

## ADAM33 Gene Polymorphisms Are Not Associated with Asthma in Turkish Children

Elçin Bora, M.D., Ph.D.,<sup>1</sup> Zeynep Arikan-Ayyildiz, M.D.,<sup>2</sup> Fatih Firinci, M.D.,<sup>2</sup> Tufan Çankaya, M.D., Ph.D.,<sup>1</sup> Özlem Giray-Bozkaya, M.D.,<sup>3</sup> Nevin Uzuner, M.D.,<sup>2</sup> and Ayfer Ülgenalp, Ph.D.<sup>1</sup>

A disintegrin and metalloprotease domain 33 (*ADAM33*) is the first asthma-susceptible gene discovered by positional cloning. The objective of this study was to determine the association between *ADAM33* polymorphisms and asthma in the Turkish children population with asthma. Four single-nucleotide polymorphisms (SNPs) previously reported to be related with asthma were genotyped in 98 cases and 100 controls. The genotyping procedure consisted of a polymerase chain reaction amplification and SNP detection of the 12433 T/C (T1), 12462 C/T (T2), 12540 C/T (T+1), and 12601 T/G (T+2) variants of the *ADAM33* gene. No significant differences were observed for T1, T2, T+1, and T+2 polymorphisms between asthmatic patients and controls. There was also no difference between the atopic and nonatopic asthma group by means of *ADAM33* polymorphisms. Our data revealed that there is no association of these 4 SNPs of the *ADAM33* gene with asthma in the Turkish children population.

### Introduction

ASTHMA IS THE MOST common chronic disease of childhood in developed countries that affects more than 150 million people all over the world.<sup>1</sup> It is a chronic respiratory disease characterized by chronic inflammation, airway obstruction, bronchial hyper-responsiveness (BHR), and airway remodeling.

The etiology of asthma is believed to be a combination of multiple genetic and environmental factors. To date, many genes have been found to be related with asthma. The first asthma susceptibility gene discovered by positional cloning was a disintegrin and metalloprotease domain 33 (*ADAM33*) gene in 2002.<sup>2</sup> The *ADAM* gene family is a subgroup of the zinc-dependent metalloprotease family, and it is located on chromosome 20p13.<sup>3,4</sup> *ADAM33* is mainly expressed by mesenchymal cells, including fibroblasts and smooth muscle cells, and it codes for the proteins that play important roles in cell fusion, cell adhesion, cell signaling, and proteolysis.<sup>5</sup> Due to this selective expression of *ADAM33* in mesenchymal cells, it has been speculated that alterations in *ADAM33* activity may be the reason of abnormalities in airway smooth muscle function and fibroblasts, which cause airway remodeling and BHR.<sup>6</sup> Several studies have demonstrated that single-nucleotide polymorphisms (SNPs) of *ADAM33* have been significantly associated with asthma after the first study documenting the positive association of *ADAM33* polymor-

phisms with asthma.<sup>2,7–14</sup> Some of them also reported associations between phenotypes of asthma and BHR.<sup>8,14</sup> However, negative associations between *ADAM33* polymorphisms and asthma have also been reported from different populations.<sup>15,16</sup> The prevalence of childhood asthma in Turkey has increased similar to the trends of Western countries in recent years.<sup>17</sup> However, the differences in the genetic background of the Turkish population may account for different asthma susceptibility genes. Therefore, to investigate whether *ADAM33* SNPs are associated with asthma in Turkish children, we genotyped 4 previously reported *ADAM33* polymorphisms and studied the possible association of these polymorphisms with asthma in a case-control study.

### Materials and Methods

#### Subjects

The study group included 98 cases and 100 controls. Asthmatic children and the control group were recruited from the Dokuz Eylül University, Pediatric Allergy Department, and Pediatric Outpatient Clinic of the same university, respectively. Asthma was diagnosed by a history of intermittent wheezing and the presence of reversible airway obstruction as defined by at least a 12% improvement in final expiratory volume in 1 s (FEV1) after bronchodilator

<sup>1</sup>Department of Medical Genetics, Faculty of Medicine, Dokuz Eylül University, Balçova, İzmir.

<sup>2</sup>Division of Pediatric Allergy, Department of Pediatrics, Faculty of Medicine, Dokuz Eylül University, Balçova, İzmir.

