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Investigating the Association of ADAM33 Single Nucleotide Polymorphisms (SNPs) with Susceptibility to Allergic Asthma in Azerbaijan Population of Iran: A Case-control Study

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ABSTRACT

Asthma, affecting a growing number of populations, is a clinical condition with complex cellular and genetic factors. Single nucleotide polymorphisms (SNPs) in gene coding for molecules, which play major roles in the immunopathogenesis of asthma have been considered recently as genetic predisposing factors to this disease. Possible association between two SNPs in a disintegrin and metalloprotease 33 (ADAM33), which participates in airway remodeling, and susceptibility to asthma was studied in this study.

190 patients with asthma and 180 healthy controls were enrolled in this case-control study. Using conventional PCR method, specific bands were amplified and the frequency of genotypes of T1 (rs2280091) and V4 (rs2787094) ADAM33 SNPs were determined by digestion with NcoI and PstI, respectively.

The results showed that the frequency of genotypes of T1 and V4 were not significantly different between patients and controls (p=0.54 and p=0.85, respectively). On the other hand, no significant differences were seen in allele frequency of both T1 and V4 SNPs (p=0.15 and p=0.47, respectively).

In agreement with some other studies in different populations, our results showed no association between frequency of genotypes or alleles of both T1 and V4 SNPs in ADAM33 gene and predisposition to asthma in Azerbaijan population of Iran. Genetic differences in different ethnic groups might be involved in such inconsistent results. More studies in populations with larger number of patients and healthy individuals are needed for concluding remarks for involvement of ADAM33 SNPs in asthma.

Keywords: Asthma; ADAM33; Single nucleotide polymorphism (SNP)

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INTRODUCTION

Asthma is an immunologic disorder with increasing morbidity and mortality worldwide. It`s defined as a clinical setting caused by exposure to some environmental agents in genetically susceptible individuals that leads to immunologic reactions and airway inflammation and obstruction. For several years, TH2 cells and it`s cytokines including IL-4, IL-5 and IL-13, mast cells, eosinophils, and IgE-producing B cells have been considered as major immunologic players. However, there is growing number of cells and molecules have been identified that function in a complex network. These include type 2 innate lymphoid cell (ILC2), IL-25, IL-33, CCL11, CCL24 and CCL26 chemokines, thymic stromal lymphopoietin (TSLP), and a disintegrin and metalloproteinase domain (ADAMs).[1,2]

ADAM family of molecules are type I glycoproteins involved in several cell processes e.g. differentiation, proliferation, adhesion and signaling. ADAM33 functions as an enzyme in cell surface to cleave and shed some molecules including TGF-β.[3] The putative role for ADAM33 in initiation or progression of asthma comes from a study that showed expression of ADAM33 in asthmatic smooth muscle and sub-epithelial fibroblasts in comparison with respiratory epithelium[4] and from an investigation which found significant higher expression of DAM33 in both patients with moderate and severe asthma compared with mild asthma patients and normal subjects.[5] Several single nucleotide polymorphisms (SNPs) of ADAM33 have been chosen and investigated in multiple studies to find possible association with clinical settings including asthma. Of them, T1 (Met764Thr) and V4 SNPs are located in exon 20 and 3` UTR of ADAM33 gene, respectively, and are thought to be important in functional activity, and transcriptional regulation of the expression of ADAM33 by several mechanisms e.g. by modifying signaling activity or via binding of specific microRNAs.[6] T1 and V4 SNPs have also been studied by several investigators in asthmatic patients in different ethnic populations.[7]

In this study we aimed to investigate the probable association of T1 (rs2280091) and V4 (rs2787094) ADAM33 SNPs with predisposition to asthma in Azerbaijan population of Northwestern Iran.

