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Original Research

Sex-Dependent Association of *SMAD3* Gene Polymorphisms With Asthma and Allergy Risk in Russian Children

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Abstract

Background: Despite significant advances in identifying susceptibility genes for bronchial asthma (BA), the immunogenetic and other molecular mechanisms underlying disease risk and progression remain incompletely understood. This study aimed to investigate the association between two common single-nucleotide polymorphisms (SNPs), rs17293632 and rs2033784, located within the *SMAD3* (SMAD family member 3) gene—previously identified in genome-wide association studies—and susceptibility to atopic asthma and allergen sensitivity in Russian children. **Methods**: This study included DNA samples from 999 unrelated children from the Kursk region, comprising 526 patients with BA (316 boys and 210 girls) and 473 healthy children. SNP genotyping was performed using the MassARRAY-4 system. **Results**: Both SNPs were associated with an increased risk of BA under the additive genetic model ($p \leq 0.001$). In the sex-stratified analysis, the rs17293632 (odds ratio (OR) = 1.79, 95% confidence interval (CI): 1.15–2.77; $p = 9.0 \times 10^{-4}$) and rs2033784 (OR = 1.80, 95% CI: 1.28–2.52; $p = 6.0 \times 10^{-4}$) variants were exclusively associated with asthma risk among girls, whereas no significant associations were observed in boys. The rs17293632T-rs2033784G haplotype was associated with an increased risk of asthma in girls (OR = 2.01, 95% CI: 1.38–2.92, $p = 3.0 \times 10^{-4}$). Notably, *SMAD3* gene polymorphisms were associated with an increased risk of asthma exclusively among children living in urban environments ($p \leq 0.0001$). Polymorphisms in the *SMAD3* gene have also been linked to specific allergies, including those to horsehair, chicken eggs, beef, wheat, oatmeal, barley, and foxtail. **Conclusions**: The present study of Russian children confirmed that the rs17293632 and rs2033784 polymorphisms of the *SMAD3* gene are significant genetic markers associated with BA and allergies. However, an association between these *SMAD3* variants and asthma was observed only among girls, marking the first time this association has been reported. Further research is necessary to determine whether the *SMAD3* gene constitutes a viable therapeutic target for treating asthma and allergic diseases.

Keywords: asthma; hypersensitivity; etiology; Smad proteins; genetic predisposition to disease; single nucleotide polymorphism

1. Introduction

Bronchial asthma (BA) is a common, clinically diverse, multifactorial disease resulting from complex interactions among multiple genetic and environmental factors that collectively contribute to its pathogenesis [1–4]. The pathogenesis of BA involves a complex interplay of multiple immune cells—including mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and dendritic cells—and their inflammatory mediators, such as cytokines, chemokines, cysteinyl leukotrienes, histamine, nitric oxide, and prostaglandin D2 [5,6]. Dysregulated T helper type 2 (Th2) inflammation, predominantly driven by cytokines IL-4, IL-5, and IL-13, underlies the core pathophysiological features of asthma: airway inflammation, hyperresponsiveness, and remodeling [6].

A causal role in asthma development within the general population has been unequivocally established for several environmental factors, including vaccination practices, reduced immune stimulation by bacterial products, occupational exposures, and contact with particulate matter [7,8]. Bronchial asthma is a genetically heterogeneous disorder with a substantial heritable component, as unequivocally demonstrated by familial aggregation, higher concordance rates in monozygotic compared to dizygotic twins, and findings from large-scale population-based genetic studies [1,3,4]. Despite significant advances in identifying asthma susceptibility genes through genome-wide association studies (GWAS) and candidate gene approaches, the immunogenetic mechanisms underlying disease risk and progression remain incompletely understood [2,3,9–11]. Multiple signaling pathways have been identified as contribut-

ing factors in the pathogenesis and progression of asthma. The Smad signaling pathway, in which the SMAD3 protein functions as an intracellular signal transducer and transcriptional modulator, is activated by transforming growth factor beta (TGF-β) and regulates cell proliferation, differentiation, and apoptosis [12,13]. Aberrant or excessive activation of SMAD3 signaling pathways can induce structural remodeling of the airways, thereby contributing to airway obstruction and hyperresponsiveness—hallmark features of asthma [13,14].

