

## Significant association between SCGB1D4 gene polymorphisms and susceptibility to adenoid hypertrophy in a pediatric population\*

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**Background/aim:** Adenoid hypertrophy (AH) is chronic enlargement of the adenoid tissue. The pathophysiology of the disease is unclear. We analyzed *SCGB1D4* gene polymorphisms in order to determine the effect of the variants or their genetic combinations on AH.

**Materials and methods:** We genotyped the *SCGB1D4* (*IIS*) gene in 167 participants (95 children with AH and 72 controls) by performing DNA sequencing in blood samples.

**Results:** We genotyped three single nucleotide polymorphisms (SNPs). In the analysis, we found that in the presence of those SNPs and the minor alleles of individual SNPs four haplotypes were associated with an increased risk of AH. In addition, those SNPs were significantly associated with asthma, allergy, sleep-disordered breathing, AH grade +4, and a high level of IgE. As indicated on multifactor dimensionality reduction analysis, single-locus (rs35328961), two-locus (rs35328961\_rs56196602), and three-locus models (rs200327820\_rs35328961\_rs56196602) had the highest synergistic interaction effect on AH. The three-factor model was also significantly associated with some genotypes of rs35328961 and allergic-asthmatic AH.

**Conclusion:** SNPs of *SCGB1D4* and their combinations are associated with an increased risk for developing AH. We highlighted the importance of genetic factors on AH and AH-related clinical phenotypes.

**Key words:** Adenoid hypertrophy, asthma, allergy, SDB, SNP, secretoglobins, *SCGB1D4*, DNA sequence analysis, PCR, MDR, haplotypes, gene

### 1. Introduction

The adenoids, also known as pharyngeal or nasopharyngeal tonsils, are important components of the Waldeyer's ring (1). Adenoid hypertrophy (AH) is enlargement of the adenoids; it is frequent in the pediatric population and can cause symptoms such as mouth breathing, nasal obstruction, hyponasal speech, snoring, and sleep-disordered breathing (SDB) (2–4). The exact mechanisms underlying AH remain extremely poorly understood, but it has been assumed that recurrent or chronic inflammation plays a role (5).

Cytokines, which are assigned to function in immunological responses, may change the progress and severity of inflammatory processes (6–8). Various genetic factors and their combinations can affect the association with various disease phenotypes, and change the severity

of chronic inflammatory diseases through altering the levels of gene expression (9–11).

Secretoglobulins (SCGBs) are newly discovered cytokines, and they are candidates to form a new cytokine family with their anti-inflammatory and immunomodulatory functions (12,13). They have been reported to play a role in the pathogenesis of inflammatory upper and lower airway diseases including asthma, allergies, and nasal polyposis (12–14). *SCGB1D4* (Secretoglobin, family 1D, member 4; *IIS*) gene is located on chromosome 11 (11q12.3), and it is expressed widely in the lymphoblasts in the airway mucosa and lymphoid tissues. The *SCGB1D4* gene, stimulated by IFN-gamma, has been reported to regulate the migration and invasion of chemotactic cells (12,14). To date, only a few studies have focused on *SCGB1D4*.

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In this study, we aimed to analyze *SCGB1D4* gene polymorphisms and determine the effect of the variants, or their genetic combinations, on AH.

## 2. Materials and methods

### 2.1. Study population

A total of 95 children with AH (39 girls and 56 boys) with a mean age of 5.27 (3–10) years were included in the study as the study group, and 72 healthy children (22 girls and 50 boys) with a mean age of 4.96 (2–11) years acted as the controls. The diagnosis of AH was based on history and physical and endoscopic examination (15). AH was graded in accordance with Parikh's classification: grade 1 indicated adenoid tissue not in contact with the adjacent structures, grade 2 indicated adenoid tissue in contact with the torus tubarius, grade 3 indicated adenoid tissue in contact with the vomer, and grade 4 indicated adenoid tissue in contact with the soft palate (16). In our study, grades 3 or 4 were regarded as AH.

The control group consisted of children admitted for their regular follow-up or minor trauma and the diagnosis of AH was ruled out by endoscopic examination. Children with craniofacial abnormalities, mental retardation, immunodeficiencies, and/or cardiovascular, pulmonary, metabolic, genetic, or neuromuscular diseases were excluded.

The diagnosis of SDB was primarily based on the clinical history obtained from the parents of the children (17). Asthma diagnosis was based on history or examination of the children in the pulmonology department (18). Skin prick testing was used to determine allergy. The tests were performed in accordance with the recommendations of the European Academy of Allergy and Clinical Immunology (19). Total serum immunoglobulin (Ig) E level was determined by nephelometric assay (Dade Behring/Siemens, Deerfield, IL, USA) (20).

