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# Association of Gasdermin B Gene *GSDMB* Polymorphisms with Risk of Allergic Diseases

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## Abstract

The *GSDMB* gene encodes gasdermin B from the family of gasdermin domain-containing proteins involved in various cellular processes related to tumor development and progression, such as differentiation, cell cycle control and apoptosis. Previously, we conducted GWAS on asthma in the Volga-Ural region of Russia and found SNPs associated with asthma with genome-wide significance (rs9303277, rs8067378, rs2290400, rs7216389, rs4795405) and located in the chromosomal region 17q12-q21, which contains *IKZF3* (IKAROS family zinc finger 3), *ZPBP2* (zona pellucida binding protein-like), *GSDMB* (gasdermin B), *ORMDL3* (orosomucoid 1-like 3) and *LRRC3C* (leucine-rich repeat-containing 3C) genes. In the present study, we investigated the association of SNPs of the *GSDMB* gene with the development of various allergic diseases and their combined manifestations in individuals of Russian, Tatar and Bashkir ethnic origin. Our results revealed that polymorphic variants rs7216389, rs2290400 and rs2305480 are associated with the development of allergic diseases as well as with asthma and asthma combined with allergic rhinitis. We did not reveal the association of rs7216389 and rs2290400 with the development of allergic rhinitis and atopic dermatitis in the groups of patients without asthma symptoms. This may reflect a more important role of these SNPs in the development of asthma.

**Keywords** Asthma · Allergic rhinitis · Atopic dermatitis · Gasdermin B gene · Polymorphism

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## Introduction

Allergic diseases are one of the most common chronic diseases, based on the body's hypersensitivity to certain environmental factors that it perceives as potentially dangerous. According to the World Allergy Organization, about 30–40% of the world's population suffers from various allergic diseases (WAO 2020). Asthma, allergic rhinitis (AR) and atopic dermatitis (AD) are three interrelated concomitant allergic diseases that often transform from one to another. Allergies have a complex multifactorial nature, the heterogeneity of which is manifested by various phenotypes of the disease in different people under the influence of genetic and epigenetic factors of constantly changing environmental conditions. Epidemiological studies allow us to classify allergic diseases as a syntropy, assuming the presence of both genes responsible for common pathogenesis (predisposition to allergies in general) and genes specific for different groups of diseases (Freidin et al. 2010; Sun et al. 2011; Ziyab et al. 2014; Garcia-Aymerich et al. 2015; Bellanti et al. 2019).

In recent years, significant progress in the study of genetic bases of allergic diseases is associated with the study of large samples within the framework of international consortiums and the active use of modern research methods: genome-wide association analysis (GWAS), whole exome, whole genome and targeted sequencing with the use of NGS technology and others. More than 300 genes coding for proteins with the function that is closely related to allergy development were detected (Holloway et al. 2010; Ortiz et al. 2015; Willis-Owen et al. 2018). More than 20 genome-wide linkage analyses of asthma, atopy, AR and AD were carried out. Chromosomal areas closely linked to the development of allergic diseases were identified, and positional candidate genes for asthma and AD were discovered (Ortiz et al. 2015).

More than 90 GWAS on asthma were performed (GWAS 2020), and more than 1000 single-nucleotide polymorphic variants (SNP) were identified. The most statistically significant associations, repeatedly confirmed in different studies on various samples, have been shown for polymorphisms of genes located in the 17q12-21 (*GSDMB, ORMDL3, GSDMA, IKZF3, ZPBP2*), 6p21.32 (*HLA-DQA1, HLA-DQ, HLA-DQB1, HLA-DQA2, HLA-DOA, HLA-DRA, HLA-DRB5, HLA-DPB1, PBX2, NOTCH4, C6orf10, BTNL2*), 9p24.1 (*IL33*), 2q12 (*IL18R1, IL1RL1, IL1RL2*) and 5q22.1 (*TSLP*) regions. The functions of the most polymorphic loci associated with asthma are related to the immune response, Th2 cell differentiation, inflammatory processes, the barrier function of the epithelium and others.

There were eight GWAS on allergic rhinitis performed, and more than 100 associated polymorphic variants were identified, many of which are also associated with other allergic diseases—*HLA-DQB1, HLA-DQA1* (6p21.32), *IL1RL1* (2q12.1), *LRRC32*, *C11orf30* (11q13.5), *GSDMB*, *ZPBP2* (17q12–21), *IL33*, *RANBP6* (9p24.1), *IL13*, *RAD50* (5q31.1) and others (Waage et al. 2018; GWAS 2020). In 10 atopic dermatitis GWAS performed so far, more than 130 polymorphic variants associated with this disease were found, and some loci coincide with those of other allergic diseases. Among them are significant polymorphisms located in the genes of *IL18R1* (2q12), *HLA-B* (6p21.32) and *FLG* (1q21.3) (GWAS 2020).