Numerous multi-ancestral GWAS have identified specific single nucleotide polymorphisms (SNPs) within the *SMAD3* gene that are associated with the development and progression of bronchial asthma by modulating airway structure, immune responses, and inflammatory pathways [15–17]. Although GWAS have produced significant findings across diverse global populations, many SNPs identified within the *SMAD3* gene require further validation in independent cohorts to confirm their clinical relevance and practical applicability. The present study aimed to investigate the association between two common SNPs, rs17293632 and rs2033784, located within the *SMAD3* gene—previously identified through GWAS [11,17]—and susceptibility to atopic asthma and sensitivity to allergens in pediatric populations from the Russian Federation.

2. Materials and Methods

2.1 Study Participants and Their Clinical Examination

The study included DNA samples from 999 unrelated children aged 1 to 16 years. This cohort comprised 526 patients with allergic bronchial asthma who were receiving inpatient treatment at the Kursk Regional Children's Clinical Hospital, and 473 unrelated children without any signs of bronchopulmonary or allergic pathology at the time of sample collection, all residing in the Kursk region. The group of patients with BA included children with intermittent, mild persistent, moderate persistent, and severe persistent asthma. The diagnosis of bronchial asthma was confirmed by qualified allergologists and pulmonologists in accordance with national guidelines [18], based on a typical clinical presentation, exclusion of other causes of bronchial obstruction, and findings from laboratory and instrumental examinations, as described previously [19–21]. Clinical examination was conducted following the standard protocol and included inspection (skin color), palpation, percussion of the lung fields and heart, and auscultation to detect abnormal breath sounds such as harsh or diminished breathing and the presence of dry rales. The severity of bronchial obstruction was clinically evaluated based on the number of choking attacks during the day, the frequency of nocturnal symptoms, and the daily number of β2-agonist inhalations. Laboratory clinical methods included general blood analysis, determination of total IgE levels, and rhinocytogram assessment (quantification of neutrophils, eosinophils, and lymphocytes). Scarification skin tests (prick tests) using non-infectious allergens were conducted and evaluated on the volar surface of the forearm. Antihistamines were discontinued 3 to 7 days prior to allergy testing. Skin prick tests were performed with standardized commercial allergens and evaluated according to a widely accepted scale [18]. The study of external respiration function parameters was conducted using computerized spirography. Based on the results, the presence, type, and severity of respiratory disorders in lung function were determined. To assess the severity of obstructive syndrome, the following spirometric parameters were calculated: vital capacity of the lungs, forced vital capacity, and forced expiratory volume in the first second. The study results were expressed as percentages of the expected values, which were calculated based on the dependence of indicators on age, sex, and anthropometric characteristics according to data from the European Respiratory Society (https://www.ersnet.org/).

2.2 Genetic Analysis

Genetic studies were conducted at Research Institute of Genetic and Molecular Epidemiology at Kursk State Medical University (Kursk, Russia). Genomic DNA was isolated from peripheral venous blood samples of the study participants using the classical phenol-chloroform extraction method. The GWAS Catalogue (https://www.ebi.ac.uk/gwas/home) was used to identify SNPs of the *SMAD3* gene associated with the isolated asthma phenotype in European populations. Associations identified in mixed populations were excluded when selecting polymorphisms. We selected SNPs associated with the following disease phenotypes (trait names): "Asthma", "Asthma (moderate or severe)", "Asthma (childhood onset)", and "Atopic asthma". As a result, we identified nine SNPs (rs10152593, rs12592283, rs12912010, rs17293632, rs28617673, rs56062135, rs56375023, rs72743461, and rs744910) with a minor allele frequency (MAF) $\geq$5% (according to the Ensembl database: https://www.ensembl.org/index.html) associated with asthma among Europeans. Additionally, SNP rs2033784 was of particular interest, as it was the only *SMAD3* polymorphism showing an association with asthma in a large cohort, the vast majority of whom were Europeans [11]. From the above SNP list, two polymorphisms, rs17293632 and rs2033784, were selected for the study because they met the criteria for joint genotyping in a multiplex panel using the MALDI-TOF iPLEX mass spectrometry platform. Genotyping of SNPs rs17293632 and rs2033784 in the *SMAD3* gene was conducted using the MassARRAY-4 system (Agena Bioscience Inc., San Diego, CA, USA), following the protocol described in a recent paper [22]. To assess the accuracy of the genotyping procedure, a random subset of 95 samples was selected for replicate genotyping across all examined loci. The replicate analyses showed complete concordance with the initial genotyping results. The molecular genetic study included 473 patients with