The parents of the children were informed about the study and the method of the study, and their verbal and written informed consents were obtained. The study protocol was approved by Hacettepe University Ethics Board, and the study was conducted in accordance with human rights and experimental ethics.

### 2.2. DNA isolation and genotyping

Blood samples were put into tubes with EDTA. Genomic DNA was extracted using a ready kit (NucleoSpin blood DNA, Macherey-Nagel GmbH & Co. Kg, Germany). Genotyping for *SCGB1D4* was performed by sequencing. First, a polymerase chain reaction (PCR) was performed to enhance the relevant gene region (SuperHot Master Mix, Bioron, GmbH, Germany). The primer pairs used in the PCR reaction were designed using Primer Designer version 2.0 (Scientific & Educational Software). For Exon I, the forward primer used was

5'-CACTCAGATGCAGAGATTCCTAAGGAG-3' and the reverse primer was 5'-CTCTAACCTTTATGAATCTTATTCAACAAGC-3'. A 443-bp fragment was amplified with this primer set. For Exon II, the forward primer used was 5'-ATGAAGCTCTTCCCTTTGTTGTGTGAC-3' and the reverse primer was 5'-CATCCCAYAGTGACCGTGTCCACTG-3'. A 609-bp fragment was amplified with this primer set. For Exon III, the forward primer used was 5'-GAAAGGCTGCTTCTCACCAGAATG-3' and the reverse primer was 5'-TTCAGGCCCCATTAGTAACAGAATGC-3'. A 476-bp fragment was amplified with this primer set. The mixture was denatured at 95 °C for 5 min and underwent 35 cycles in a thermocycler PCR system under the following conditions: denaturation at 95 °C for 1 min, annealing at 60 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min.

A ready kit was used for purification of the amplified products (NucleoFast 96 PCR, Macherey-Nagel GmbH & Co. Kg, Germany). A sequence reaction was done in the purified PCR products with a ready kit (BigDye Terminator V3.1 Cycle Sequencing, Applied Biosystems, USA). The nucleotide changes in the *SCGB1D4* gene were investigated using the data obtained from the ABI PRISM 3130 Genetic Analyzer capillary automatic sequence equipment.

### 2.3. Statistical analysis

The statistical analysis was carried out with SPSS 16.0 (SPSS Inc, Chicago, IL, USA). Comparisons of quantitative variables such as age and IgE levels were made with parametric or nonparametric tests depending on the normality of the distribution. The chi-squared test or Fisher's exact test was used to assess the difference between the *SCGB1D4* polymorphisms and the clinical phenotypes. The frequency of each genotype was tested for concordance with the Hardy-Weinberg equilibrium (HWE) using  $\chi^2$  (21). The effective sample size and statistical power were computed using a web browser program, Genetic Power Calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc/>).

SNPstats (<http://bioinfo.iconcologia.net/index.php?module=Snpsstats>) was used to determine the degree of pairwise linkage disequilibrium (LD), and genotype and haplotype analysis (22). This software provides the odds ratio (OR) and 95% confidence interval (95% CI). The software was regressed in a logistic model, assuming the codominant (major homozygotes versus heterozygotes versus minor homozygotes), the dominant (major homozygotes versus heterozygotes plus minor homozygotes), and the recessive (major homozygotes plus heterozygotes versus minor homozygotes) models of inheritance with covariates.

The Multifactor Dimensionality Reduction (MDR) software package (version 1.0.0, available at [www.epistasis.org](http://www.epistasis.org)) is a new statistical and computational tool described by Hahn et al. We used it to address possible genotype-genotype and genotype-phenotype relationships (24). MDR is a nonparametric (i.e. no parameters are estimated) novel approach, and it is free of any assumed genetic model. It can detect and characterize nonlinear patterns of gene-gene, SNP-SNP, and SNP-phenotype interactions in genetic association studies, and it is more powerful than the parametric statistical approaches. This method reduces data dimensionality by pooling genotypes from multiple SNPs into either high-risk or low-risk groups for a disease, thereby circumventing the problem of high-order genotype combinations with a low number of observations. It was shown that it successfully identified combinations of multilocus genotypes and discrete environmental factors that are associated with diseases (25–27).  $P < 0.05$  was considered as significant in all statistical analyses.