<sup>3</sup>Division of Pediatric Genetics, Department of Pediatrics, Dokuz Eylül University, Balçova, İzmir.

administration, and asthma severity grading was made according to Global Initiative for Asthma (GINA) guidelines.<sup>18</sup> Briefly, the severity of a patient's asthma was classified into mild, moderate, or severe categories based on the clinical features present before treatment had begun. When the patient had already been on treatment, the classification of severity was based on the clinical features present and the step in the daily medication regimen that the patient was currently on. Thus, a patient with ongoing symptoms of mild persistent asthma, despite being on the appropriate maintenance treatment for this step, was regarded as having moderate persistent asthma. Subjects with mild and moderate asthma were included in the study, and severe asthmatics were excluded. The control group consisted of subjects who denied any respiratory symptoms and had no personal and family history of asthma or allergy. Asthmatic patients with any other chronic diseases and the ones who experienced an acute asthma attack within the previous month were excluded from the study.

Asthmatic subjects underwent skin prick testing with a panel of aeroallergens (Allergopharma), including house dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), weed mix (*Artemisia vulgaris*, *Urtica dioica*, *Taraxacum vulgare*, and *Plantago lanceolata*), grass and cereal mix (*Holcus lantus*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Festuca pratensis*, *Hordeum vulgare*, *Avena sativa*, *Secale cereale*, and *Triticum sativum*), tree mix (*Alnus glutinosa*, *Corylus avellana*, *Populus alba*, *Ulmus scabra*, *Salix caprea*, *Betula verrucosa*, *Fagus silvatica*, *Quercus robur*, and *Platanus orientalis*), mold mix (*Alternaria alternata*, *Botrytis cinerea*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium moniliforme*, *Helminthosporium halodes*, *Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans*, and *Serpula lacrymans*), animal dander mix (Golden hamster, dog, cat, rabbit, and guinea pig epithelia), and olea and pine tree. Histamine hydrochloride 10 mg/mL and NaCl 9 mg/mL were used as positive and negative controls, respectively. A mean wheal diameter of 3 mm was considered positive. Asthmatic patients were classified as atopic or nonatopic according to the skin prick test results.

This study was approved by the ethics committee of the Dokuz Eylul University Faculty of Medicine. All participants provided written informed consent.

### Genotyping

Genomic DNA was extracted from peripheral whole blood using AccuPrep® Genomic DNA Extraction kit according to the manufacturer's instructions (Bioneer). The genotyping procedure consisted of a polymerase chain reaction (PCR) amplification and SNP detection of the 12433 T/C and 12462 C/T (exon 20), 12540 C/T and 12601 T/G (intron 20) variants of ADAM33 gene using the following pair of primers<sup>19</sup>: Forward: 5'-TGG ACT CTT ATC ACG TTG CTC-3' and Reverse: 5'-GGG AAG AAA CTT CCA AGC TGC-3' by direct sequencing. PCR amplification was carried out in a volume of 25 µL containing 100 ng DNA; 100 µM of each dATP, dCTP, dGTP, and dTTP; 1×buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 1 mM DTT, and pH -7.5 at 25°C); 5×of Tune Up solution; 25 nM of each primer; and 1 U of Taq polymerase (Nanohelix). The amplification protocol conditions were selected as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of

denaturation at 94°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 45 s, and a final extension at 72°C for 7 min. PCR products were purified using the PureHelix™ PCR Purification Kit according to the manufacturer's instructions (Nanohelix) and subjected to automatic sequence analysis (Automated sequencer ABI 3130; Applied Biosystems) by the BigDye terminator reaction according to the supplier's instructions (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits Version 3.1; Applied Biosystems) using Forward: 5'-TGG ACT CTT ATC ACG TTG CTC-3' primer. The obtained sequences were analyzed using BioEdit software, version 7.0.5.3 (www.mbio.ncsu.edu/bioedit/bioedit.html).

### Statistics

The Hardy-Weinberg equilibrium was tested using the  $\chi^2$  test. The  $\chi^2$  test was used to evaluate differences between cases and controls and individual subgroups. Data were analyzed with Scientific Package for Social Sciences (SPSS) software (Version 15.0; SPSS, Inc.). A value of  $P=0.05$  or less was considered significant.

### Results

The demographics of the cases and controls are presented in Table 1. All the SNPs investigated in the study were in Hardy-Weinberg equilibrium in both cases and controls ( $P>0.05$ ). None of the SNPs under study was significantly associated with asthma in Turkish asthmatic children (Table 2). The statistical  $P$  values were 0.083, 0.113, 0.083, and 0.238 respectively for 12433 T/C, 12462 C/T, 12540 C/T, and 12601 T/G variants of the ADAM33 gene.

