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Investigating the Association of *Orosomucoid 1-like 3 (ORMDL3)* Gene Polymorphism (rs12603332) with Susceptibility to Allergic Asthma in Iranian Northwestern Azeri Population

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ABSTRACT

Orosomucoid 1-like 3 (ORMDL3) gene, located on chromosome 17q21, is an asthma candidate gene that encodes ORMDL3. This molecule has been reported to play a role in airway remodeling and bronchial hyper-responsiveness. In this study, we aimed to investigate the possible association of ORMDL3 single nucleotide polymorphism (SNP) (rs12603332) with susceptibility to allergic asthma in Iranian Northwestern Azeri population.

193 asthmatic patients and 185 normal individuals were included. Genomic DNA was extracted and genotyping was performed by standard restriction fragment length polymorphism-polymerase chain reaction RFLP-PCR method using BstUI restriction enzyme.

Our results showed dominant presence of TC genotype and C allele in both patients (49.2% and 59.8%, respectively) and controls (48.6% and 60%, respectively). Frequency of genotypes and alleles showed no significant difference between two groups (p=0.994 and p=1.00, respectively). None of alleles could be defined as risk allele for allergic asthma (OR=0.99, 0.88-1.12, 95% CI).

We failed to show significant association between ORMDL3 rs12603332 with predisposition to allergic asthma in Iranian Northwestern Azeri population. More studies with larger number of participants should be done to find more reliable results for such association.

Keywords: Asthma; Orosomucoid 1-like 3; Restriction fragment length polymorphism-polymerase chain reaction; rs12603332; Single nucleotide polymorphism

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INTRODUCTION

The mammalian orosomucoid like (ORMDL) proteins are orthologues of the yeast Orm proteins

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(Orm1/2), which are key negative regulators of serine palmitoyltransferase (SPT) that is located in the endoplasmic reticulum (ER).[1,2] The enzyme homeostatically is regulated by cellular levels of sphingolipid.[3] Sphingolipids represent an important structural component of cell membranes play a key role in regulation of membrane fluidity.[1] Sphingolipids can be rapidly metabolized to ceramide or further to sphingosine and its more active metabolite sphingosine-1-phosphate (S1P). S1P receptor (S1PR) activation is necessary for initiation of lung immune responses by mature antigen-presenting cells (APCs).[4] Changes in S1P concentrations at tissue-specific sites and in the blood have been investigated in asthma.[5] ORMDL3 is a 153-aa ER-localized protein with two predicted transmembrane domains that is coded by ORMDL3.[6,7] ORMDL3 is capable of inhibiting sphingolipid synthesis by forming a complex with SPT.[8] Overexpression of ORMDL3 regulates ceramide homeostasis in cells in a complex manner in which small increases in ORMDL3 expression decrease ceramide levels, while higher expression in lung epithelial cells and macrophages in vitro and in vivo results in increased ceramide production.[9] STAT6 has been reported to play a role in regulating the expression of human ORMDL3.[10] It is widely expressed in both fetal and adult mammalian tissues including lung epithelial cells. In mouse lungs, expression can be increased under influence of stimuli, including allergens, tobacco smoke, and lipopolysaccharides.[11] ORMDL3 alters cytoplasmic calcium levels and ER Ca^{2+} release in lymphocytes, thus plays a role in activation of lymphocyte. This finding provides a functional link between the genetic associations of the ORMDL3 gene with autoimmune and/or inflammatory diseases.[12] ORMDL3 regulates IL-3-induced expression of CD48, and CD48-mediated eosinophil degranulation. Indeed, ORMDL3 ability to regulate eosinophil trafficking, recruitment and degranulation elucidates a role for this molecule in allergic asthma.[13] Associations between ORMDL3 polymorphisms and human diseases including pediatric-onset inflammatory bowel disease,[14] atherosclerosis,[15] and allergic rhinitis[16] have been investigated in different populations and the results suggest that ORMDL3 may be involved in dysregulation of the immune system.[17] Although the underlying etiologies for asthma remain incompletely understood, it is known that the genetic predisposition can be pivotal for asthma development.[18] Genome-wide association studies (GWAS) have established a strong correlation between elevated expression of the endoplasmic reticulum protein ORMDL3 and risk for childhood asthma.[19] In this study, we aimed to investigate the possible association of ORMDL3 rs12603332 SNP with allergic asthma in north-western Azeri population of Iran.