Previously, we conducted GWAS on asthma in the Volga-Ural region of Russia (Karunas et al. 2011, 2015). We found polymorphic loci associated with asthma with genome-wide significance (rs9303277, rs8067378, rs2290400, rs7216389, rs4795405) and located in the chromosomal region 17q12–q21, which contains *IKZF3* (IKAROS family zinc finger 3), *ZPBP2* (zona pellucida binding protein-like), *GSDMB* (gasdermin B), *ORMDL3* (orosomucoid 1-like 3) and *LRRC3C* (leucine-rich repeat-containing 3C) genes. The association of asthma with this region was first identified in children from Germany and the UK (Moffatt et al. 2007). To date, a large number of studies have identified an association between SNPs on 17q12–q21 and asthma in various European and Asian populations (Moffatt et al. 2010; Wan et al. 2011; Das et al. 2017; Demenais et al. 2018; GWAS 2020).

In our study sample the most significant SNP associated with asthma development was rs7216389 (c.236–1199C > T) ($p = 1.0 \times 10^{-7}$). This SNP is located in intron 1 of the *GSDMB* gene encoding the gasdermin domain-containing protein gasdermin B, which is expressed in the epithelial cells of bronchi, stomach, intestines, T-lymphocytes and others (Das et al. 2016, 2017).

As a whole, the genome-wide association analysis and the further replication study on an independent sample established the statistically significant association of SNPs and haplotypes on 17q12-21 region with the development of asthma in Russian, Tatar and Bashkir individuals (Karunas et al. 2011). In the present study, we investigated the association of SNPs of the *GSDMB* gene with the development of various allergic diseases, asthma and other syntropic diseases (AR and AD) and their combined manifestations, in individuals of Russian, Tatar and Bashkir ethnic background.

## Material and Methods

We performed an analysis of three SNPs of *GSDMB* gene (rs7216389, rs2290400, rs2305480) in an independent cohort of patients with various allergic diseases (asthma, AR and AD) and a control group of different ethnic origin (Russians, Tatars and Bashkirs) from the Republic of Bashkortostan located in the Volga-Ural region of Russia. The study involved 661 unrelated individuals (386 males, 275 females) with various allergic diseases (asthma, AR and AD) aged 1 to 67 (median age 20.45 years). The examined individuals were patients of the Paediatric Department of the Bashkir State Medical University Clinic, Pulmonary and Allergy Departments of the City Clinical Hospital No.21 of Ufa, Allergy Department of the Republican Children's Clinical Hospital of Ufa. The diagnosis of diseases was established by qualified physicians by the criteria of guidance documents for the diagnosis, treatment and prevention of diseases. The total sample of allergic diseases patients also was divided into groups depending on the presence of particular allergic diseases (asthma, AR, AD) and their combined manifestations (Table 1).

The control group was composed of 343 healthy individuals (male, female) aged 2 to 66 (median age 24.01 years) with no manifestations of allergic diseases and having no family history of allergies (144 Russians, 109 Tatars, 90 Bashkirs).

**Table 1** Characteristics of patients included in various clinico-pathogenetic groups and controls

| | Total sample | Russians | Tatars | Bashkirs |
|---|---|---|---|---|
| Total sample of patients with allergic diseases | 661 | 364 | 211 | 86 |
| Patients with combined manifestations of asthma, AR and AD | 102 | 60 | 32 | 10 |
| Patients with combined manifestations of asthma and AD (no AR) | 22 | 9 | 12 | 1 |
| Patients with combined manifestations of asthma and AR (no AD) | 173 | 85 | 57 | 31 |
| Patients with combined manifestations of AR and AD (no asthma) | 71 | 46 | 21 | 4 |
| Patients with asthma (no AR and AD) | 139 | 71 | 38 | 30 |
| Patients with AR (no asthma and AD) | 48 | 37 | 9 | 2 |
| Patients with AD (no asthma and AR) | 106 | 56 | 42 | 8 |
| Control group | 343 | 144 | 109 | 90 |

DNA was isolated from 5–8 ml of peripheral blood by phenol–chloroform extraction (Mathew 1984). Genotyping of the *GSDMB* gene polymorphisms rs7216389 (c.236–1199C > T), rs2290400 (c.408-63A > G), rs2305480 (c.865G > A, p.Pro289Ser) was performed using real-time PCR and PCR-RFLP analysis, as described earlier (Karunas et al. 2011).

