly and positively associated with the SNPs ($P < 10^{-22}$ for rs7216389) in the disease-associated locus, and rs7216389 was the marker most strongly associated with the disease in combined genome-wide association analysis.² *ORMDL3* is a member of a gene family that encodes transmembrane proteins anchored in the endoplasmic reticulum; however, the gene function is unclear.³ It was reported that *ORMDL3* was expressed in many tissues, particularly in liver and peripheral blood lymphocytes.² In the induction of asthma, the important role of respiratory tract infections has been recognized.^{4,5} Clinical studies suggest that the proportion of virus-induced asthma exacerbations is likely to be around 80% to 85% in school-age children.⁶ To assess whether the genetic variations regulating *ORMDL3* expression contribute to the susceptibility in childhood atopic asthma in a Japanese population, we conducted association studies using the marker rs7216389. A total of 545 children with atopic asthma were recruited, and all subjects with asthma were diagnosed according to the criteria of the National Institutes of Health (mean age, 9.5, 3.6-17 years; male:female ratio, 1.49:1.0).⁷ The mean $\log_{10}$ (total IgE [IU/mL]) of patients was 2.75. A total of 738 healthy subjects who had neither respiratory symptoms nor a history of asthma-related diseases (mean age, 49, 19-78 years; male:female ratio, 2.68:1.0) were recruited after being interviewed by physicians regarding whether they had been diagnosed with asthma, atopic dermatitis, or nasal allergies. The profiles of patients with asthma and controls were provided in our recent report.^{8,9} All individuals were unrelated Japanese and gave written informed consent to participate in the study according to the rules of the process committee at the Institute of Physical and Chemical Research. Genotyping of the SNP was performed by the TaqMan allele-specific amplification (TaqMan-ASA) method (Applied Biosystems, Foster City, Calif). rs7216389 was genotyped by using TaqMan(R) Pre-Designed SNP Genotyping Assays, C_29062108_10. We calculated allele frequencies and tested agreement with Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test. We then compared differences in the allele frequencies and genotype distribution of the polymorphism between case and control subjects by using a contingency χ^2 test and calculated odds ratios (ORs) with 95% CIs. All genotype frequencies are shown in Table I. We found a significant association between the rs7216389 genotype and children with atopic asthma (T allele vs C allele frequency, OR, 1.44; 95% CI, 1.20-1.73; $\chi^2 = 15.6$; $P = .000080$;

TABLE I. Genotype frequencies for rs7216389 and childhood atopic asthma susceptibility

Genotype	Cases (%) (n = 545)	Controls (%) (n = 738)	P value OR (95% CI)			
			TT vs TC vs CC	TT vs TC + CC	TT + TC vs CC	T allele vs C allele
TT	338 (62.0)	370 (50.1)	.00013	.000023	.181	.000080
TC	176 (32.3)	312 (42.3)		1.62		1.44
CC	31 (5.7)	56 (7.6)		(1.30-2.03)		(1.20-1.73)
T	852 (78.2)	1052 (71.3)				
C	238 (21.8)	424 (28.7)				

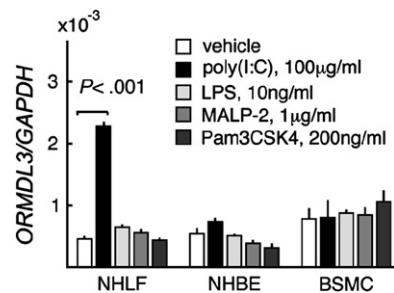


FIG 1. Induction of *ORMDL3* expression in NHLF in response to poly(I:C). Quantitative RT-PCR assay of *ORMDL3*. Normal human bronchial epithelial cells, NHLF, and bronchial smooth muscle cells were stimulated with 10 μ g/mL poly(I:C), 100 ng/mL LPS, 1 μ g/mL macrophage-activating lipopeptide 2 (MALP-2), or 200 ng/mL Pam3CSK4 for 24 hours. We used the following primer sets for RT-PCR: 5'-AAGGCGAGGCTGCTAAC-3' and 5'-TGTA-GAAGCTGGTGAGGAAGTACA-3'. Data represent means $\pm$ SDs of triplicate samples. mRNA expression analysis was performed with the Student *t* test. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

TT vs TC+CC, OR, 1.62; 95% CI, 1.30-2.03; $\chi^2 = 17.9$; $P = .000023$; Table I). In our study, the T nucleotide allele at rs7216389 had a frequency of 78% among subjects with asthma compared with 71% in subjects without asthma, and the direction of association was similar to findings in a previous study.² Serum total IgE levels were analyzed as quantitative levels, and we investigated associations between these levels and genetic variation within patients with childhood asthma. Multiple comparisons were first analyzed by the Kruskal-Wallis test, and correlations were analyzed by the Spearman test. However, we could not find any association between the genetic variant and serum IgE levels of patients with asthma in this study. We next assessed whether the *ORMDL3* could be induced in lung-derived cells by some pathogen-associated microbial patterns recognized by Toll-like receptors and other pattern-recognition receptors as described.¹⁰ We investigated the *ORMDL3* expression in lung-derived primary cells, including normal human bronchial epithelial cells, normal human lung fibroblasts (NHLF), and normal human bronchial smooth muscle cells (Fig 1). In NHLF, the expression level of *ORMDL3* was strongly induced for 24 hours by stimulation with polyinosine-polycytidylic acid but not by LPS, macrophage-activating lipopeptide 2, or Pam3CSK4 (Fig 1). These results implied that the high induction of *ORMDL3* in lung fibroblasts might play a role in viral respiratory infections.

We found a significant association between susceptibility to childhood atopic asthma and the polymorphism regulating *ORMDL3* expression in a Japanese population. Our data strongly support the important role of *ORMDL3* in childhood asthma. Further investigation of the functional role of the variant during allergic events may provide additional targets for therapeutic interventions and would be helpful to clarify the etiology of childhood asthma.

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