MATERIALS AND METHODS

Study Populations

193 patients with asthma and 185 normal individuals were studied. Informed consent letter was signed by all participants, and the study was approved by Medical Ethics Committee of Tabriz University of Medical Sciences (N. 5.4.9735). Normal population had no history of any inflammatory, infectious, autoimmune diseases, and especially allergy and asthma.

Patients were diagnosed and selected according to the laboratory and clinical findings by a clinical immunology and allergy specialist, family history, and the International Study of Asthma and Allergies in Childhood (ISAAC) guideline and Global Initiative for Asthma (GINA). Demographic features of studied groups have been shown in Table 1.

Genotyping of ORMDL3 rs12603332 SNP

Genomic DNA was extracted from vein peripheral blood according to the previously described protocol.[20] Extracted DNAs were qualified and quantified using agarose gel electrophoresis and UV spectrophotometry. Flanking regions of rs12603332 SNP were amplified using conventional PCR method by primers [21] and conditions described in Table 2. Reaction mixtures were 25 µL PCR including 50 ng template DNA, 2.5 µL 10×PCR buffer, 1.5 µL MgCl2 (25 mM), 1 µL dNTPs (10 mM) (Thermo Scientific Inc. USA), 2 µl of both forward and reverse primers (10 pM) (Bioneer Inc., South Korea), 0.4 µL Taq DNA polymerase (5 u/µL) (Thermo Scientific Inc., USA). PCR products were seen and verified after 2% agarose gel electrophoresis under UV illuminator, and in comparison with a standard DNA size marker. After that, PCR products were subjected to the digestion with 10 units BstUI restriction endonuclease (Thermo Scientific Inc., USA). Digestion patterns corresponding to each genotype were evaluated by 1% agarose gel electrophoresis (Figure 1). Some random samples from

each genotype were verified by direct sequencing method. Because of upstream location of SNP in PCR product, sequencing was done in reverse order, and sequencing results were in reverse and complement format (Figure 2).

Statistical Analysis

Allele and genotype frequencies for studied SNP were analyzed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA), and Chi-squared and logistic regression tests. *p*-value less than 0.05 was considered as statistically significant.

RESULTS

Amplification of flanking regions of ORMDL3 rs12603332 SNP by conventional PCR method and using specific primers led to specific 204 bp bands. Digestion of all PCR products with BstUI restriction enzyme that recognizes CGˆCG sequence showed different patterns of gel electrophoresis according to the genotype of each sample (Figure 1). Restriction site recognized by the eBstUI enzyme is Undigested PCR product (204 bp) represented TT genotype. Inversely, CC genotype was revealed as digested one (174 and 30 bp). Samples with both digested and undigested patterns (204, 174 and 30 bp) showed TC heterozygote genotype. Genotyping of some samples were verified by direct sequencing of PCR product, as shown in Figure 2. Important point is that rs12603332 SNP was located in the upstream of PCR products, and sequencing was performed in reverse orientation. Thus, representative results for sequencing are reverse and complement, and presence of G and A nucleotides was interpreted as C and T, respectively. Statistical analysis showed that TC heterozygote genotype is dominant in both patients and control study groups (n=95, 49.2%, and n=90, 48.6%, respectively). However, the frequency of genotypes are not significantly different (p=0.994). C allele was also more frequent than T allele in both studied groups (n=231, 59.8% vs. n=155, 40.2% in patients, and n=222, 60.0% vs. n=148, 40.0% in controls) and showed no significant difference in frequency (p=1.00, OR=0.99, 0.88-1.12, 95% CI). More detailed data has been shown in Table 3.

