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

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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.

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
ADAM33 polymorphisms in pediatric asthma patients

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 (FEF25-75% 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 Table 1. 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 χ² 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 | $P$ |
|-------------------|--------|---------|-----|
| Genotype          | (75.4%) | (76.75%) | 0.7874 |
| An Allele         | 95     | 132     |     |
| G Allele          | (24.6%)| (23.25%)|     |
| AA                | (58.7%)| (59.1%) |     |
| AG                | (33.33%)| (23.65%)| 0.5584 |
| GG                | (7.9%) | (9.6%)  |     |

### Table 3. ADAM33 rs2280091 associated with asthma subgroups

| Groups                  | Genotypes | $P$  |
|-------------------------|-----------|------|
| Control                 | GG        |      |
| n=86                    | 9         | 22   |
| non-atopic asthma       | GG        |      |
| n=49                    | 5         | 13   | 0.9924 |
| Atopic Asthma           | GG        |      |
| n=11                    | 0         | 7    | 0.0288* |
| High Eosinophil%        | GG        |      |
| n=33                    | 4         | 6    | 0.6925 |
| Normal Eosinophil%      | GG        |      |
| n=27                    | 1         | 14   | 0.0328* |
| Normal IgE level        | GG        |      |
| n=43                    | 1         | 16   | 0.1396 |
| High IgE levels >200 UI/ml | GG    |      |
| n=17                    | 4         | 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 | $P$  |
|-------------------|----------|--------------|------|
| FEV1(% predicted) | AA       | 93.14±14.23  | 88.1-98.1 | 0.0219* |
|                   | AG       | 81.75±13.3   | 75.93-87.57 |      |
|                   | 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.

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