Mathematical processing of results was performed using MS Office Excel 2003 (Microsoft) and PLINK 1.06 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) software packages. The differences in allelic and genotypic frequencies between the patients and controls were compared by a Chi-square ($\chi 2$) test with the Yates correction. The strength of associations was assessed by the odds ratio (OR) values. A meta-analysis of the results was performed using the WinPepi v.11.32 program (Abramson et al. 2011), considering fixed effects (Mantel–Haenszel method) and random effects models (DerSimonian–Laird method). The level of heterogeneity was determined using $I^2$ estimates (percentage of variation due to the heterogeneity of samples) (Higgins and Thompson 2002).

## Results

Taking into account the statistically significant association between asthma and polymorphisms in the 17q12-q21 region, we have analyzed three SNPs in the *GSDMB* gene (rs7216389, rs2290400, rs2305480) with various clinical manifestations and combinations of allergic diseases in order to determine the role of polymorphic variants of this chromosomal region in the development not only of asthma but also of other syntropic diseases (AR and AD) and their combined manifestations.

### Association Analysis of GSDMB Gene rs7216389, rs2290400 Polymorphisms with the Development of Allergic Diseases

The results of the association analysis of rs7216389 located in 1 intron of the *GSDMB* gene and associated with asthma at the highest level of significance in GWAS in the Volga-Ural region of Russia are presented in Table 2. Statistically significant differences in the distribution of alleles and genotypes frequencies of this polymorphism were found in Russians when comparing the controls with the group of patients with three combined allergic diseases (asthma, AR and AD), with the group of patients having asthma and concomitant AR and the group of patients with clinical manifestations of asthma only. The markers of increased risk of these diseases are rs7216389*TT genotype and rs7216389*T allele (Table 2).

The rs7216389*T allele was found with the highest frequency in Russians with combined manifestations of all three allergic diseases (asthma, AR and AD) – in 65.74% of patients ($p=0.001$; $OR=2.14$ (CI 95% 1.35–3.40)), and the rs7216389*TT genotype was determined in 42.59% of patients ($p=0.004$; $OR=2.60$ (CI 95% 1.33–5.06)). In patients with combined clinical manifestations of asthma and AR, the rs7216389*T allele was detected with a frequency of 61.94% ($p=0.005$; $OR=1.82$ (CI 95% 1.20–2.76)), the rs7216389*TT genotype—with a frequency of 40.30% ($p=0.006$; $OR=2.36$ (CI 95% 1.26–4.42)). Analysis of the distribution of alleles and genotypes of the rs7216389 in patients with clinical manifestations of any of these allergic diseases (AD or AR or AD) showed the presence of a statistically significant association between this SNP and asthma development. In patients with asthma only the rs7216389*TT genotype was determined with a frequency of 38.33% ($p=0.02$; $OR=2.18$ (CI 95% 1.13–4.18)).

A study of the rs7216389 in individuals of Tatar ethnicity revealed statistically significant differences in the distribution of allele frequencies of this SNP between controls and patients with asthma and concomitant AR. In patients, the allele frequency of rs7216389*T was higher than in the controls and equals 58.51% ($p=0.03$; $OR=1.70$ (CI 95% 1.04–2.77)).

The analysis of *GSDMB* gene rs7216389 polymorphism in Bashkirs with allergic diseases and the corresponding control group revealed statistically significant differences between the total sample of allergic diseases patients and the controls. The allele frequency of rs7216389*T in patients was significantly higher than in the control group (56.41% and 41.11%, respectively) ($p=0.005$; $OR=1.85$ (CI 95% 1.2–2.86)). Statistically significant differences in the distribution of alleles and genotypes frequencies of this SNP were found between controls and the patients with asthma only and the patients with combined manifestations of asthma and AR. The allele and genotype frequencies in these two groups of patients were similar. The frequency of rs7216389*T allele in patients with asthma was 60.71% ($p=0.01$; $OR=2.21$ (CI 95%1.2–4.09)), in patients with asthma and AR—59.62% ($p=0.02$; $OR=2.11$ (CI 95% 1.13–3.96)).

Thus, the analysis of the rs7216389 polymorphism in three ethnic groups showed the association of this SNP with the development of asthma and combined forms of

**Table 2** Distribution of genotype and allele frequencies of *GSDMB* rs7216389, rs2290400 gene polymorphisms in patients with allergic diseases and control group