### 3. Results

In the study group, 68 (71%) patients had grade 3 and 27 (29%) patients had grade 4 AH. The ages ( $P = 0.181$ ) and sexes ( $P = 0.163$ ) of the study and the control groups were similar. The rates for SDB, allergy, and asthma were significantly higher in the study group ( $P < 0.05$ ). Similarly, total IgE level was significantly higher in the study group compared to the controls ( $P < 0.05$ ) (Table 1).

#### 3.1. Genetic analyses

We identified three SNPs (rs200327820 (NM\_996881.1:p.Val5=), rs35328961 (NM\_206998.1:c.243-9\_243-8insA) and rs56196602 (NM\_206998.1:c.\*13G>A)). Those

SNPs and were previously reported, and registered in the dbSNP database (Short Genetic Variations Database at <http://www.ncbi.nlm.nih.gov/snp>) (27,28). All genotype distributions of the control subjects were consistent with those expected from the HWE (all  $P > 0.05$ ).

Individual SNP analysis revealed that the frequencies of the three SNPs were significantly different between the study and the control groups. The analysis of the individual SNPs revealed that there was a significant association of rs200327820 and rs35328961 with presence of asthma ( $P = 0.001$  and  $P = 0.002$ , respectively) and grade 4+ AH ( $P = 0.023$  and  $P = 0.014$ , respectively). A similar association of the rs56196602 was found with allergy and SDB ( $P = 0.040$  and  $P = 0.012$ , respectively). The rs35328961 showed a significant association with IgE level ( $P = 0.02$ ). However, sex and age did not show any associations with SNPs of *SCGB1D4* ( $P > 0.05$ ).

The genotype analysis showed that the genotype frequencies of the three SNPs were not similar in the study and the control groups (Table 2). The analyses revealed that rs200327820 GA, AA, and GA-AA genotypes were associated with a significantly higher risk of AH compared to the controls ( $P = 0.0051$ ,  $P = 0.0012$ , and  $P = 0.00233$ , respectively). In addition, the frequency of rs200327820 GA-AA genotype was higher in asthmatic children with AH when compared to the asthmatic controls ( $P = 0.0011$ ). The rs35328961 -A, AA, -A-AA, and -- genotypes increased the risk of AH significantly when compared to the controls ( $P = 0.008$ ,  $P = 0.008$ ,  $P = 0.023$ , and  $P = 0.036$ , respectively) and in AH patients that had higher IgE levels when compared to the controls ( $P = 0.0079$ ,  $P = 0.0069$ ,  $P = 0.0021$ , and  $P = 0.036$ , respectively). The frequencies of

**Table 1.** Characteristics of the study population.

Variables	Study group (n: 95) n (%)	Control group (n: 72) n (%)	P-value*
Age (years) (mean ± SD)	5.27 (1.46913; 3–10)	4.96 (1.56983; 2–11)	0.181
Sex	56 (59)/39 (41)	50 (69)/22 (31)	0.163
AH grade + 3	68 (71)		
+ 4	27 (29)		
SDB (+)	71 (75)	10 (10)	<0.001
IgE, µg/L	11.3700 (10.78601; 0.38–49.30)	9.0550 (6.33587; 0.38–32.90)	<0.001
Asthma (+)	23 (24)	5 (7)	0.003
Allergy (+)	28 (29)	7 (10)	0.003

Values are presented as median ± SD or numbers (%) unless otherwise specified; SD = Standard deviation; SDB = Sleep-disordered breathing; n (%) = Frequency

\*Derived from chi-squared or Fisher exact test for comparison of discrete variables and unpaired Student's t-test for continuous variables.

