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ORMDL3 variants associated with bronchiolitis susceptibility in a Chinese population

X.T. Liu[1], L. Ren[1], L.L. Zhou[1], Q.Y. Xiao[1], Y. Deng[2] and E.M. Liu[2]

[1]Ministry of Education Key Laboratory of Child Development and Disorders, Key Laboratory of Pediatrics in Chongqing, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Chongqing, China
[2]Department of Respiratory Medicine, Children's Hospital of Chongqing Medical University, Chongqing, China

Corresponding author: E.M. Liu
E-mail: emliu186@hotmail.com

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ABSTRACT. Recent studies revealed common genetic risks for both viral bronchiolitis and asthma. Genome-wide association studies revealed that rs7216389 in the ORMDL3 gene is associated with childhood asthma. We conducted a case-control study examining the associations between *ORMDL3* polymorphisms (rs7216389, rs12603332, and rs11650680) and bronchiolitis susceptibility/viral findings among 247 infant bronchiolitis cases and 190 healthy controls. We genotyped single nucleotide polymorphisms by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry and detected respiratory viruses with multiplex reverse transcriptase-polymerase chain reaction. Only the genotype and allele frequencies of rs7216389 significantly differed between bronchiolitis and controls. The frequencies of the TT homozygote and the T allele of rs7216389 were significantly higher in the bronchiolitis patients (P = 0.0325; P = 0.0089, respectively). Polymorphisms were not associated with bronchiolitis severity. Cases were further stratified by viral infection, but no significant differences

in the *ORMDL3* genotype between the virus-detected group (e.g., respiratory syncytial virus alone, respiratory virus alone, virus detected) and no-virus-detected group were observed. Bronchiolitis is associated with the ORMDL3 gene in Chinese children, and there were no significant associations between genetic variations and disease severity or respiratory viruses. The TT homozygote and the T allele of rs7216389 in ORMDL3 increased bronchiolitis risk. The rs7216389 polymorphism may be a predictor for identifying infants with predisposition to virus-induced wheezing to persistent asthma.

Key words: Bronchiolitis; Chinese population; ORMDL3 gene; Respiratory viruses; Single nucleotide polymorphisms

INTRODUCTION

Infants hospitalized with bronchiolitis have a 2-3-fold increase in the risk of developing asthma later in childhood (Singh et al., 2007); however, the underlying mechanism of this association is not clear. Previous studies have suggested that viral bronchiolitis and asthma may share common genetic risk factors (Bartlett et al., 2009; Stensballe et al., 2009; Thomsen et al., 2009). In 2006, a genome-wide association study of asthma in children revealed that genetic variants on chromosome 17q21 are associated with the risk of childhood asthma. The disease-associated variants are associated with expression levels of 2 genes, *GSDMB* and *ORMDL3* (Çalışkan et al., 2013), and the strongest signal was detected for the single-nucleotide polymorphism (SNP) rs7216389 near *ORMDL3*. Nevertheless, whether the gene polymorphism of this locus is associated with bronchiolitis susceptibility remains unknown.

Bronchiolitis is a viral lower respiratory tract infection, and the most common viruses identified to be associated with this condition include respiratory syncytial virus (RSV) and human rhinovirus (HRV). More recently, HRV wheezing illnesses have been recognized as a stronger predictor of school-age asthma than RSV (Jackson et al., 2008). Nearly 90% of children who had HRV wheezing illnesses at 3 years of age had asthma at 6 years of age (Stensballe et al., 2009). The aim of this study was to assess the genetic association between ORMDL3 gene polymorphisms and bronchiolitis and determine the relationships between the genetic variants and specific viral findings in bronchiolitis patients.