asthma and 465 control subjects, while the remaining patients were excluded due to poor quality of isolated DNA, which was unsuitable for genotyping. Genotyping failed for the rs17293632 polymorphism in one control subject and for the rs2033784 SNP in two asthma patients.

2.3 Statistical Analysis

The sample size of patients for the study was determined through statistical power calculations performed for each phase of the association analysis. Statistical power calculations were performed using the Genetic Association Study (GAS) power calculator (https://csg.sph.umich.edu/abecasis/gas_power_calculator/). Assuming a significance level of $\alpha = 0.05$ and an average MAF of 0.25, our study has a power between 85% and 98% to detect a genotype relative risk (GRR) ranging from 1.39 to 1.48 in the overall association analysis, and a GRR between 1.70 and 2.00 in the subgroup analyses. Fisher's exact test was used to evaluate the genotype frequency distribution according to Hardy-Weinberg equilibrium. Logistic regression analysis assessed the associations between *SMAD3* gene polymorphisms and the risk of bronchial asthma, as well as binary clinical phenotypes. Overall odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate SNP-phenotype associations. Associations between SNPs and continuous phenotypes were analyzed using linear regression, estimating mean differences between genotypes along with 95% confidence intervals, utilizing SNPstats software (https://www.snpstats.net/start.htm) [23]. Dominant, recessive, additive, overdominant, and codominant genetic models were applied to assess SNP-disease associations. The optimal genetic model was determined based on the criterion of the lowest *p*-value.

3. Results

3.1 Baseline and Clinical Characteristics of the Study Patients

The baseline and clinical characteristics of the study patients are summarized in Table 1. The group of patients with bronchial asthma was matched with the control group by gender ($p = 0.72$). The mean age of the BA patients was 8.1 years, compared to 8.2 years in the control group. The mean age at disease onset was 5.1 years. Concomitant allergic conditions—including food allergy, atopic dermatitis, allergic rhinitis, pollinosis, and acute exogenous urticaria—were observed in 402 children within the study group. The distribution of asthma severity was as follows: mild BA in 264 patients (50.2%), moderate BA in 184 patients (35%), and severe BA in 78 patients (14.8%). Among the study group, 214 children lived in urban areas, while 312 resided in rural areas.

3.2 Association of SMAD3 Gene Polymorphisms With the Risk of Asthma

The genotype frequencies of rs17293632 and rs2033784 polymorphisms in the *SMAD3* gene were in Hardy-Weinberg equilibrium in both patient groups ($p > 0.05$). We analyzed the associations between *SMAD3* gene polymorphisms and asthma risk in the overall cohort as well as in groups stratified by sex. Table 2 presents the genotype frequencies for the overall group and the sex-stratified subgroups. As shown in Table 2, both polymorphisms, rs17293632 and rs2033784, were strongly associated (under the additive genetic model) with an increased risk of asthma in Russian children ($p \leq 0.001$). Gender-stratified analysis showed that the rs17293632 and rs2033784 variants were exclusively associated with asthma risk in girls ($p \leq 0.0001$), whereas no significant associations of these polymorphisms were observed in boys.

Notably, we also found that polymorphisms of the *SMAD3* gene were significantly associated with an increased risk of asthma in children living in urban areas, whereas no such association was observed in those from rural regions (Table 3). Specifically, the rs17293632 variant showed an odds ratio (OR) of 2.00 with a 95% confidence interval (CI) of 1.32–3.03 ($p \leq 0.0001$) under a dominant genetic model, while the rs2033784 variant exhibited an OR of 1.73 (95% CI: 1.25–2.39, $p = 7.0 \times 10^{-4}$) under an additive model.