**Table 2.** Frequencies of *SCGB1D4* SNPs genotype and allele.

SNPs	Genotype/Allele	Study group (n: 95)	Controls (n: 72)	Models	OR (95 CI)*	P-value**
		n (%)	n (%)			
rs200327820	GG	86 (91)	72 (100)	Codominant	1.00 (reference)	0.0051
	GA	8 (8)	0 (0)		8.0051 (4.01–12.02)	
	AA	1 (1)	0 (0)		1 (0.00–1.5)	
	GG	86 (91)	72 (100)	Dominant	1.00 (reference)	0.0012
	GA-AA	9 (9)	0 (0)	9 (4.50–13.5)		
	GG-GA	94 (99)	72 (100)	Recessive	1.00 (reference)	0.29
	AA	1 (1)	0 (0)		1 (0.00–1.5)	
	GG-AA	87 (92)	72 (100)	Overdominant	1.00 (reference)	0.0023
	GA	8 (8)	0 (0)		8 (4.60–12.60)	
A‡	0.053	0			0.003**	
rs35328961	--	33 (35)	42 (59)	Codominant	1.00 (reference)	0.0008
	-A	47 (49)	24 (33)		2.49 (1.27–4.87)	
	AA	15 (16)	6 (8)		3.18 (1.11–9.10)	
	--	33 (35)	42 (58)	Dominant	1.00 (reference)	0.0023
	-A-AA	62 (65)	30 (42)	2.63 (1.40–4.94)		
	---A	80 (84)	66 (92)	Recessive	1.00 (reference)	0.14
	AA	15 (16)	6 (8)		2.06 (0.76–5.61)	
	---AA	48 (51%)	48 (67)	Overdominant	1.00 (reference)	0.036
	--	47 (49)	24 (33)		1.96 (1.04–3.69)	
A‡	0.41	0.26			0.005	
rs56196602	GG	84 (88)	70 (93)	Codominant	1.00 (reference)	0.56
	GA	9 (10)	2 (7)		1.40 (0.45–4.38)	
	AA	2 (2)	0 (0)		1 (0.00–2.5)	
	GG	84 (88)	70 (97)	Dominant	1.00 (reference)	0.026
	GA-AA	11 (12)	2 (3)	4.58 (0.98–21.37)		
	GG-GA	93 (99)	72 (100)	Recessive	1.00 (reference)	0.13
	AA	2 (2)	0 (0)		1 (0.00–1.5)	
	GG-AA	86 (91)	70 (97)	Overdominant	1.00 (reference)	0.07
	GA	9 (9)	2 (3)		3.66 (0.77–17.50)	
A‡	0.08	0.02			0.013	

n (%) = Frequency; NA = Not available; SNP = Single nucleotide polymorphism; OR = Odds ratio; CI = Confidence interval

\*According to chi-squared test

\*\*According to Fisher's exact test;

‡ Assumed risk alleles.

the rs35328961 --, -A-AA genotypes were higher in AH patients with SDB when compared to the controls with SDB (for rs35328961 -- -- P = 0.0019; for rs35328961 -A-AA P = 0.0022). The frequency of rs56196602 GA-AA genotype was significantly higher in the children with AH

(P = 0.026), and it was also higher in allergic children with AH when compared to the allergic controls (P = 0.027).

There were significant differences between the study and control groups for the frequencies of rs200327820 A, rs35328961 A, and rs56196602 A alleles (P = 0.003, P =

0.005, P = 0.013, respectively) (Table 2). In order to reveal the potential interactions of those three SNPs with the risk of AH, we combined them based on the numbers of variant (risk) alleles. In the logistic regression model, “A” alleles together at rs200327820 and rs35328961 had 4.076-fold increased risk for AH (P = 0.03).

We found that the rs35328961 and rs56196602 had the highest pairwise LD compared to other pairwise LD (D’= 0.3341, D’= 0.218, and D’= 0.0121). Haplotype analysis including three SNPs was performed, and it was seen that there were significant associations among some haplotypes and AH (P = 0.0052) (Table 3). The frequencies of GAG, G-A, AAG and A-G haplotypes were significantly higher in the study group (P = 0.002, P = 0.001 and P = 0.001, respectively), but the frequency of GAA haplotype was significantly higher in the controls when compared to the common haplotype G-G (P = 0.031) (*SCGB1D4*, three SNPs were ordered from 5’ to 3’). We also found that the frequency of the GAG haplotype was significantly higher in asthmatic-AH, allergic-AH, and AH with SDB in the study group when compared to the controls (P = 0.0026 for asthma, P = 0.0001 for allergy, and P = 0.0001 for SDB). Similarly, the frequency of the GAG haplotype was higher in patients with a high IgE level when compared to the controls (P = 0.0025).

**3.2. SNP–SNP interactions**

We used MDR, an analytical data-mining approach, to explore the potential interactions of three individual SNPs in the *SCGB1D4* gene (27,28). The best model across all possible combinations was assessed by testing balanced accuracy (TBA), cross-validation consistency (CVC), and significance level. MDR analysis indicated four predictive models for AH. The single-locus model (rs35328961) was

one of the best attributes for risk prediction of AH with a TBA of 61.8% and CVC of 10/10 (P = 0.002) (Figure 1). Homozygous individuals for the wild-type allele in the SNP were located in the low-risk group while the genotypes with “A” allele were located in high-risk group for AH. Among all predicted models, the two-locus model (rs35328961\_rs56196602) was the second best significant attribute for predicting the risk of AH, with a TBA of 64.4% and a CVC of 10/10 (P = 0.002). In those SNPs, the combination of homozygous genotype for the wild-type was higher in the control group, and associated with 1.5-fold more protection against AH. The three-locus model (rs56196602\_rs200327820\_rs35328961) was the third best significant attribute for prediction of AH risk, with a TBA of 64.4% and a CVC of 10/10 (P = < 0.0001). The combination of homozygous genotype for the wild-type in all three SNPs was placed in the low-risk group, and was associated with 1.7-fold increased protection against AH.

