Association of *ADAM33* T1 Polymorphism With Subgroups of Pediatric Asthma Patients in Iran

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Received: 08 Mar. 2019; Accepted: 21 Oct. 2019

Abstract- There is strong evidence on the interaction of several genetic variations and environmental conditions in the etiology of asthma. Association of a disintegrin and metalloproteinase 33 (*ADAM33*) with asthma risk is not clear and shows diversity between nations and ethnicities. Several single nucleotide polymorphisms (SNP) of the *ADAM33* gene are introduced and studied according to the disease onset and characteristics. The aim of our study is to determine the association of *ADAM33* rs2280091 polymorphism and pediatric asthma in the Iranian population. A total of 63 asthma patients (aged 6-18) and 86 healthy controls were enrolled in our study. Asthma type, classification, and severity were defined. SNPs of the *ADAM33* gene at rs2280091 (T1) were analyzed. Pulmonary function tests, total blood eosinophil count, and IgE count were also assessed. T1 genotype and allele frequencies were not associated with asthma risk in Iranian pediatric asthma. Atopic asthma subgroup and patients with normal eosinophil count showed association with *ADAM33* rs2280091. Moreover, asthma patients with AG genotype showed lower pulmonary functions.

Acta Med Iran 2019;57(11):635-639.

Keywords: Asthma; *ADAM33*; rs2280091; Polymorphisms

Introduction

Asthma is a complex disorder of airway narrowing, which manifests as a broad spectrum of symptoms. Due to its obstructive characteristics, spirometry results may indicate the severity of asthma (1). Exacerbation of asthma episodes may be attributed to several factors. For instance, eosinophilia (>4% or 350,000 absolute counts) may account for frequent asthma attacks, whereas normal blood eosinophil is 2-3% (2). Previous studies have introduced various gene polymorphisms involved in disease susceptibility. However, candidate genotypes and their correlation with asthma have shown diversity between nations and even ethnicities within a country. This indicates that environmental conditions interact differently with genetic alterations (3). Previous linkage studies have introduced many single nucleotide polymorphisms (SNPs) as possible genetic variants in the etiology of asthma. Airway remodeling and its regulating genes also participate in disease pathogenicity (4). A disintegrin and metalloproteinase 33 (*ADAM33*) have been first identified in a positional cloning strategy. *ADAM33* is located on chromosome 20p13 and expressed within the smooth muscle cells and fibroblasts in airways (5). *ADAM33* is involved in several molecular mechanisms due to its multifunctional

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domains. For example, it mediates several cell-matrix and cell-cell interactions in airway smooth muscles and also lung-developing progenitor cells (6,7). .It is reported to be involved in airway hyper-responsiveness, asthma, and chronic obstructive pulmonary disorder (8). Several studies have indicated a correlation between *ADAM33* polymorphisms and asthmatic phenotypes such as lung dysfunction, progressive wheezing, modulating chemo-attractant activities, etc. (9). More than a hundred *ADAM33* SNPs are reported to be related to asthma. Therefore, many studies are performed to investigate the association of *ADAM33* with Asthma in various populations. For instance, the association of *ADAM33* polymorphisms with asthma phenotypes were found to be significant in Korean and German asthma patients (10) (11). However, no significant difference was observed in Latino nations in comparison with healthy controls (12). A meta-analysis on studies from India, Saudi Arabia, Brazil, Portugal, Czech, Netherlands, Egypt, and China indicated that rs2280091 polymorphism was associated with the increased risk of childhood asthma (13). No study has been conducted to assess the association of *ADAM33* polymorphisms at T1 locus (rs2280091) in the Iranian pediatric asthma population. Therefore, we designed a prospective case-control study to analyze the distribution of different genotypes of *ADAM33* at rs2280091 in children with asthma and healthy controls, which were age/sex-matched.