| rs7216389, rs2290400* | Genotypes | | | Alleles | | |
|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | n (%) | n (%) | N |
| | CC | CT | TT | C | T | |
| *Russians* | | | | | | |
| Total sample of patients with allergic diseases | 75 (23.08) | 154 (47.38) | 96 (29.54) | 304 (46.77) | 346 (53.23) | 325 |
| Patients with asthma, AR and AD | 6 (11.11)<br>$p=0.01$; $OR=0.33$ | 25 (46.3) | 23 (42.59)<br>$p=0.004$; $OR=2.60$ | 37 (34.26)<br>$p=0.001$; $OR=0.47$ | 71 (65.74)<br>$p=0.001$;<br>$OR=2.14$ | 54 |
| Patients with asthma and AR | 11 (16.42) | 29 (43.28) | 27 (40.30)<br>$p=0.006$; $OR=2.36$ | 51 (38.06)<br>$p=0.005$; $OR=0.55$ | 83 (61.94)<br>$p=0.005$;<br>$OR=1.82$ | 67 |
| Patients with AR and AD | 13 (28.89) | 22 (48.89) | 10 (22.22) | 48 (53.33) | 42 (46.67) | 45 |
| Patients with asthma | 15 (25.00) | 22 (36.67) | 23 (38.33)<br>$p=0.02$; $OR=2.18$ | 52 (43.33) | 68 (56.67) | 60 |
| Patients with AR | 11 (30.56) | 22 (61.11) | 3 (8.33) | 44 (61.11) | 28 (38.89) | 36 |
| Patients with AD | 18 (32.14) | 30 (53.57) | 8 (14.29) | 66 (58.93) | 46 (41.07) | 56 |
| Control group | 40 (27.78) | 72 (50.00) | 32 (22.22) | 152 (52.78) | 136 (47.22) | 144 |
| *Tatars* | | | | | | |
| Total sample of patients with allergic diseases | 45 (23.44) | 95 (49.48) | 52 (27.08) | 185 (48.18) | 199 (51.82) | 192 |
| Patients with asthma, AR and AD | 14 (43.75) | 12 (37.5) | 6 (18.75) | 40 (62.50) | 24 (37.50) | 32 |
| Patients with asthma and AR | 9 (19.15) | 21 (44.68) | 17 (36.17) | 39 (41.49)<br>$p=0.03$; $OR=0.59$ | 55 (58.51)<br>$p=0.03$;<br>$OR=1.70$ | 47 |
| Patients with AR and AD | 9 (33.33) | 12 (44.44) | 6 (22.23) | 30 (55.56) | 24 (44.44) | 27 |
| Patients with asthma | 7 (23.33) | 15 (50.00) | 8 (26.67) | 29 (48.33) | 31 (51.67) | 30 |
| Patients with AD | 15 (35.71) | 17 (40.48) | 10 (23.81) | 47 (55.95) | 37 (44.05) | 42 |

**Table 2** (continued)

| rs7216389, rs2290400* | Genotypes | | | Alleles | | |
|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | n (%) | n (%) | N |
| | CC | CT | TT | C | T | |
| Control group | 35 (32.11) | 49 (44.95) | 25 (22.94) | 119 (54.59) | 99 (45.41) | 109 |
| *Bashkirs* | | | | | | |
| Total sample of patients with allergic diseases | 16 (20.51)<br>$p=0.015$; $OR=0.43$ | 36 (46.15) | 26 (33.34) | 68 (43.59)<br>$p=0.005$; $OR=0.54$ | 88 (56.41)<br>$p=0.005$; $OR=1.85$ | 78 |
| Patients with asthma and AR | 4 (15.38)<br>$p=0.03$; $OR=0.30$ | 13 (50.00) | 9 (34.62) | 21 (40.38)<br>$p=0.02$; $OR=0.47$ | 31 (59.62)<br>$p=0.02$; $OR=2.11$ | 26 |
| Patients with asthma | 4 (14.29)<br>$p=0.02$; $OR=0.27$ | 14 (50.00) | 10 (35.71) | 22 (39.29)<br>$p=0.01$; $OR=0.45$ | 34 (60.71)<br>$p=0.01$; $OR=2.21$ | 28 |
| Control group | 34 (37.78) | 38 (42.22) | 18 (20.00) | 106 (58.89) | 74 (41.11) | 90 |

N is the number of individuals; n is the sample size; allele and genotype frequencies are shown in brackets, %; and p values were calculated from Chi-square test and are shown in the case of statistical significance ($p<0.05$)

*OR* odds ratio

*The rs2290400 SNP is in close linkage disequilibrium with rs7216389 (D` > 0.99, r2 > 0.99)

allergic pathology—asthma with AR in Russians, Tatars and Bashkirs, asthma with concomitant AR and AD in Russians and allergic diseases in the Bashkirs.

The rs2290400 SNP is in close linkage disequilibrium with rs7216389 (D` > 0.99, r2 > 0.99). The alleles and genotypes frequencies of the rs2290400, observed during the study of three ethnic groups (Russians, Tatars and Bashkirs), were practically identical with the frequencies of alleles and genotypes of the rs7216389; therefore, the results of the association analysis of the rs2290400 are not described further. The rs2290400*T allele and the rs2290400*TT genotype are markers of increased risk of allergic diseases and are also associated with the development of asthma and combined forms of allergic diseases (asthma, AR and AD; asthma and AR).