MATERIALS AND METHODS

Patients and Controls

190 individuals with diagnosed asthma and 180 healthy controls were enrolled in this study. Patients were diagnosed by a clinical immunology and allergy specialist according to clinical symptoms, laboratory findings, clinical examinations, family history, and global initiative for asthma (GINA) and the international study of asthma and allergies in childhood (ISAAC) guideline. Normal individuals showed no symptoms of asthma, allergy, autoimmunity and inflammatory diseases. Both study groups were also negative for parasitic diseases. More detailed demographic features have been shown in Table 1. This study was approved by Medical Ethics Committee of Tabriz University of Medical Sciences (No. 93.2-7.18), and informed consent letter was signed by participants.

Genotyping of ADAM33 SNPs

One mL vein peripheral blood was collected in tubes containing ethylene diamine tetraacetic acid (EDTA) anticoagulant. Using standard salting out method,[8] DNAs were extracted and evaluated quantitatively and qualitatively by spectrophotometry and agarose gel electrophoresis. Flanking regions of both ADAM33 SNPs, T1 and V4, were amplified by conventional PCR method using specific primers listed in Table 2.[9] Twenty-five µL PCR reaction mixture was prepared from 2.5 µL 10× PCR buffer, 1 µL dNTPs (10 mM) (Thermo Scientific Inc., USA), 1.5 µL $MgCl_2$ (25 mM), 1 µL each forward and reverse primers (10 pM) (Bioneer Inc., South Korea), 0.5 µL Taq DNA polymerase (5u/ µL) (Thermo Scientific Inc., USA) and 50 ng template DNA. PCR programs were run in 35 cycles in cluding 58°C and 60°C for annealing temperature of T1 and V4, respectively. Specific PCR bands were seen under UV gel documentation system after 1.5% agarose gel electrophoresis using 1× Tris-borate-EDTA (TBE) buffer for 1 hour, and staining with DNA safe stain (Sinaclone, Tehran, Iran). Band sizes were compared to 50-bp DNA size marker (Thermo Scientific Inc., USA). To determine the genotype
of each sample, amplified bands were subjected to digestion with 10 units NcoI and PstI restriction endonucleases (Thermo Fisher Scientific Inc., USA) incubated at 37°C for 16 hours for T1 and V4 SNPs, respectively. Results were seen after 2%

Table 1. Demographics and history of allergic reactions in controls and patients with allergic asthma to investigate association between ADAM33 SNPs and predisposition to asthma

	Asthma patients (n=190)	Control subjects (n=180)
Gender		
Mean age ± SD (years)	25.84±15.08	27.43±15.64
Male	79 (41.4%)	74 (41.1%)
Female	111 (58.6%)	106 (58.9%)
Eosinophil count per µL (%)	363 (4.24%)	116 (1.5%)
History of allergic reactions		
Allergic rhinitis	55 (28.8%)	-
Conjunctivitis	43 (22.5%)	-
Atopic dermatitis	23 (12%)	-
other	69 (36.6%)	-

Table 2. Primer sequences and digestion patterns of ADAM33 T1 and V4 SNPs to investigate their possible association with asthma

SNP	primer sequence	restriction enzyme	digestion pattern of genotypes (bp)
T1 (rs2280091)	F:5´-ACTCAAGGTGACTGGGTGCT-3´	NcoI	G/G: 400
	R: 5´-GAGGGCATGAGGCTCACTTG-3´		G/A: 400/260/140
			A/A: 260/140
V4	F: 5´-ACACACAGAATGGGGGAGAG-3´	PstI	C/C: 374
(rs2787094)	R: 5´-CCAGAAGCAAAGGTCACACA-3´		G/C: 374/206/168
			G/G: 206/168

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

agarose gel electrophoresis using 1×TBE buffer for 1 hour. The pattern of digestion and corresponding allele and genotype are listed in Table 2.

Genotyping of samples were verified by direct sequencing of some random samples and analysis using Chromas 2.6.2 software.

Statistical Analysis

Frequency of alleles and genotypes were compared by Chi-squared and logistic regression tests using SPSS software Version 16, SPSS Inc., Chicago, IL, USA). A *p*-value less than 0.05 was considered as statistically significant.