In order to clarify whether atopy would affect the association between ADAM33 polymorphisms and asthma, we analyzed the genotype of the patients with or without atopy, but we could not find any association (Table 3).

### Discussion

The identification of genetic factors in multifactorial complex diseases such as asthma may give promise for the development of individualized interventional strategies, including planning for treatment options and prediction of the prognosis. This promise has led to the study of many asthma susceptibility genes worldwide among different populations. ADAM33 is one of the most important genes in this context, and it has a significant impact on the study of the genetics of bronchial asthma. This study evaluated 4 polymorphisms of ADAM33 (12433 T/C (T1), 12462 C/T (T2), 12540 C/T (T+1), and 12601 T/G (T+2)) to investigate the association between these polymorphisms and asthma in the Turkish children population.

The initial report by Van Eerdewegh et al.<sup>2</sup> identified 37 SNPs in the ADAM33 gene, and 15 polymorphisms have been genotyped in the United Kingdom and U.S. populations. No

TABLE 1. DEMOGRAPHICS OF THE STUDY GROUP

|               | Patients with<br>asthma (n=98) | Controls<br>(n=100) |
|---------------|--------------------------------|---------------------|
| Age (mean±SD) | 9.30±2.93                      | 10.8±3.14           |
| Gender (F/M)  | 36/62                          | 51/49               |

TABLE 2. ASSOCIATION BETWEEN THE ADAM33 POLYMORPHISMS AND ASTHMA IN TURKISH CHILDREN

| Genotype      | Asthma patients,<br>n=98 (%) | Controls,<br>n=100 (%) | P<br>value |
|---------------|------------------------------|------------------------|------------|
| T1-12433 T/C  |                              |                        | 0.083      |
| T/T           | 78 (79.5)                    | 67 (67)                |            |
| T/C           | 20 (20.5)                    | 32 (32)                |            |
| T2-12462 C/T  |                              |                        | 0.113      |
| C/C           | 78 (79.5)                    | 68 (68)                |            |
| C/T           | 20 (20.5)                    | 31 (31)                |            |
| T+1 12540 C/T |                              |                        | 0.083      |
| C/C           | 78 (79.5)                    | 67 (67)                |            |
| C/T           | 20 (20.5)                    | 32 (32)                |            |
| T+2 12601 T/G |                              |                        | 0.238      |
| T/T           | 69 (70.4)                    | 80 (80)                |            |
| T/G           | 26 (26.5)                    | 19 (19)                |            |

Cells of the double mutants of the studied SNPs are collapsed, because they have counts <5.

ADAM33, a disintegrin and metalloprotease domain 33; SNP, single-nucleotide polymorphisms.

single SNP was associated with asthma in both the U.S. and United Kingdom cohort, but it has been found that SNPs I1, L-1, M+1, T1, T2, and T+1 were significantly associated with asthma in the U.S. cohort, while SNPs F+1, Q-1, S1, S2, ST+4, V-1, and V4 were significant in the United Kingdom cohort. They also stated that ADAM33 polymorphisms were associated with BHR. This conclusion was also shared by Lee et al.<sup>14</sup> Howard et al.<sup>8</sup> studied 8 SNPs of ADAM33, including T1 in 4 different populations and stated that there was an association of at least 1 SNP with asthma in each population. Studies from Thai, Indian, Japanese, and Australian populations also reported significant associations between ADAM33 polymorphisms and asthma.<sup>9-11,20</sup>

In contrast, no associations were reported from Puerto Rican, German, Mexican, and Chinese populations.<sup>15,16,21</sup> Different reasons such as population heterogeneity, differences in case definitions among the studies, and gene-environment interactions may contribute to these inconsistencies in replication.<sup>16</sup> Most of the studies reporting negative associations are noted to originate mainly from

asthmatic children populations.<sup>15,16</sup> This raises the suspicion about the association of childhood asthma and ADAM33 polymorphisms, but studies are also inconclusive in this issue. Simpson et al.<sup>22</sup> reported that F+1 SNP of ADAM33 was associated with reduced lung function at the age of 3 years and F+1, M+1, T1, and T2 SNPs were associated with decreased FEV1 and suggested that the ADAM33 gene is related with early life lung function. Another study from Japan suggested that S+1, ST+4, and T2 SNPs were associated with childhood asthma, and an Indian study also concluded that the ADAM33 gene polymorphisms could modify individual susceptibility to develop childhood asthma in the Indian population.<sup>10,11</sup> However, larger studies investigating childhood asthma and ADAM33 polymorphisms did not find any significant association.<sup>15,16,23</sup>