## Association Analysis of GSDMB Gene rs2305480 Polymorphism with Development of Allergic Diseases

The study of rs2305480, located in exon 6 of the *GSDMB* gene, was conducted in patients with allergic diseases and the control group (Table 3). The analysis of rs2305480 in individuals of Russian ethnic background did not reveal statistically significant differences in the distribution of allele and genotypes frequencies of this locus between the total sample of allergic diseases patients and the control group. When considering groups of patients with different clinical manifestations, the association of this SNP with the development of three concomitant allergic diseases (asthma, AR and AD) as well as combined manifestations of asthma and AR was found. In patients with three allergic diseases, the rs2305480*G allele frequency was significantly higher than in controls (66.98% vs 53.47%) ($p = 0.02$; $OR = 1.77$ (CI 95% 1.11–2.81)). The frequency of homozygous rs2305480*GG genotype in patients of this group (43.40%) was also higher than in the controls (28.47%) ($p = 0.04$; $OR = 1.93$ (CI 95% 1.0–3.7)). In the group of Russian patients with asthma and AR, the rs2305480*G allele was found with higher frequency of 68.66% ($p = 0.003$; $OR = 1.91$ (CI 95% 1.24–2.94)), and the rs2305480*GG genotype was found in 46.26% of patients with asthma and AR ($p = 0.01$; $OR = 2.16$ (CI 95% 1.19–3.95)).

Statistically significant differences in the distribution of allele and genotypes frequencies of the rs2305480 polymorphism were found between the controls and patients with concomitant asthma and AR of Tatar ethnic background. The rs2305480*G allele of increased risk was found with a frequency of 63.83% in patients of this group, and in 51.38% of controls ($p = 0.04$; $OR = 1.67$ (CI 95% 1.02–2.75)). The frequency of rs2305480*GG genotype in patients with asthma and AR was 44.68%, in controls – 25.68% ($p = 0.02$; $OR = 2.34$ (CI 95% 1.14–4.79)).

The analysis of rs2305480 in individuals of Bashkir ethnicity showed statistically significant differences in the allele frequencies distribution between the control group and the total sample of patients with allergic diseases and patients with clinical manifestations of asthma only. The rs2305480*G allele was detected more frequently in patients with allergic diseases than in the control group (57.69% vs 44.44%) ($p = 0.015$; $OR = 1.70$ (CI 95% 1.11–2.63)). In patients with clinical

**Table 3** Distribution of genotype and allele frequencies of *GSDMB* rs2305480 gene polymorphism in patients with allergic diseases and control group

| rs2305480 | Genotypes | | | Alleles | | |
|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | n (%) | n (%) | N |
| | AA | AG | GG | A | G | |
| *Russians* | | | | | | |
| Total sample of patients with allergic diseases | 60 (18.93) | 152 (47.95) | 105 (33.12) | 272 (42.90) | 362 (57.10) | 317 |
| Patients with asthma, AR and AD | 5 (9.43) | 25 (47.17) | 23 (43.40)<br>$p=0.04$; $OR=1.93$ | 35 (33.02)<br>$p=0.016$; $OR=0.57$ | 71 (66.98)<br>$p=0.016$;<br>$OR=1.77$ | 53 |
| Patients with asthma and AR | 6 (8.96)<br>$p=0.025$; $OR=0.36$ | 30 (44.78) | 31 (46.26)<br>$p=0.01$; $OR=2.16$ | 42 (31.34)<br>$p=0.003$; $OR=0.52$ | 92 (68.66)<br>$p=0.003$;<br>$OR=1.91$ | 67 |
| Patients with AR and AD | 8 (18.60) | 19 (44.19) | 16 (37.21) | 35 (40.70) | 51 (59.30) | 43 |
| Patients with asthma | 15 (24.59) | 24 (39.34) | 22 (36.07) | 54 (44.26) | 68 (55.74) | 61 |
| Patients with AR | 8 (25.00) | 21 (65.62) | 3 (9.38)<br>$p=0.02$; $OR=0.26$ | 37 (57.81) | 27 (42.19) | 32 |
| Patients with AD | 17 (31.48) | 29 (53.70) | 8 (14.82) | 63 (58.33) | 45 (41.67) | 54 |
| Control group | 31 (21.53) | 72 (50.00) | 41 (28.47) | 134 (46.53) | 154 (53.47) | 144 |
| *Tatars* | | | | | | |
| Total sample of patients with allergic diseases | 40 (21.27) | 87 (46.28) | 61 (32.45) | 167 (44.41) | 209 (55.59) | 188 |
| Patients with asthma, AR and AD | 4 (13.33) | 19 (63.33) | 7 (23.34) | 27 (45.00) | 33 (55.00) | 30 |
| Patients with asthma and AR | 8 (17.02) | 18 (38.30) | 21 (44.68)<br>$p=0.02$; $OR=2.34$ | 34 (36.17)<br>$p=0.04$; $OR=0.60$ | 60 (63.83)<br>$p=0.04$;<br>$OR=1.67$ | 47 |
| Patients with AR and AD | 6 (28.57) | 6 (28.57) | 9 (42.86) | 18 (42.86) | 24 (57.14) | 21 |
| Patients with asthma | 6 (20.00) | 14 (46.67) | 10 (33.33) | 26 (43.33) | 34 (56.67) | 30 |
| Patients with AD | 11 (27.50) | 18 (45.00) | 11 (27.50) | 40 (50.00) | 40 (50.00) | 40 |