MATERIAL AND METHODS

Study population

In total, 247 children with physician-diagnosed bronchiolitis were enrolled at Children's Hospital of Chongqing Medical University from June 2009 to December 2012. Clinical diagnosis of bronchiolitis (breathlessness, chest wall recession, and inspiratory crepitations on auscultation, and requirement for oxygen and/or nasogastric feeds during hospital admission) were used as the inclusion criteria (American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis, 2006). Patients with known risk factors for RSV disease (prematurity, chronic lung disease, congenital heart disease, and Down syndrome or with neurologic, ophthalmic,

endocrine, or immunologic diseases) were excluded from the study. Of the 247 patients, 80% wheezed for the first time. In total, 190 outpatient children who suffered from non-asthmatic diseases and with no symptoms or history of asthma or other allergic diseases, such as rhinitis and eczema, no symptoms or history of other pulmonary diseases, and no first-degree relatives with a history of asthma, were enrolled as the control group. A 2-mL venous blood sample was collected from controls and used for genotyping assays. Nasopharyngeal aspirate samples were collected from bronchiolitis patients when they were admitted to hospital and used for respiratory virus detection and genotyping. The study was approved by the Ethics Committee of Children's Hospital of Chongqing Medical University, and all subjects' parents gave written informed consent.

Respiratory virus detection

Both viral and human DNA and RNA were extracted from the nasopharyngeal aspirate by using the QIAmp MinElute Virus Spin kit (Qiagen, Hilden, Germany). The RNA was used as a template for cDNA synthesis with the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). The DNA and RNA extractions and cDNA products were used for subsequent testing of 16 respiratory viruses. All samples were analyzed using a detection kit (TaKaRa, Shiga, Japan) according to the manufacturer instructions. Real-time reverse transcription-polymerase chain reaction (PCR) was used to detect human rhinovirus (HRV A/HRV C) and human BoCa virus; nested PCR assays were used to detect human RSV subtypes A and B, influenza virus A, influenza virus B, influenza virus C, human coronaviruses, human metapneumovirus, parainfluenza virus 1-4, and adenovirus (Xu et al., 2000; Coiras et al., 2003; Coiras et al., 2004; Allander et al., 2007; Denlinger et al., 2011).

SNP selection and genotyping

The 3 SNPs (rs7216389, rs12603332, and rs11650680) with the most significant P values according to Moffatt et al. (2007) were examined. SNP rs7216389 was very strongly ($P < 10^{-22}$) associated with ORMDL3 expression. Genomic DNA was extracted from peripheral venous blood (controls) and nasopharyngeal aspirate (bronchiolitis patient) by using a DNA extraction kit (Qiagen), according to manufacturer instructions. 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) (Yang et al., 2012).

Statistical analysis

All SNPs were tested for deviations from Hardy-Weinberg equilibrium (HWE), and a locus was considered to be in HWE if $P > 0.001$. The genotype and allele frequencies were obtained by direct counting. Differences in genotype and allele distributions between the cases and controls were analyzed by the chi-square test. The associations between allele frequencies of the ORMDL3 gene and bronchiolitis were estimated by computing the OR and 95%CI. All statistical analyses were performed using SPSS version 11.5 (SPSS, Inc., Chicago, IL, USA) and $P < 0.05$ was considered statistically significant.

RESULTS

Study population

In total, 437 subjects including 247 bronchiolitis patients and 190 controls were included in our study. The patient group consisted of 169 males and 78 females, with a median age of 4 months (range = 1-20 months). The control group consisted of 110 males and 80 females, with an average of 6 years (6.0 ± 2.5 years).

Genotype and allele frequencies of *ORMDL3*

All genotypes and alleles of SNPs were in accordance with HWE in each group. There were significant differences in the genotype frequencies of rs7216389 between bronchiolitis patients and controls (P = 0.035), and the bronchiolitis children showed a higher TT genotype frequency. No significant differences in genotype frequencies of the other 2 SNPs were observed between bronchiolitis children and controls (P > 0.05; Table 1). Compared with controls, bronchiolitis children showed a significantly higher T allele frequency (P = 0.0089; OR = 1.489; 95%CI = 1.104-2.007) in rs7216389. No significant difference was detected in the distribution of the alleles in the other 2 SNPs among bronchiolitis children when compared with controls (P > 0.05; Table 2).