The other interaction model that was determined empirically by permutation testing was the three-factor model, in which rs35328961, allergy, and asthma were combined. This model had a TBA of 58.4% and a CVC of 7/10, and it may be associated with a high risk and a low risk for AH, depending on the presence of asthma (P ≤ 0.0001) (Figure 2A). The combinations of the rs3532896 -A, AA genotypes, and allergic asthma were placed in the high-risk group. The dendrograms provided by MDR were examined to help visualization and interpretation of the potential genotype–genotype and genotype–phenotype interactions (29). The dendrogram demonstrates the nature of the interactions between the rs35328961, asthma, and allergy, and the synergistic association of those three factors for predicting susceptibility to AH (Figure 2B).

**Table 3.** Associations between the risk for adenoid hypertrophy and the frequencies of haplotypes based on *SCGB1D4* variants.

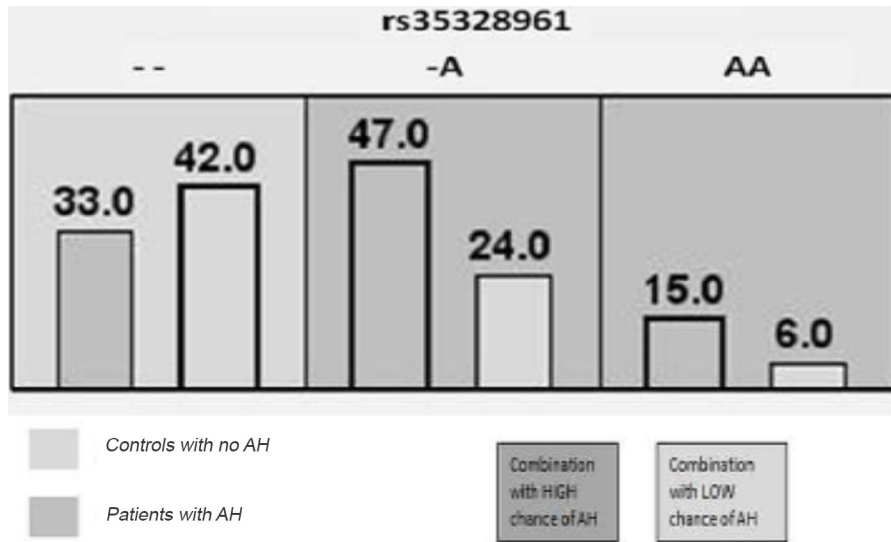
No.	Haplotypes*			Haplotype frequencies		Cure OR (95% CI)**	P-value***
	rs200327820	rs35328961	rs56196602	Study group	Controls		
1	G	-	G	0.5516	0.7263	1.00 (reference)	---
2	G	A	G	0.3516	0.239	2.31 (1.37–3.88)	.002
3	G	-	A	0.0523	0	1.15 (0.18–6.05)	<0.001
4	G	A	A	0.0139	0.0141	2.91 (0.38–22.26)	0.31
5	A	A	G	0.0256	NA	NA	<0.001
6	A	-	G	0.0238	NA	NA	<0.001

Values are presented as median ± SD or numbers (%) unless otherwise specified; SD = Standard deviation; SDB = Sleep-disordered breathing; OR = Odds ratio; CI = Confidence interval; NA = Not available,

\*The alleles of haplotypes were arrayed as the location of in the SNPs *SCGB1D4*,

\*\*In logistic regression model,

\*\*\*Global haplotype association P-value = 0.0052.



**Figure 1.** Distribution of the rs35328961 genotype in the study and control groups on MDR. Single-locus model demonstrating the effect of the rs35328961, which is able to correctly predict AH with 61.8% accuracy. The rs35328961 GA and AA genotypes had a 1.9-fold and 2.5-fold increased risk for AH, respectively. For each genotype, the number of cases is displayed in the histogram on the left in each cell while the number of controls is displayed in the histogram on the right. Darker shade indicates the high-risk group (TBA: 0.618, CVC 10/10, OR 2.6303, CI 1.3998–4.9426, P = 0.002).