**Table 3** (continued)

| rs2305480 | Genotypes | | | Alleles | | |
|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | n (%) | n (%) | N |
| | AA | AG | GG | A | G | |
| Control group | 25 (22.94) | 56 (51.38) | 28 (25.68) | 106 (48.62) | 112 (51.38) | 109 |
| *Bashkirs* | | | | | | |
| Total sample of patients with allergic diseases | 16 (20.51) | 34 (43.59) | 28 (35.90) | 66 (42.31)<br>$p=0.015$; $OR=0.59$ | 90 (57.69)<br>$p=0.015$; $OR=1.70$ | 78 |
| Patients with asthma and AR | 4 (15.38) | 13 (50.00) | 9 (34.62) | 21 (40.38) | 31 (59.62) | 26 |
| Patients with asthma | 4 (14.29) | 14 (50.00) | 10 (35.71) | 22 (39.29)<br>$p=0.03$; $OR=0.52$ | 34 (60.71)<br>$p=0.03$; $OR=1.93$ | 28 |
| Control group | 30 (33.33) | 40 (44.44) | 20 (22.22) | 100 (55.56) | 80 (44.44) | 90 |

N is the number of individuals; n is the sample size; allele and genotype frequencies are shown in brackets, %; and p values were calculated from Chi-square test and are shown in the case of statistical significance ($p<0.05$)

*OR* odds ratio

manifestations of asthma only the higher frequency of rs2305480*G allele (60.71%) was also found ($p = 0.03$; $OR = 1.93$ (CI 95% 1.05–3.56)).

Thus, the analysis of the rs2305480 showed the association of this SNP with the development of three concomitant allergic diseases (asthma, AR and AD) in Russians, with combined manifestations of asthma and AR in Russians and Tatars and with allergic diseases in total and asthma as the only manifestation of allergy in Bashkirs.

### Meta-Analysis of GSDMB Gene Polymorphisms Study Results

A meta-analysis of the association analysis results on the studied SNPs was carried out in order to generalize the obtained data and identify common and specific markers of allergic disease risk. According to the conducted meta-analysis (Table 4) of the results on three SNPs of *GSDMB* gene (rs7216389, rs2290400, rs2305480), obtained for the samples of three ethnic groups (Russians, Tatars and Bashkirs), we revealed allelic variants of genes, which can be considered as genetic risk markers of various clinical manifestations and combinations of allergic diseases for the general population of Bashkortostan. Using meta-analysis, it was found that rs7216389*T and rs2290400*T alleles of the *GSDMB* gene are risk markers of the development of allergic diseases ($p = 0.001$; $OR = 1.38$) and asthma as the only clinical manifestation of allergy ($p = 0.003$; $OR = 1.56$). At the same time, the heterogeneity level of the total sample of allergic diseases according to these loci is moderate ($I^2 = 11.3$) and heterogeneity in the asthma sample was not detected ($I^2 = 0.0$).

According to the meta-analysis results, rs2305480*G allele is associated with the development of three accompanying allergic diseases (asthma, AR and AD) ($p = 0.003$; $OR = 1.67$), combined manifestations of asthma and AR ($p = 5.4 \times 10–5$, $OR = 1.81$), as well as with allergic diseases as a whole ($p = 0.001$; $OR = 1.38$). The heterogeneity level of the samples with three accompanying allergic diseases (asthma, AR and AD) and allergic diseases as a whole for this locus is moderate ($I^2 = 50.0$ and $I^2 = 11.3$, respectively), and no heterogeneity was detected in the sample with asthma and AR ($I^2 = 0.0$).

Thus, there was a significant association of rs7216389, rs2290400 and rs2305480 of *GSDMB* gene with the development of allergic diseases in general, rs7216389 and rs2290400 with the development of asthma only, and rs2305480 with combined forms of allergic pathology—asthma with AR, asthma with AR and AD, established.