Table 1. Genotype frequencies of all SNPs between bronchiolitis patients and controls.

	Genotype	Bronchiolitis	Controls	χ^2	Pearson P
rs7216389	CC	14 (0.057)	21 (0.111)		
	TT	143 (0.579)	90 (0.474)		
	CT	90 (0.364)	79 (0.416)	6.8536	0.0325
rs12603332	CC	141 (0.571)	101 (0.532)		
	TT	13 (0.053)	10 (0.053)		
	CT	93 (0.377)	79 (0.4 16)	0.7199	0.6977
rs11650680	CC	157 (0.636)	115 (0.605)		
	TT	6 (0.024)	9 (0.047)		
	CT	84 (0.340)	66 (0.347)	1.8418	0.3982

A = adenine; C = cytosine; G = guanine; T = thymine.

Table 2. Allele frequencies of all SNPs between bronchiolitis patients and controls.

	Allele	Bronchiolitis	Controls	χ^2	Pearson P	OR	95%CI
rs7216389	T	376 (0.761)	259 (0.682)				
	C	118 (0.239)	121 (0.318)	6.842	0.0089	1.489	1.104-2.007
rs12603332	T	119 (0.241)	99 (0.261)				
	C	375 (0.759)	281 (0.739)	0.4423	0.506	0.901	0.662-1.226
rs11650680	T	96 (0.194)	84 (0.221)				
	C	398 (0.806)	296 (0.779)	0.9377	0.3329	0.85	0.612-1.181

ORMDL3 gene and severity of bronchiolitis

According to the criterion of severity classification (Wang et al., 1992), bronchiolitis infants were divided into 2 groups based on clinical manifestation, including a mild group (N = 75, total score

<4.9) and a moderate to severe group (N = 170, total score ≥5), except for 2 cases for whom clinical characteristics were not complete. There were no significant differences in genotype frequencies of all SNPs between the mild group and the moderate to severe group (P > 0.05; Table 3).

Table 3. Genotype frequencies of all SNPs between mild group and moderate to severe group.

	Genotype	Mild (N = 75)	Moderate to severe (N = 170)	χ^2	P
rs12603332	CC	43 (0.573)	96 (0.565)		
	TT	3 (0.040)	10 (0.059)		
	CT	29 (0.387)	64 (0.376)	0.3686	0.8317
rs7216389	CC	3 (0.040)	11 (0.065)		
	TT	45 (0.600)	96 (0.565)		
	CT	27 (0.360)	63 (0.371)	0.6844	0.7102
rs11650680	CC	50 (0.667)	106 (0.624)		
	TT	2 (0.027)	4 (0.024)		
	CT	23 (0.307)	60 (0.353)	0.5019	0.7781

ORMDL3 variants and viral findings of bronchiolitis

Viral etiology was identified in 214 (87%) of the 247 bronchiolitis cases. The types of viruses detected included RSV (single detection) (87; 35%), HRV (single detection) (13; 5%), and RSV coinfection with HRV (CO) (29; 12%), while no virus was detected in 33 subjects. Because RSV and RV were most frequently identified as the cause of bronchiolitis, the analysis focused on these 2 viruses. The genotype frequencies are shown in Table 4. There were no significant differences in genotype frequencies of 3 SNPs between the virus-detected group and no-virus-detected group [rs12603332 (χ^2 = 1.7483; P = 0.4172), rs7216389 (χ^2 = 1.8822; P = 0.3902), rs11650680 (χ^2 = 1.3025; P = 0.5214)]. In addition, no significant differences were observed between the RSV-single detected group and no-virus detected group [rs12603332 (χ^2 = 1.3435; P = 0.5108), rs7216389 (χ^2 = 1.0062; P = 0.6047), rs11650680 (χ^2 = 1.7766; P = 0.4113)]. Comparison of the HRV-single-detected group and the no-virus-detected group showed that there were no significant differences in the genotype frequencies of all SNPs [rs12603332 (χ^2 = 0.6648; P = 0.7172), rs7216389 (χ^2 = 0.5514; P = 0.759), rs11650680 (χ^2 = 5.3372; P = 0.0693)].