Table 1. Demographic features of studied asthmatic and normal Iranian north-western populations for possible association of ORMDL3 rs12603332 SNP with allergic asthma

	Patients with asthma (n=193)	Control subjects (n=185)
Gender		
Mean age ± SD (years)	26.84±14.7	26.73±14.8
Male	80 (41.45%)	75 (40.54%)
Female	113 (58.56%)	110 (59.46%)
Eosinophil count per µL (%)	363 (4.24%)	116 (1.5%)
History of allergic reactions		
Allergic rhinitis	56 (29.01%)	
Conjunctivitis	44 (22.79%)	
Atopic dermatitis	24 (12.43%)	
other	69 (35.75%)	

Table 2. Primer sequences and digestion patterns of restriction enzyme (BstUI) considered for genotyping of ORMDL3 rs12603332 single nucleotide polymorphism (SNP) in asthmatic and normal Iranian north-western populations for possible association with allergic asthma

SNP	primer sequence	restriction enzyme	digestion pattern (bp)	PCR conditions
ORMDL3 (rs12603332)	F:5'-GAGTGTCTGGCATACTGGCTGG-3' R:5'-CCGAAAACTTCTGCTGCCATAGCTGGCACG-3'	BstUI	T/T:204 T/C:204/174/30 C/C: 174/30	35 cycles 58°C

F: Forward, R: Reverse, bp: base pair

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Table 3. Genotype and allele distribution of ORMDL3 rs12603332 single nucleotide polymorphism (SNP) in asthmatic and normal Iranian north-western populations for possible association with allergic asthma

ORMDL3 SNP	Frequency (percent)		*p*-value	Odds ratio,(95% CI)
	patient group (n=193)	normal group (n=185)		
rs12603332 *genotype*				
C/C	68 (35.2%)	66 (35.7%)		
T/C	95 (49.2%)	90 (48.6%)		
T/T	30 (15.5%)	29 (15.7%)	0.994	
allele				
C	231 (59.8%)	222 (60.0%)		
T	155 (40.2%)	148 (40.0%)	1.00	0.99 (0.88-1.12)

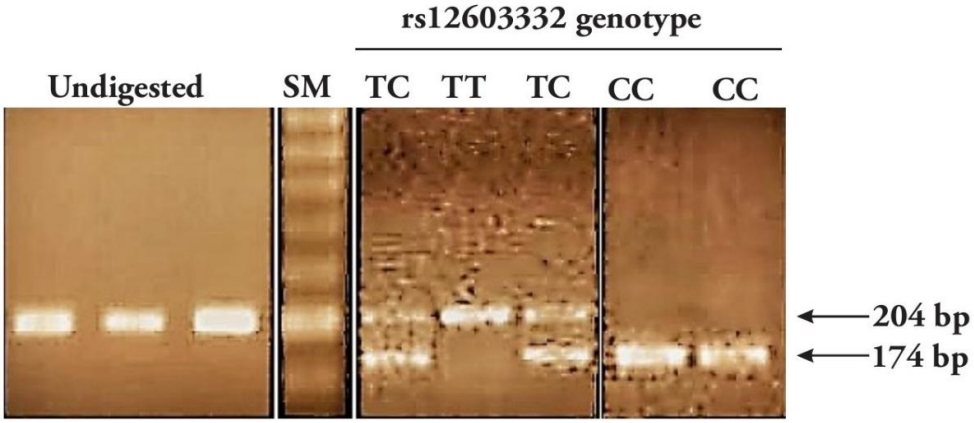

Figure 1. Representative results for genotyping of ORMDL3 rs12603332 single nucleotide polymorphism (SNP) using RFLP-PCR method in asthmatic and normal Iranian north-western populations for possible association with allergic asthma.
PCR products before digestion with BstUI restriction endonuclease showed specific 204 bp bands. After digestion, different patterns were seen according to the genotype of any sample. SM, size marker; bp: base pair.