## Discussion

Pathogenesis of allergic diseases is complex because of numerous genetic and environmental factors, which together cause extensive phenotypic heterogeneity of allergic diseases. Identification of genetic risk variants of individual allergic phenotypes provides an understanding of the pathogenesis of these diseases. We had carried out the study of polymorphisms of *GSDMB* gene (rs7216389, rs2290400, rs2305480) in the samples of patients with different clinical manifestations of allergic diseases

**Table 4** Meta-analysis of association between *GSDMB* rs7216389, rs2290400, rs2305480 gene polymorphisms and allergic diseases

| SNP | Groups | Alleles | Fixed-effect model | | Random-effect model | | $I^2$, % |
|---|---|---|---|---|---|---|---|
| | | | p | OR | p (R) | OR (R) | |
| rs7216389. rs2290400* | Patients with asthma, AR and AD | C | – | – | 0.263 | – | 82.3 |
| | | T | | | | | |
| | Patients with asthma and AD | C | 0.755 | – | – | – | 0.0 |
| | | T | | | | | |
| | Patients with asthma and AR | C | – | – | 0.072 | – | 96.6 |
| | | T | | | | | |
| | Patients with AR and AD | C | 0.58 | – | – | – | 16.2 |
| | | T | | | | | |
| | Patients with asthma | C | 0.003 | 0.64 | – | – | 0.0 |
| | | T | 0.003 | 1.56 | | | |
| | Patients with AR | C | 0.139 | – | – | – | 0.0 |
| | | T | | | | | |
| | Patients with AD | C | 0.385 | – | – | – | 0.0 |
| | | T | | | | | |
| | Total sample of patients with allergic diseases | C | 0.001 | 0.73 | – | – | 11.3 |
| | | T | 0.001 | 1.38 | | | |
| rs2305480 | Patients with asthma, AR and AD | A | 0.003 | 0.60 | – | – | 50.0 |
| | | G | 0.003 | 1.67 | | | |
| | Patients with asthma and AD | A | 0.810 | – | – | – | 0.0 |
| | | G | | | | | |
| | Patients with asthma and AR | A | $5.4\times10^{-5}$ | 0.55 | – | – | 0.0 |
| | | G | $5.4\times10^{-5}$ | 1.81 | | | |
| | Patients with AR and AD | A | – | – | 0.801 | | 57.0 |
| | | G | | | | | |
| | Patients with asthma | A | 0.086 | – | – | – | 11.9 |
| | | G | | | | | |
| | Patients with AR | A | 0.067 | – | – | – | 0.0 |
| | | G | | | | | |
| | Patients with AD | A | 0.385 | – | – | | 0.0 |
| | | G | | | | | |
| | Total sample of patients with allergic diseases | A | 0.001 | 0.73 | – | – | 11.3 |
| | | G | 0.001 | 1.38 | | | |

*p* p value fixed effects (Mantel–Haenszel); *p (R)* p value random effects (DerSimonian–Laird); *OR* odds ratio fixed; *OR(R)* odds ratio random; and $I^2$ heterogeneity index

and controls of three ethnic groups (Russians, Tatars and Bashkirs) of the population of Bashkortostan. An association of these SNPs with asthma and with combined manifestations of three (asthma, AR and AD) or two allergic diseases (asthma and AR) was found in Russians. An association with the development of asthma and

concomitant AR was found in individuals of Tatar ethnicity. In Bashkirs, in a general sample of patients, an association was found with the development of allergy, as well as with the development of asthma itself. The association of polymorphic variants rs7216389, rs2290400 and rs2305480 with the development of AR and AD in the groups of patients without clinical manifestations of asthma was not revealed, and this signals a more important role of these SNPs in the development of asthma. In order to generalize the results, to reveal heterogeneity of the studied samples, general and specific risk markers, a meta-analysis of the studied SNPs was conducted. A meta-analysis of *GSDMB* gene SNPs (rs2305480, rs2290400 and rs7216389) confirmed the results of the association study of separate polymorphisms with different clinical manifestations and combinations of allergic diseases. A significant association of *GSDMB* gene SNPs (rs7216389, rs2290400 and rs2305480) with the development of allergic diseases in general was established, as well as of rs7216389 and rs2290400 with development of asthma only, and of rs2305480 with combined forms of allergic pathology—asthma with AR, asthma with AR and AD.

To date, association studies of polymorphic loci located in the region of 17q12-q21 with asthma and other allergic diseases have been conducted in various populations around the world (Moffatt et al. 2007, 2010; Wan et a. 2011; Das et al. 2017; Demenais et al. 2018). There is a cluster of genes in the region: *IKZF3, ZPBP2, GSDMB, ORMDL3, LRRC3C*. The *ORMDL3* gene encodes the transmembrane endoplasmic reticulum protein ORMDL3, a member of the family of orosomucoid-like proteins that are expressed in many tissues, including liver cells and blood lymphocytes. ORMDL proteins regulate the biosynthesis of sphingolipids involved in important cellular processes (growth, differentiation, proliferation and apoptosis) (Das et al. 2017). Increased expression of ORMDL3 in epithelial cells of bronchi induces expression of chemokines and metalloprotease (Miller et al. 2012, 2014). In addition, ORMDL3 participates in the activation of the signaling pathway of transcription factor ATF6α, which regulates the expression of SERCA2b (sarcoplasmic reticulum ATPase) and IL-6 (interleukin 6) involved in the pathogenesis of asthma (Miller et al. 2012).