Table 4. Genotype frequencies of all SNPs among different virus-detected bronchiolitis patients.

	Genotype	Virus detected (N = 214)	RSV (N = 87)	HRV (N = 13)	CO (N = 29)	Others (N = 30)	No-virus detected (N = 33)
rs12603332	CC	119 (0.556)	48 (0.552)	7 (0.538)	19 (0.655)	16 (0.533)	22 (0.667)
	TT	11 (0.051)	6 (0.069)	1 (0.077)	0 (0.000)	1 (0.033)	2 (0.061)
	CT	84 (0.393)	33 (0.379)	5 (0.385)	10 (0.345)	13 (0.433)	9 (0.273)
rs7216389	CC	11 (0.051)	6 (0.069)	1 (0.077)	0 (0.000)	1 (0.033)	3 (0.091)
	TT	122 (0.570)	49 (0.563)	7 (0.538)	20 (0.690)	15 (0.500)	21 (0.636)
	CT	81 (0.379)	32 (0.368)	5 (0.385)	9 (0.310)	14 (0.467)	9 (0.273)
rs11650680	CC	137 (0.640)	54 (0.621)	7 (0.538)	21 (0.724)	16 (0.533)	20 (0.606)
	TT	6 (0.028)	4 (0.046)	2 (0.154)	0 (0.000)	0 (0.000)	0 (0.000)
	CT	71 (0.332)	29 (0.333)	4 (0.308)	8 (0.276)	14 (0.467)	13 (0.394)

*Virus detected: more than one virus was detected. RSV = only respiratory syncytial virus (RSV) was detected. HRV = only human rhinovirus (HRV) was detected. Coinfection (CO) = only RSV and HRV were detected. Others = viruses other than RSV and HRV were detected.

DISCUSSION

It is well known that infants hospitalized with bronchiolitis are at a significantly increased

risk for both recurrent wheezing and childhood asthma. In Sweden, 30% of former bronchiolitis patients had asthma at 10 years of age and 43% at 17-18 years of age (Goksör et al., 2006). In China, the number ranges from 25-50%, which is much higher than the natural prevalence rate of asthma in healthy children (0.25-4.63%) (Chen, 2003). One crucial determinant is the genetic susceptibility in the process of asthma after viral bronchiolitis. The common genetic risks for bronchiolitis and asthma have recently been reviewed (Bartlett et al., 2009). These genes include those involved in the innate/Th1 response to infection [e.g., Toll-like receptors and CD14 (Hong et al., 2007; Kormann et al., 2008; Bjornvold et al., 2009)], and those encoding for interferons [e.g., interferon-γ gene (Kumar and Ghosh, 2008), interferon regulatory factor-1 gene (Schedel et al., 2008)], as well as the interleukin-13 and interleukin-14 genes (Forton et al., 2009). The sequence variant rs7216389 in *ORMDL3* was shown to be associated with the risk of childhood asthma in a genome-wide association study of asthma (Moffatt et al., 2007). Additionally, a meta-analysis of 5 published studies in 9 populations_reported an OR for asthma of 1.44 (95%CI, 1.35-1.54, P < 0.00001) for rs7216389 (Wu et al., 2009). Recently, the association between this polymorphism and childhood asthma was confirmed in a Chinese population (Yang et al., 2012).