RESULTS

Amplification of flanking regions of T1 and V4 SNPs yielded to 400 bp and 374 bp specific bands (Figure 1), which showed unique band patterns after digestion with NcoI and PastI restriction enzymes (Table 2 and Figure 1). Direct sequencing confirmed results for genotyping by restriction endonucleases (Figure 2). After genotyping of DNA samples using RFLP-PCR method, the most frequent genotype of T1 and V4 SNPs were AA and GG in both patients and controls, with no statistically significant differences (p=0.54 and p=0.85, respectively). Accordingly, the most frequent alleles were A and G for two studied SNPs, that did not show significant differences between patients and controls (p=0.15 and p=0.47, ,respectively). Statistical analysis also showed that none of A and G alleles could be risk allele for predisposition to asthma (OR=1.2 and OR=1.02, respectively). More detailed frequency of genotypes and alleles are shown in Table 3. There was also no significant difference in any possible haplotype, combinations of T1 and V4 alleles, between two study groups (data not shown).

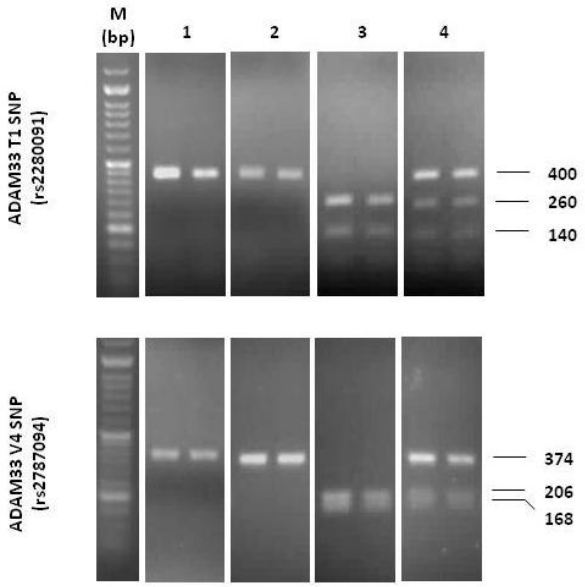

Figure 1. Representative results for digestion pattern of T1 and V4 SNPs of ADAM33 gene by RFLP-PCR method to investigate their possible association with asthma. Digestion of PCR products by NcoI and PstI determined three digestion patterns corresponding to the three different genotypes of T1 and V4, respectively. Lanes numbered as 1, 2, 3 and 4 for T1 SNP are undigested PCR product, GG, AA, and GA genotypes, respectively. For V4 SNP, these lanes correspond to undigested PCR product, CC, GG, and GC, respectively. All bands are in base pair (bp). M: DNA size marker.