The results of the *SMAD3* haplotype association analysis are shown in Table 4. All three *SMAD3* haplotypes had frequencies greater than 5%. The rs17293632 and rs2033784 polymorphisms exhibited strong linkage disequilibrium (D' >0.99, $p < 0.0001$). As shown in Table 3, the haplotype H2 (rs17293632T-rs2033784G) of the *SMAD3* gene was strongly associated with an increased risk of bronchial asthma in girls (OR = 2.01, 95% CI: 1.38–2.92, $p = 3.0 \times 10^{-4}$), but not in boys ($p = 0.17$).

3.3 Association of SMAD3 Gene Polymorphisms With Allergy Types and Laboratory and Instrumental Data

Data from skin prick tests were used to assess the association between *SMAD3* gene polymorphisms and allergy types in children with asthma. To address the issue of multiple comparisons, when analyzing associations with multiple allergens, we adjusted for multiple testing using the false discovery rate (FDR) procedure via an online calculator (https://www.sdmproject.com/utilities/?show=FDR). Table 5 presents a summary of the observed associations between *SMAD3* gene polymorphisms and various allergy types in children with bronchial asthma. Both polymorphisms of *SMAD3* were associated with various types of allergies; however, the nature and direction of these associations varied depending on the specific allergic condition. In particular, the SNP rs2033784 was associated with increased sensitivity to horse hair (FDR = 0.016), chicken

Table 1. Baseline and clinical characteristics of the study patients.

Characteristics	Children with BA n = 526	Control group n = 474	*p*-value
Age ± standard deviation, years	8.1 ± 3.5	8.2 ± 3.3	0.35
Boys, n (%)	316 (60)	279 (59)	0.72
Girls, n (%)	210 (40)	195 (41)	
Place of residence (Urban/Countryside), n (%)	214 (41)	188 (39)	**0.03**
	312 (59)	286 (61)	
Concomitant pathology			
Food allergy, n (%)	306 (58.2)	-	-
Atopic dermatitis, n (%)	12 (2.28)	-	-
Allergic rhinitis, n (%)	123 (23.38)	-	-
Pollinosis, n (%)	12 (2.28)	-	-
Insect allergy, n (%)	64 (12.16)	-	-
Urticaria, n (%)	76 (14.45)	-	-

BA, bronchial asthma. Bold indicates statistically significant values ($p < 0.05$).

Table 2. Associations of *SMAD3* gene polymorphisms with asthma in overall and sex-stratified groups.

SNP ID	Genotype/Allele	Gene frequency (%)		OR (95% CI)[1]
		Control group	Children with BA	p[2]
		Overall groups		
	C/C	298 (63)	255 (48.5)	1.48 (1.18–1.85)
rs17293632	C/T	153 (32.3)	189 (36)	**6.0×10^{-4}**
	T/T	14 (4.7)	28 (12.5)	
	A/A	254 (53.7)	216 (41.1)	1.39 (1.13–1.70)
rs2033784	A/G	183 (38.7)	214 (40.7)	**0.001**
	G/G	26 (7.6)	43 (18.2)	
		Boys		
	C/C	162 (59)	153 (53)	1.23 (0.92–1.64)
rs17293632	C/T	100 (37)	118 (41)	0.15
	T/T	11 (4)	15 (6)	
	A/A	134 (49)	129 (45)	1.17 (0.90–1.52)
rs2033784	A/G	119 (44)	134 (47)	0.25
	G/G	19 (7)	25 (8)	
		Girls		
	C/C	136 (70)	102 (55)	1.79 (1.15–2.77)
rs17293632	C/T	53 (28)	71 (38)	**9.0×10^{-4}**
	T/T	3 (2)	13 (7)	
	A/A	120 (63)	87 (47)	1.80 (1.28–2.52)
rs2033784	A/G	64 (33)	80 (43)	**6.0×10^{-4}**
	G/G	7 (4)	18 (10)	

[1] Odds ratio and 95% confidence intervals.

[2] p-level for SNP-BA association. Bold indicates statistically significant values ($p < 0.05$).