ORMDL3 gene and bronchiolitis

In the present study, we found significant differences in the TT genotype and T allele frequencies of rs7216389 between bronchiolitis patients and controls (P = 0.0325 and 0.0089, respectively). rs7216389 in the ORMDL3 gene may give rise to the bronchiolitis phenotype. Children carrying the T allele had a high risk for bronchiolitis (OR = 1.489; 95%CI = 1.104-2.007). Increasing data support the notion that genetic polymorphisms in potentially numerous genes determine the susceptibility to both viral bronchiolitis and asthma (Carroll et al., 2009). Similarly, our results confirmed that both bronchiolitis and asthma share similar genetic contributions. We showed that the ORMDL3 gene was a common genetic risk factor for both asthma and bronchiolitis. The TT homozygote and T allele of rs7216389 not only increased the risk of childhood asthma, but also the risk of bronchiolitis.

rs7216389 is located in an intron of the GSDML gene and is associated with asthma by affecting the expression level of the nearby ORMDL3 gene. The ORMDL3 gene encodes a protein functioning in the endoplasmic reticulum membrane, where it regulates endoplasmic reticulum-mediated calcium signaling and the unfolded-protein response (Cantero-Recasens et al., 2010). The induction and manipulation of unfolded-protein-response signaling are mechanisms through which viruses protect host cells from death mediated by endoplasmic reticulum stress (Trujillo-Alonso et al., 2011). Over-expression of *ORMDL3* may increase the efficiency of infection or viral replication in respiratory epithelial cells and possibly reduce the ability of these cells to repair themselves after virus infection (Çalışkan et al., 2013). The GSDML gene belongs to the cancer-associated gasdermin domain-containing protein family and may be involved in secretory pathways and stem cell proliferation in normal epithelia (Saeki et al., 2009).

ORMDL3 gene and viral infections

Viral etiologies were identified in 87% of bronchiolitis patients in the present study, which is nearly consistent with the results of a previous study (Jackson et al., 2008). The types of virus-induced wheezing episodes are thought to influence the risk of subsequent asthma. Studies have typically focused on RSV, but a number of recent studies have suggested that other respiratory pathogens, including HRV, may also contribute to the incidence of asthma after bronchiolitis. The

Childhood Origins of Asthma cohort consisted of 259 children followed prospectively from birth. They found that wheezy lower respiratory tract infection with RSV (OR = 2.6), HRV (OR = 9.8), or both (OR = 10) was associated with asthma at 6 years of age (Jackson et al., 2008). Thus, the ORMDL3 gene and other viral infections may be associated.

However, we found no significant associations between the ORMDL3 gene and viral infection. There were no significant associations between rs7216389 and susceptibility to RSV/RV infection in bronchiolitis patients. This may be because the site itself does not determine susceptibility to a virus, or because a larger sample size is needed. Abnormalities in innate immune may be responsible for lower respiratory infection in early life (Martinez, 2009). Other studies reported that several genetic factors modify the risk of RSV-induced wheezing, including polymorphisms in genes encoding surfactant proteins, cytokines, and chemokines (Singh et al., 2007), while polymorphisms in interleukin-10 may be associated with HRV infections (Helminen et al., 2008).

Three theories may explain the progression of asthma after bronchiolitis. First, viral respiratory infections may damage the airways and initiate asthma. Second, the relationship is not causal, but a virus may reveal a preexisting tendency for asthma. A third possibility, which combines elements of the first 2, is a “2 hit hypothesis”, in which viral infections promote asthma mainly in predisposed children (Gern, 2009). Our preliminary results suggest that the same genetic contribution exist for both bronchiolitis and asthma, which indirectly supports the important role of a virus in identifying infants with predisposition.

Çalışkan et al. (2013) observed significant interactions between 17q21 genotypes and HRV wheezing illness with respect to the subsequent risk of asthma. Interactions between genetic and environmental factors may also be important in understanding the influence of bronchiolitis on asthma and should be examined in future studies.

In conclusion, this case-control study supports that genetic polymorphisms in the ORMDL3 gene were associated with bronchiolitis but not with disease severity or respiratory viruses in a Chinese population. The TT homozygote and T allele of rs7216389 in *ORMDL3* increased the risk of bronchiolitis, providing a predictor that may be useful in future targeted research aimed at preventing asthma.

Conflicts of interest

The authors declare no conflict of interest.

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