Materials and Methods

Study group

Patients were recruited from Masih Daneshvari hospital, Tehran, Iran (n=63). The study criteria included the patients with physician-diagnosed asthma, according to GINA 2014, and aged 6-18 years. The following exclusion criteria were also used: patients with severe alternative diseases such as cystic fibrosis, congenital lung diseases, etc. Parents of all children signed an informed written consent to participate in the study. Each subject underwent pulmonary function tests, including predicted forced expiratory volume at 1 second (FEV1 % predict), forced vital capacity (FVC % predict), and forced expiratory flow at 25-75% of the pulmonary volume (FEF% predict). Total IgE levels and eosinophil counts were also measured on obtained blood samples individually. Atopic asthma type was defined when at least one skin prick test was positive in patients. Asthma classification and severity were determined, and demographic characteristics are summarized in Table1. Age/sex-matched control subjects (n=86) without any history of atopy, asthma, and other respiratory disorders were also enrolled. Approval was obtained from the research ethics committee of Shahid Beheshti University of Medical Sciences.

Table 1. Patients' characteristics.

Characteristics		Patients
Sex (M/F)		38/22
Mean (SD) age		10/45 ± 3/23
Mean(SD) total IgE		185.2833±201.2572
Mean (SD) eosinophil count		488±880
Severity	Mild intermittent	1
	Mild continuous	50
	Severe intermittent	7
	Severe continuous	1
Asthma classification	Continuous early wheezing	31
	Delayed wheezing	29

Genotyping

Blood samples (10 ml) were obtained from all subjects into EDTA containing tubes, and genomic DNA was isolated using the phenol-chloroform method (14). In order to study the association of T1 *ADAM33* gene SNP, the polymorphic region was amplified using Real-time PCR according to previous studies (15).

Statistical analysis

The correlation between gene polymorphisms and asthma in general and subgroups were examined using Fisher's exact test or χ^2 test. In order to compare the values for FEV1 %, FVC%, and FEF 25-75 %, an ANOVA test was used.

Results

Correlation of *ADAM33* gene and asthma in the general population and subgroups

No association was observed between asthma and

rs2280091 genotypes and alleles in Table 2. However, comparing the genotype frequencies between different asthma subgroups and controls showed an association between *ADAM33* T1 and pediatric atopic asthma and patients with normal blood eosinophil count whereas strong similarity (*P*=0.99) was observed in the non-atopic group and healthy subjects. No association was evaluated based on the levels of IgE Table 3.

Table 2. Association of *ADAM33* rs2280091 with Asthma

Genotype rs2280091	Asthma	Control	*P*
An Allele	(75.4%) 95	(76.75%) 132	
G Allele	(24.6%) 31	(23.25%) 40	0.7874
AA	(58.7%) 37	(59.1%) 55	
AG	(33.33%) 21	(23.65%) 22	0.5584
GG	(7.9%) 5	(9.6%) 9	

Table 3. *ADAM33* rs2280091 associated with asthma subgroups

Groups	Genotypes			*P*
	GG	AA	AG	
Control N=86	9	55	22	
non-atopic asthma N=49	5	31	13	0.9924
Atopic Asthma N=11	0	4	7	0.0288*
High Eosinophil% N=33	4	23	6	0.6925
Normal Eosinophil% N=27	1	12	14	0.0328*
Normal IgE level N=43	1	25	16	0.1396
High IgE levels >200 UI/ml N=17	4	10	4	0.3904

Correlation between SNP of *ADAM33* and asthma phenotypes

The enrolled patients were mostly diagnosed with mild severity asthma whose predicted FEV1% results were: 89.06667+-14.94835. The rs2280091 SNP itself had a significant influence on the predicted FEV1%, FVC%, and FEF% within our asthmatic subjects using ANOVA test Table 4. Significant differences were found for mean FEV1 with *ADAM33* AG heterozygote patients exhibiting significantly lower FEV1 values when compared to *ADAM33* AA homozygotes patients (*P*=0.0161). Similarly, a significantly lower FVC % predicted was found amongst patients with *ADAM33* AG heterozygote when compared to *ADAM33* AA homozygotes (*P*=0.0217). The findings for FEF % predicted were also significant (*P*=0.0217).