In this study, we investigated 4 previously reported ADAM33 polymorphisms: 12433 T/C (T1), 12462 C/T (T2), 12540 C/T (T+1), and 12601 T/G (T+2). We observed that T1, T2, and T+1 polymorphisms have almost identical distribution of alleles, and it seems that T1, T2, and T+1 are in tight linkage disequilibrium in our population. The results of our study showed no association between these 4 SNPs in ADAM33 and asthma in the Turkish population. SNPs T1, T2, and T+1 were significantly associated with asthma in the U.S. cohort of Van Eerdewegh et al.,<sup>2</sup> while the following studies yielded different results. Either T1 or T2 SNP was associated with asthma in some studies,<sup>7,8,11,13</sup> but no relation was found in the studies of Wang et al.,<sup>21</sup> Lind et al.,<sup>15</sup> Schedel et al.,<sup>16</sup> and in the meta-analysis study of Blakey et al.<sup>12</sup> Raby et al.<sup>23</sup> studied T1, T2, and T+1 T+2 in their asthmatic children group and stated that T1 and T+1 were only marginally associated with asthma in the Hispanic cohort. T2 SNP was also found to be overtransmitted to asthma-affected offspring in the Japanese population.<sup>11</sup> Associations between atopy and ADAM33 SNPs were not extensively studied earlier. In the study of Blakey et al.,<sup>12</sup> no association was detected between ADAM33 SNPs and atopy in the Icelandic population. Total IgE levels, skin test responsiveness, and blood eosinophilia were studied in limited studies, and T1 and T2 SNPs were found to be related with positive skin tests in U.S. white and Hispanic populations.<sup>8,23</sup> SNP T+2 demonstrated an evidence of association with blood eosinophil levels among white subjects, while T1 and T+1 SNPs were associated with serum IgE and eosinophil levels among Hispanic subjects in another study.<sup>23</sup> A comparison of our results with mentioned studies indicates that no single SNP was universally associated with asthma and genetic differences among populations and subtypes of asthma (pediatric, atopic, and nonatopic); sample size and criteria for the definition of asthma in studies could be regarded as the reasons of these different results.

In conclusion, we studied the ADAM33 polymorphisms in the Turkish asthmatic population for the first time, but we did not detect a significant association between 4 SNPs of the ADAM33 gene and asthma. Larger studies that will also investigate many more SNPs of the ADAM33 gene will lead to an understanding of the role of the ADAM33 gene polymorphisms in childhood asthma in the Turkish population.

### Authors' Contributions

This article was prepared by a team led by Ayfer Ülgenalp and Nevin Uzuner. Elçin Bora and Zeynep Arik-

TABLE 3. ASSOCIATION OF ADAM33 GENE POLYMORPHISMS WITH ATOPIC STATUS OF THE ASTHMATIC PATIENTS

| Genotype      | Atopic,<br>n=51 (%) | Nonatopic,<br>n=47 (%) | P<br>value |
|---------------|---------------------|------------------------|------------|
| T1-12433 T/C  |                     |                        | 0.293      |
| T/T           | 38 (74.5)           | 40 (85.1)              |            |
| T/C           | 13 (25.5)           | 7 (14.9)               |            |
| T2-12462 C/T  |                     |                        | 0.293      |
| C/C           | 38 (74.5)           | 40 (85.1)              |            |
| C/T           | 13 (25.5)           | 7 (14.9)               |            |
| T+1 12540 C/T |                     |                        | 0.293      |
| C/C           | 38 (74.5)           | 40 (85.1)              |            |
| C/T           | 13 (25.5)           | 7 (14.9)               |            |
| T+2 12601 T/G |                     |                        | 0.967      |
| T/T           | 35 (68.6)           | 34 (72.3)              |            |
| T/G           | 14 (27.4)           | 12 (25.5)              |            |

Cells of the double mutants of the studied SNPs are collapsed, because they have counts <5.

Ayyildiz have been actively involved in preparing and contributing during at all stages of this article. Zeynep Arikan-Ayyildiz also contributed to patient selection and material collection along with Fatih Firinci. Tufan Çankaya and Özlem Giray-Bozkaya were involved in the genetic study of the work.

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## Author Disclosure Statement

None of the authors have a conflict of interest.

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Address correspondence to:  
Zeynep Arikan-Ayyildiz, M.D.  
Division of Pediatric Allergy  
Department of Pediatrics  
Faculty of Medicine  
Dokuz Eylul University  
Balcova 35340  
Izmir

E-mail: zeynep.ayyildiz@deu.edu.tr

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