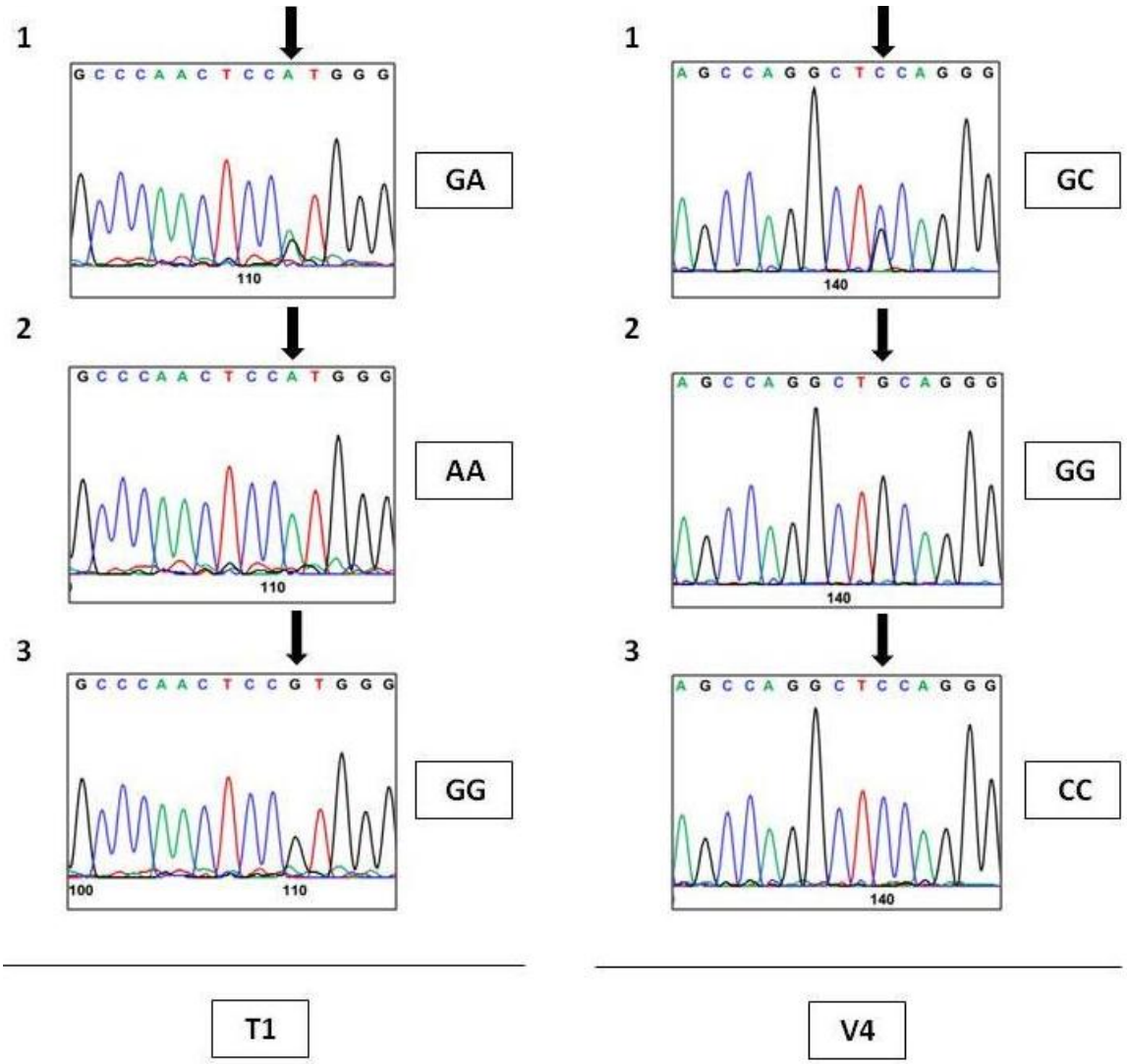

Figure 2. Representative results for direct sequencing of T1 and V4 SNPs in ADAM33 genes to investigate their possible association with asthma. Selected samples from any genotype were sequenced and confirmed as GA (1), AA (2) and GG (3) for T1 in NcoI restriction site (CCATGG), and GC (1), GG (2) and CC (3) for V4 in PstI restriction site (CTGCAG). Arrow shows the site of SNP.

Table 3. Genotype and allele frequencies of ADAM33 T1 (rs2280091) and V4 (rs2787094) in asthmatic and normal groups

ADAM33 SNP	Frequency (percent)		*p*-value	Odds ratio, (CI 95%)
	patient group (n=190)	normal group (n=180)		
T1(rs2280091)				
genotype				
G/G	9(4.7%)	11(6.1%)		
G/A	64(33.7%)	68(37.8%)		
A/A	117(61.6%)	101(56.1%)	0.54	
allele				
A	298(78.4%)	270(75.0%)		
G	82(21.6%)	90(25.0%)	0. 15	1.2 (0.86 -1.7)
V4(rs2787094)				
genotype				
C/C	11(5.8%)	12(7.1%)		
G/C	80(42.4%)	74(40.8%)		
G/G	99(51.8%)	94(52.2%)	0.85	
allele				
G	278(73.0%)	267(72.6%)		
C	102(27.0%)	101(27.4%)	0.47	1.02 (0.74 -1.41)