#### 4. Discussion

We investigated the effects of the genetic variants of the *SCGB1D4* gene, and conducted an association study. To the best of our knowledge, this is the first study that correlated *SCGB1D4* polymorphisms, haplotypes, and SNP-SNP interactions with AH in a Turkish population. In our study, three polymorphisms (rs200327820, rs35328961, and rs56196602) were detected. We found that the presence of those SNPs and minor alleles in each SNP and their combinations were associated with an increased risk for AH. In addition, those SNPs were significantly associated with asthma, allergy, SDB, and high IgE levels in children with AH.

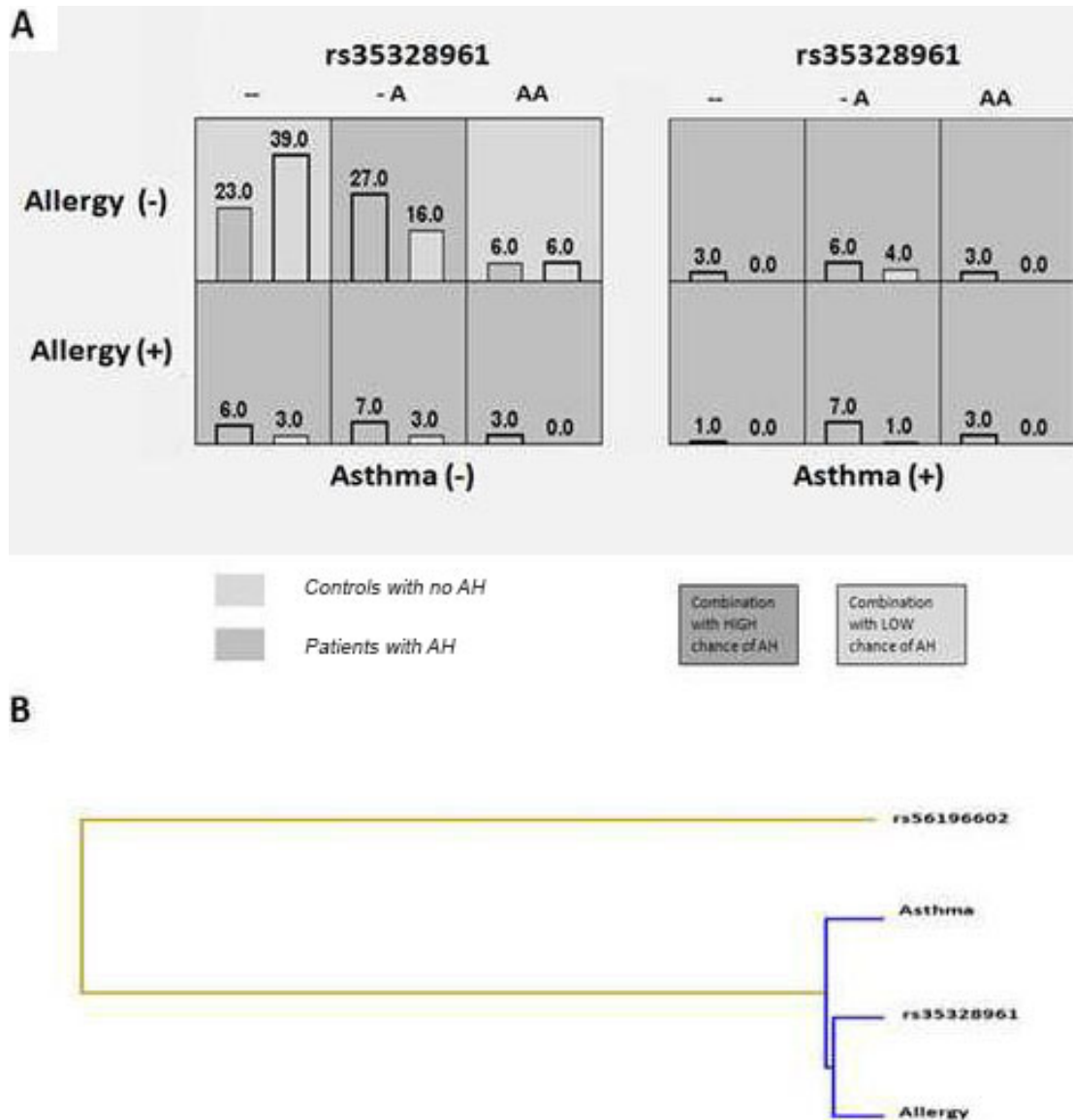
rs200327820 is located in the second exon of the gene (27). It alters the 5th codon from GTG to ATG, both of which code for the amino acid valine. We found that all inheritance models except recessive genotypes with presence of a minor allele at rs200327820 were associated with an increased risk for AH. Furthermore, we found that carriers of the minor allele at rs200327820 had a higher risk for AH when compared to the noncarriers. In the present study, we showed that AH was associated with rs200327820 GA, AA, and GA-AA genotypes in Turkish children. The presence of GA-AA genotype increased risk for asthma +AH 15.3-fold.