Table 4. Genotypes of *ADAM33* rs2280091 associate with the mean value of spirometry test results

Genotype		Mean± SD	95% CI for M	*P*
	AA	93.14±14.23	88.1-98.1	
FEV1(%predicted)	**AG**	81.75±13.3	75.93-87.57	0.0219*
	GG	89.8±8.28	82.54-97.06	
	AA	85.94±13.46	81.61-90.27	
FVC(% predicted)	**AG**	72.85±13.59	66.57-79.13	0.0022**
	GG	88.4±11.97	77.9-98.89	
	AA	96.45±25.52	88-104.9	
FEF(% predicted)	**AG**	75.83±24.42	64.55-104.11	0.0245*
	GG	97.00±33.61	67.54-126.46	

Discussion

Our study did not show a significant difference between the general frequency of rs2280091 genotypes in asthmatic pediatrics and healthy controls. It has been reported that patients with mild asthma have lower *ADAM33* expression levels in comparison with severe asthma. It is also documented that *ADAM33* is mostly associated with persistent severe asthma (16). Due to the overall mild disease of patients in our study, it is suggested that further studies on more severe states of asthma population might demonstrate different results (17). Our results are consistent with previous studies conducted on the Azeri ethnicity of Iran, Venezuelan, Punjabi population of Pakistan, etc. However, rs2280091 has been reported to be correlated with increased pediatric asthma risk in Egypt and also adulthood asthma in Mongolian and Han groups (15,18,19). Furthermore, previous analyses on fourteen studies reported significant associations of T1 polymorphism with asthma risk among Asian children (20). These contrasting results may be attributed to various interactions of *ADAM33* with environmental factors, which may result in asthma. For example, the soluble form of *ADAM33* due to loss of its regulatory cytoplasmic domain is beneficiary, whereas abnormal localization of *ADAM33* results in lung dysfunction due to airway obstruction. Besides, the discrepancies in studies may be attributed to the different subgroups of asthma whose associations are not considered differentially. In our study, This SNP alone was not associated with asthma risk. However, comparing atopic patients with healthy controls revealed an association between *ADAM33* and atopic asthma. This finding is consistent with the previous study on populations of African Americans, US white, Dutch white, and US Hispanic (21). However, contraindicating reports are also documented (22). This suggests that *ADAM33* T1 polymorphism may modulate the atopic phenotype in Iranian children. Moreover, considering patients with normal blood eosinophil percentage also showed a significant association of rs2280091 with Asthma. This may suggest a role for *ADAM33* T1 genotypes in regulating eosinophil recruitment from blood to the airway walls, which requires further investigations considering total eosinophil counts both in the asthmatic airways and peripheral blood. However, the association between this polymorphism and total IgE in the asthmatic population could not be confirmed. Further studies investigating the role of *ADAM33* polymorphism on atopic asthma patients with normal blood eosinophil count may reveal novel interactions of this factor. Our findings indicated that *ADAM33* T1 polymorphisms might influence the outcome of asthma based on spirometry results. This finding is consistent with previous reports on the potential roles of *ADAM33* on exacerbating lung functions, especially early in life (23,24). According to our results, AG genotype was associated with lower respiratory functions, which may indicate a possible role of this heterozygote genotype in promoting airway wall thickening. This hypothesis needs further investigations as the downstream effects of *ADAM33* polymorphisms are complex. In conclusion, our study demonstrated significant differences in spirometry values within rs2280091 genotypes. We also reported an association of *ADAM33* T1 polymorphism with atopic and normal eosinophil count subgroups of asthma despite the overall similarity of the genotype frequencies between pediatric asthma population and healthy controls.

Acknowledgments

This study was funded by Shahid Beheshti University of Medical Sciences.

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