DISCUSSION

Asthma is a clinical setting with immunologic and non-immunologic causes. Genetic and environmental factors are important in predisposition of individuals to asthma. SNPs in genes which are playing important roles in immunopathogenesis of asthma have been implicated as one category of genetic factors.[1,2] Several SNPs have been identified in different regions of ADAM33 gene with possible roles in controlling or modifying the function or expression of ADAM33. ADAM33 has important roles in different cellular processes and have potential role in immunopathogenesis of asthma.[3] We aimed in this study to investigate possible association of two well-studied SNPs in ADAM33 gene, T1 and V4, with susceptibility to asthma in north-western Azerbaijan population of Iran. We found no significant association among genotype and allele frequency of T1 and V4 SNPs and asthma.

Consistent with our findings, some other investigators also found no association of ADAM33 SNPs with asthma. Raby et al studied 474 white, 66 African-American and 47 Hispanic North American population and found no significant association in asthmatic patients in comparison with control subjects.[10] Similar results were showed by Lind et al in 190 Mexican and 183 Puerto Rican patients,[11] Lee et al in 326 Korean population,[12] Blakey et al in 384 Icelandic asthmatic population,[13] Werner et al in 48[14] and Schedel et al in 624 German patients,[14,15] Wang et al in 296 Chinese population,[16] Kedda et al in 612 Australian asthmatics,[17] Thongngarm et al in 200 Thai population.[18] Vergara et al in 429 Colombians asthmatic patients,[19] and Zand Karimi et al in 95 northeasters of Iran.[20] In contrary, some investigators found significant association of both T1 and V4 ADAM33 SNPs with asthma. Such result has been shown in 181 asthmatic patients of Chinese Han[9] and 126 patients of Uygur populations.[21] Both studies introduced T1 G and V4 G alleles as risk alleles for asthma. This is in contrary to our study that showed more frequency (but not significant) of T1 A allele in asthma patients, and similar frequency of V4 alleles in both study groups. In parallel, some studies showed significant association of only one of T1 and V4 SNPs with asthma. T1 was found as significant SNP by Van Eerdewegh et al in US population,[6] Howard et al in 220 US white patients (T1 A allele as more frequent allele),[22] and Raby et al in 47 Hispanic asthmatics.[10] On the other hand, V4 has been found to be significant SNP in studies by Howard et al in 180 Dutch whites, with V4 G allele was more frequent allele in patients.[22] Also, Awasthi et al in 211 northern Indian patients[23] and Tripathy et al in 175 Indians[24] found V4 G allele as risk allele.

It`s noteworthy that other ADAM33 SNPs were also studied in some above-mentioned studies. The results for them and studies considering other SNPs, but not T1 and V4, were omitted because of brevity. Collectively, predisposition for asthma in different ethnic groups showed correlation with different SNPs and also with different alleles of a defined SNP, although V4 G allele showed consistent results in different Studies. Because of location T1 and V4 SNPs in exon 20 and 3`-UTR of ADAM33 gene, it`s predicted that T1 and V4 SNPs alter functional activity or expression level of ADAM33, respectively, that eventually make an individual susceptible to asthma. However, all data presented here didn`t support such claim at least for T1 SNP. The possible role for V4 G allele could be attributable to higher expression and more activity of ADAM33 in comparison with V4 A allele.

In conclusion, we failed to show association between any genotype and allele of T1 and V4 SNPs in ADAM33 gene with susceptibility to asthma. Main limitation of our study was lower number of participants in both groups in comparison with the majority of previous studies. Therefore, it is necessary to study more SNPs of ADAM33 gene and also other genes involved in the immunopathogenesis of asthma in higher numbers of study groups. High throughput studies including genome-wide association studies (GWAS) using novel technique will provide more valid and accurate results.

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