Similar to other secretory proteins, *SCGB1D4* has a hydrophobic N-terminal region with a 20 amino acid signal peptide sequence, which is required for its

channeling into the target organelle. ([http://www.uniprot.org/uniprot/Q6XE38#section\\_seq](http://www.uniprot.org/uniprot/Q6XE38#section_seq)). The minor allele of this synonymous SNP (sSNP), which does not change the amino acid sequence, might lead to the vitiated signal activity of the protein through influencing gene expression biology and posttranscriptional regulation mechanisms involving conformation and stability of pre-mRNA, translation initiation, and stability and folding of protein (30,31). Therefore, the gene may be a modifying factor for AH. However, further functional studies should be carried out to determine the clinical significance of the SNP on AH.

The rs35328961 is an intronic SNP (27). In this study, we found that the subjects with presence of the minor allele of rs35328961 in all inheritance models except recessive one were significantly associated with AH. Moreover, we found that carrier insertion of A allele at rs35328961 was higher in patients with AH and in patients with higher IgE levels compared to noncarriers, and in patients with AH with SDB when compared to the controls with SDB. Similarly, MDR analysis showed that the insertion of A allele at rs35328961 genotypes was associated with AH.

Introns are elements that include intron splice enhancers and silencers, as well as the cis-acting RNA elements that regulate alternative splicing. Some intronic SNPs are known to have the potential to confer susceptibility to disease, and these may be much more common than hitherto realized (32). Approximately



**Figure 2.** Distribution of the rs35328961 genotype, asthma, and allergy combinations in the study and the control groups on MDR. A) The rs35328961 genotypes, asthma, and allergy combination, which are able to correctly predict AH with 58.4% accuracy. The *SCGB1D4* -, -A, and AA genotypes had a ∞-fold, 7-fold, and ∞-fold increased risk of allergic-asthmatic AH, respectively. For each genotype combination, the number of cases with asthma and allergy (positive skin prick test) or without asthma and allergy (negative skin prick test) is displayed in the histogram on the left in each cell while the number of controls with asthma and allergy (positive skin prick test) or without asthma and allergy (negative skin prick test) is displayed by the histogram on the right. Darker shade indicates the high-risk group. The pattern of high and low risk for the allergy presence (negative or positive skin prick test) differs depending on the presence of the asthma and value of the rs35328961 (TBA 0.584, CVC 7/10, OR 3.79, CI 1.9866–7.2425, P = < 0.0001). B) The dendrogram demonstrates the nature of the interactions between the rs35328961, asthma, and allergy included in the genetic classifier obtained by MDR. There was a synergistic interaction, with the strongest interaction between the rs35328961, allergy, and asthma.

15% of disease-causing SNPs directly affect pre-mRNA splicing. Single base substitutions localized at the exon-intron boundaries can impair one of the cis-transcriptional elements known as exonic splicing enhancers, and thereby

affect normal pre-mRNA splicing (33). However, proper interpretation of the effects of polymorphisms might be difficult, especially when they result in noncoding variants (34). We suggest that the minor allele of this intronic SNP

may impact upon the exon splicing ability, and contribute to the risk of AH. However, in vitro assays should be performed to elucidate the potential clinical significance of the SNP.

The rs56196602 is located in the 3'UTR region of the gene (27). In the present study, we showed that AH was associated with the rs56196602 GA-AA genotype more when compared to those possessing other genotypes, and it was more common in cases of allergy + AH. Additionally, we found that the carriers of the minor allele at rs56196602 had AH more frequently when compared to noncarriers. As this SNP is located within the 3' flanking region, it does not cause a residual change in the SCGB1D4 protein. The intrinsic stability of mRNA species is determined by cis-acting sequences located within the mRNA (often in the 3-UTR), as well as trans-acting RNA binding proteins (34). As such, the SNPs may alter the binding of these trans-acting factors and stability of pre-mRNA polyadenylation site selection, and thus influence mRNA half-life to contribute to the pathogenesis of AH. However, further functional analyses should be carried out to verify the effect of the variant in gene expression.