The *GSDMB* gene encodes gasdermin B from the family of gasdermin domain-containing proteins involved in various cellular processes related to tumor development and progression, such as differentiation, cell cycle control and apoptosis (Das et al. 2017). The *GSDMB* gene SNPs (rs7216389, rs2290400 and rs2305480) are associated with expression levels of ORMDL3 and GSDMB in human lymphoblastoid cell lines (Moffatt et al. 2007; Verlaan et al. 2009), expression levels of ORMDL3 and GSDMB in blood eQTL databases (Westra et al. 2013; Blood eQTL browser 2021), with expression of GSDMB and ORMDL3 in whole blood, lung and cells—EBV-transformed lymphocytes (Single-Tissue eQTLs for chr17) (Consortium GT 2015; GTEx Portal 2021). In vitro studies show that the expression of *GSDMB* gene in bronchial epithelial cells is associated with increased expression of chemokines and heat shock proteins (Hou et al. 2011; Das et al. 2016). The increased expression of *GSDMB* gene in transgenic mice was accompanied by increased expression of proteins involved in remodeling of lung tissues (TGF-β1, 5-LO, MMP-9) and cysteinyl leukotrienes, key participants of asthma pathogenesis (Miller et al. 2014; Das et al. 2017).

Numerous studies and meta-analyses have confirmed that the association of the chromosomal region 17q12-q21 with asthma is the most replicable and statistically significant (Stein et al. 2018). It is obvious that the region 17q12-q21 is associated with childhood asthma. The association has been identified in Japanese, Chinese, Koreans, Mexicans, French, Scottish, Danish, Icelandic, English patients and individuals of European origin from various countries in the combined sample of the GABRIEL consortium (Moffatt et al. 2010; Wan et al. 2011; Das et al. 2017; Demenais et al. 2018; Stein et al. 2018). Polymorphic variants of this locus have shown a contradictory association with different allergic disease phenotypes. There was no association of the 17q12-q21 region with allergic sensitization, atopic dermatitis, elevated IgE (Paternoster et al. 2012; Bonnelykke et al. 2013; Stein et al. 2018). Several works confirmed the association of AR with SNPs in the 17q12-q21 region, while the subsequent GWAS and a number of association works did not reveal the above (Stein et al. 2018). The association of the 17q12-q21 chromosomal region has been confirmed with a number of autoimmune diseases, which indicates the important role of this region in immunity development (Stein et al. 2018). In most studies, the most significant association of asthma was identified with the rs7216389. This polymorphism is located in a conservative sequence coding highly homologous transcription factor C/EBPb (CCAAT/enhancer-binding protein b) that involved in the regulation of cell differentiation, cell cycle and expression of cytokine genes (Moffatt et al. 2007). In addition, the risk allele of rs7216389 disrupts the CpG-site and, as a consequence, changes the methylation pattern in the 5′ UTR of the gene (Acevedo et al. 2015).

## Conclusions

In our study, polymorphic variants rs7216389, rs2290400 and rs2305480 are associated with the development of allergic diseases in general, as well as with the development of asthma combined with AR, and asthma as the only clinical manifestation of allergic diseases. The established association of the studied SNPs with the combined manifestation of asthma and AR is reasonable and expected, given the commonality of morpho-functional features of the upper and lower respiratory tract. The association of rs7216389 and rs2290400 polymorphisms with the development of AR and AD in the groups of patients without asthma symptoms has not been revealed, and this signals a more important role of these SNPs in the development of asthma.

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**Data Availability** All data generated or analyzed during this study are included in this published article.

**Declarations**

**Conflict of interest** The authors declare no conflicts of interest.

**Ethical Approval** The study was also approved by the Ethics Committee of the Institute of Biochemistry and Genetics of the Ufa Scientific Center of the Russian Academy of Sciences (IBG USC RAS) (Protocols dated November 5, 2007, and December 21, 2007). Informed voluntary consent was obtained from each of the participants included in the study. Parental consent to participate in the study for children under the age of 18 was obtained as well. All samples were collected after obtaining informed consent. All research involving humans complies with the ethical standards of the Institutional and National Research Ethics Committee and the Declaration of Helsinki (1964) and its subsequent amendments or comparable ethical standards.

**Consent to Participate** Informed voluntary consent was obtained from each of the participants included in the study. Parental consent to participate in the study for children under the age of 18 was obtained as well. All samples were collected after obtaining informed consent.

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