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

eggs (FDR = 0.03), wheat (FDR = 0.03), oatmeal (FDR = 0.04), and foxtail (FDR = 0.03). Nonetheless, this variant demonstrated a protective effect against certain allergy types, including barley (FDR = 0.05) and beef (FDR = 0.01). The polymorphism rs17293632 showed a significant association with increased sensitivity in children to wheat (FDR = 0.03), timothy grass (FDR = 0.03), oatmeal (FDR = 0.03), and foxtail (FDR = 0.03). Meanwhile, this variant was protective against beef allergy (FDR = 0.004). We found no significant associations between the rs17293632 and rs2033784 polymorphisms in the *SMAD3* gene and the severity of bronchial asthma, total IgE levels, or pulmonary function parameters ($p < 0.05$).

4. Discussion

The present study found, for the first time, that the rs17293632 and rs2033784 polymorphisms of the *SMAD3*

Table 3. Associations of *SMAD3* gene polymorphisms with asthma based on children's place of residence*.

SNP ID	Genotype/Allele	Gene frequency (%)		OR (95% CI)[1]
		Control group	Children with BA	p^2
Children living in rural regions				
	C/C	177 (63.4)	163 (58)	1.29 (0.91–1.82)
rs17293632	C/T	91 (32.6)	108 (38.4)	0.15
	T/T	11 (4)	10 (3.6)	
	A/A	150 (54.1)	136 (48.4)	1.26 (0.90–1.76)
rs2033784	A/G	110 (39.7)	127 (45.2)	0.17
	G/G	17 (6.2)	18 (6.4)	
Children living in urban areas				
	C/C	121 (65)	92 (48.2)	2.00 (1.32–3.03)
rs17293632	C/T	62 (33.3)	81 (42.4)	$\mathbf{p \leq 0.0001}$
	T/T	3 (1.7)	18 (9.4)	
	A/A	104 (55.9)	80 (41.7)	1.73 (1.25–2.39)
rs2033784	A/G	73 (39.2)	87 (45.3)	$\mathbf{7.0 \times 10^{-4}}$
	G/G	9 (4.9)	25 (13)	

[1] Odds ratio and 95% confidence intervals.

[2] p-level for SNP-BA association.

* Data on the place of residence were available for 937 individuals. Bold indicates statistically significant values ($p < 0.05$).

Table 4. Association analysis of the of *SMAD3* haplotypes with the risk of asthma.

Haplotype	SNP		Control group	Children with BA	OR (95% CI)[1]	p^2
	rs17293632	rs2033784				
Overall groups						
H1	C	A	0.746	0.682	1.00	-
H2	T	G	0.195	0.259	1.48 (1.18–1.86)	$\mathbf{8.0 \times 10^{-4}}$
H3	C	G	0.059	0.058	1.10 (0.74–1.62)	0.64
Boys						
H1	C	A	0.711	0.681	1.00	-
H2	T	G	0.224	0.258	1.22 (0.92–1.63)	0.17
H3	C	G	0.064	0.061	0.99 (0.60–1.63)	0.97
Girls						
H1	C	A	0.797	0.685	1.00	-
H2	T	G	0.153	0.261	2.01 (1.38–2.92)	$\mathbf{3.0 \times 10^{-4}}$
H3	C	G	0.045	0.054	1.28 (0.69–2.39)	0.44

[1] Odds ratio and 95% confidence intervals.

[2] p-level for haplotype-BA association. Bold indicates statistically significant values ($p < 0.05$).

gene are significant markers of increased genetic susceptibility to bronchial asthma in Russian children in a sex-specific manner. However, both polymorphisms were found to be associated with asthma risk exclusively in girls. Furthermore, the observed associations were exclusively present among children residing in urban environments, while in children from rural areas, these genetic variants showed no significant influence on disease risk. Furthermore, we identified significant correlations between polymorphisms in the *SMAD3* gene and multiple allergy phenotypes, demonstrating increased susceptibility to certain allergens while concurrently exhibiting reduced sensitivity to others.

Multiple polymorphisms within the *SMAD3* gene have been identified as being associated with an increased risk of asthma and other allergic disorders, including atopic dermatitis, allergic rhinitis, and hay fever [15–17,24,25]. *SMAD3* gene polymorphisms, including those examined in this study, are also linked to the development of various immune-mediated diseases such as ankylosing spondylitis, Crohn's disease, psoriasis, ulcerative colitis, autoimmune thyroid disease, systemic lupus erythematosus, and type 1 diabetes mellitus [26,27].