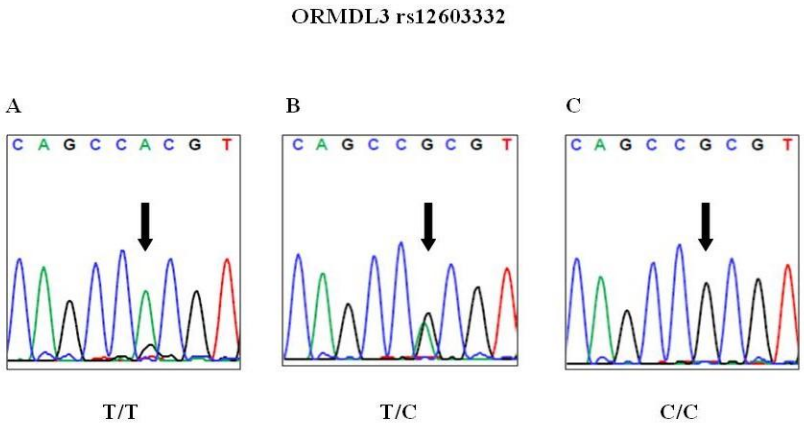

Figure 2. Representative data for direct sequencing of ORMDL3 rs12603332 single nucleotide polymorphism (SNP) for three possible genotypes.
Arrows show the exact SNP site for ORMDL3 rs12603332. Note that because of reverse orientation of sequencing, showed alleles are the complement alleles of SNP, i.e. G and A for C and T, respectively.

DISCUSSION

ORMDL3 protein has attracted increased attention since the discovery of SNPs in the chromosome 17q12-q21 region that were associated with onset of asthma especially in childhood. The relation between polymorphisms within this chromosomal region and the development of asthma has now been replicated in a number of independent studies including different ethnicities. Asthma-associated polymorphisms of 17q21 were shown to alter transcription levels of ORMDL3.[22] ORMDL3 rs12603332 SNP located in the first intron of ORMDL3 gene which may affect mRNA splicing has been investigated for its possible association with asthma in different populations. Our study on 193 patients with asthma and 185 normal individuals to explore probable association between ORMDL3 rs12603332 SNP with susceptibility to asthma in Iranian Northwestern Azeri population was showed no significant association. Also, no C and nor T allele was introduced as risk allele for asthma.

The locus on chromosome 17q21, that encompasses the ORMDL3 gene, was considered a susceptibility locus associated to asthma and polymorphisms within this locus were thought to be associated to an increased expression of the ORMDL3 gene.[23-25] Association between ORMDL3 SNPs and asthma has been reported in Australian population (rs6503525),[26] Mexico City population (rs4378650),[27] Slovenian population (rs4795405),[28] and Chinese population (rs7216389).[29,30] The association of rs12603332 (-2352C/T) another ORMDL3 SNP with asthma was investigated in Chinese population and it has been found that individuals with T allele in rs12603332 are protected from asthma.[31] rs12603332 SNP which is located in the first intron lies in a conserved element across species with a high correlation to the consensus target sequence for the transcription factor E47. This factor has been linked to development of T- and B-cell, and the C allele substitution in the position of rs12603332 leads to the reduction in the transcription factor score of this region.[21,32]

The possible association between rs12603332 and predisposition to asthma in different ethnic groups and found significant association in Mexicans (n=301, p=0.021) and African Americans (n=261, p=0.001) was studied.[33] Significant association was also found in two another studies in Chinese (194 asthmatic patients and 153 control subjects, p=0.002) and Chinese Han (384 patients and 298 healthy individuals, p=0.07) populations.[21,34] The latter study introduced the C allele as a risk allele for asthma (p=0.06). In contrary to described studies, and in line with our study, in one study the possible association of four ORMDL3 SNPs with asthma in Czech population (337 patients and 331 normal subjects) was investigated and found no association for rs12603332 SNP (p=0.17).[32]

Our results showed that there is no significant association between ORMDL3 rs12603332 with predisposition to allergic asthma in Iranian Northwestern Azeri population. However, to provide more reliable results larger case and control groups should be included. Moreover, investigating the association of other SNPs of ORMDL3 with asthma would provide more reliable results. Moreover, studying other ORMDL3 gene polymorphisms in larger number of patient and control groups, and also grouping of asthmatic patients into mild, moderate, or severe sub-groups will lead to the more defined results. On the other hand, because of interaction of several molecules during allergic asthma, including the all possible major molecules in one larger study is recommended.

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