Allergy is an important risk factor for AH in children (5). On MDR analysis, we found that combination of the rs35328961-A genotype and nonallergic asthma increased the risk of AH 1.5-fold, but the combination of rs35328961-A genotype and allergic asthma increased the risk of AH 7-fold. Since those phenotypes affect airways where mucosa presents similarities, they likely share a common genetic background (35). Therefore SCGB1D4 rs35328961 gene expressed remarkably in the lymphoid tissues may be a modifying factor for AH and allergic asthma with AH. On the other hand, environmental influence may be a crucial factor in the progression of AH and related clinical phenotypes (3–5). Therefore, further studies focusing on the gene–environment interactions of AH, allergy, and asthma are needed to further enlighten the pathophysiology. Recent studies found that SDB (3), asthma (36), and allergy (5) were associated with each other. Increased airway inflammation is evident in those phenotypes, which promotes AH (37). Therefore, associations of similar genotypes, haplotypes, and their combinations with AH and related clinical phenotypes such as SDB, asthma, and allergy are not surprising, and we showed the role of SCGB1D4's SNP in the molecular mechanisms underlying AH.

Haplotypes are more powerful discriminators between the study and control groups in disease association studies, and they may provide more information regarding the effect of genetic interactions on disease phenotypes rather than genotypes (38). On haplotype analysis, we found that the haplotypes GAG, G-A, AAG, and A-G were more frequent in the study group and associated with AH in

the population studied, but the haplotype GAA seemed to protect the individual from AH. We found that the frequency of the haplotype GAG, which was defined by the common allele at rs200327820, and a rare allele at rs35328961 was associated with AH. We also found that the haplotype AAG, which was defined by the rare alleles of rs200327820 and rs3532896, was also associated with AH. The haplotype AAG, which was defined by rare alleles of those SNPs, was only observed in the study group. Notably, genotypes defined by the rare alleles at rs200327820 and rs35328961 were associated with an increased risk of AH and AH-related phenotypes. The GAG haplotype was significantly associated with allergy, asthma, high IgE level, and SDB in children with AH. Those results are consistent with each other, and show the potential clinical significance of rs200327820 and rs35328961 in AH. However, the functions of the SNPs and haplotypes remain to be elucidated.

Multiple genetic variants might have synergistic effects beyond main effects or pairwise effects. In the current study, we used a data mining approach, MDR, which is able to detect interactions in the absence of main effects, where LD and other traditional approaches are not able to detect and characterize combinations of attributes that interact to influence AH and AH-related phenotypes (1 + 1 = 3 principle) (23–26). Based on the MDR method, four predictive models for AH were observed with a P value of 0.05, suggesting that the power of those SNPs very strongly predicted the susceptibility to AH. When tested independently, those three SNPs were found to be associated with AH. Those polymorphisms may play a role in the pathogenesis of AH.

Among the models with different loci tested, the best genotype–genotype and genotype–phenotype models identified were single-locus, two-locus, and three-locus models including three SNPs, and the three-factor model including rs35328961, asthma, and allergy. The two-locus model included rs35328961 and rs56196602. The LD between rs35328961 and rs56196602 was stronger than other pairwise LDs. The three-locus model included rs200327820, rs35328961, and rs56196602. We found that the haplotypes GAG and G-A were more frequent in the study group, and associated with AH on haplotype analysis. On MDR, those findings were consistent with each other. It was also observed that all SNPs included in the study had synergistic effects with each other. This further corroborates that the pathogenesis of AH involves interplay of a variety of susceptible alleles and external stimuli, which, in turn, may influence disease severity and natural history. Individuals may also differ in their susceptibility to environmental risk factors, and hence the role of gene–environment interaction on AH needs to be clarified. However, as different populations have distinct genetic backgrounds, it is necessary to validate or



replicate such associations with independent and larger samples collected especially from other ethnic groups or populations.

There are a number of possible extensions of this study in which we intended to collect representative data and to consider in our future work. Thus, analysis of haplotypes and LD, and use of MDR analysis in the present study gives an insight into the possible interactions between the polymorphisms of *SCGB1D4* and susceptibility to AH. Furthermore, the studies on *SCGB1D4* are somewhat scarce, and the *SCGB1D4*'s SNPs potential relationships with AH and AH-related clinical phenotypes have been studied for the first time in the current study.

Grasso et al. studied MBL2 genetic polymorphisms in Italian children with adenotonsillar hypertrophy,

and found that the MBL2 00 genotype could be used as a prognostic marker in subjects with adenotonsillar hypertrophy.

Studies suggested that gene interaction is not only possible but is probably ubiquitous for determining susceptibility to complex human diseases (11). Further studies on the interactions are therefore warranted to elucidate their possible underlying role in the pathogenesis and delineation of pathways leading to AH.

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