The availability of bioinformatics tools and genomic-transcriptomic databases, such as the GTEx Portal and the eQTLGen Consortium, which are derived from large pop-

Table 5. Association analysis between *SMAD3* gene polymorphisms and allergy types in children with asthma.

SNP	Genotype	OR (95% CI)[1]	p[2]	FDR
Horse hair				
rs17293632	C/C-T/C T/T	0.87 (0.47–1.12)	0.09	0.10
rs2033784	A/A G/G	0.63 (0.47–0.85)	0.002	**0.02**
Chicken eggs				
rs17293632	C/C-T/C T/T	1.94 (0.89–3.16)	0.12	0.12
rs2033784	A/A-G/A G/G	2.26 (1.19–4.30)	0.01	**0.03**
Wheat				
rs17293632	C/C-T/C T/T	2.92 (1.35–6.31)	0.007	**0.03**
rs2033784	A/A-G/A G/G	2.14 (1.12–4.07)	0.02	**0.03**
Barley				
rs17293632	C/C-T/C T/T	0.69 (0.46–1.09)	0.08	0.10
rs2033784	A/A G/A-G/G	0.65 (0.43–0.98)	0.04	**0.05**
Beef				
rs17293632	C/C-T/T T/C	0.56 (0.37–0.83)	0.004	**0.02**
rs2033784	A/A G/A-G/G	0.52 (0.35–0.76)	8.0×10^{-4}	**0.01**
Timothy-grass				
rs17293632	C/C-C/T T/T	2.41 (1.07–5.44)	0.02	**0.03**
rs2033784	A/A G/A-G/G	2.17 (0.95–6.04)	0.09	0.10
Oatmeal				
rs17293632	C/C-T/T C/T	1.65 (1.45–1.94)	0.02	**0.03**
rs2033784	A/A-G/G A/G	1.67 (1.47–1.97)	0.03	**0.04**
Foxtail				
rs17293632	C/C-C/T T/T	2.81 (1.24–6.35)	0.009	**0.03**
rs2033784	A/A-A/G G/G	2.04 (1.07–3.86)	0.02	**0.03**

[1] OR – odds ratio and 95% confidence intervals;

[2] p-level for SNP-allergy type association.

FDR, false discovery rate. Bold indicates statistically significant values ($p < 0.05$).

ulation samples, enables the assessment of the functionality of genetic polymorphisms in relation to their impact on gene expression. This, in turn, facilitates the interpretation of genotypic correlations. The studied polymorphisms are located in intronic regions of the *SMAD3* gene. The functional effects of the polymorphisms were evaluated using genome-transcriptome data available at the GTEx Portal (https://gtexportal.org). The asthma-associated alleles rs17293632-T and rs2033784-G are associated to decreased *SMAD3* gene expression in thyroid tissue and esophageal mucosa, and, to a lesser extent, in the lung (only for the rs17293632-T allele). Additionally, a decreased *SMAD3* gene expression in the carriers of the rs17293632-T and rs2033784-G alleles was observed in blood, according to data from the eQTLGen Consortium portal (https://www.eqtlgen.org/cis-eqtls.html). According to Ensembl data (https://www.ensembl.org/) on genes and regulation obtained from the ENCODE database, SNP rs17293632 participates in epigenetic regulatory functions within an enhancer region located in the lung and airway tissues. This activity is particularly prominent in bronchial epithelial cells, which are characterized by the presence of H3K27me3, H3K4me3, CTCF, and DNase I markers. It is also notable in lung fibroblasts, which exhibit markers such as H3K27ac, H3K27me3, H3K4me1, H3K4me3, CTCF, and DNase I.

We hypothesize that the association between *SMAD3* gene polymorphisms and susceptibility to asthma and sensitization to food allergens may result from a disruption of TGF-β1 signaling mediated by *SMAD3*. Transforming growth factor-beta plays a critical role in regulating immune responses triggered by allergens, thereby contributing to allergic and asthmatic inflammation [28,29]. It controls key cellular processes involved in allergic reactions, including cell differentiation, proliferation, and migration. Smad proteins, particularly Smad3, mediate TGF-β signaling. Impaired Smad3 signaling is known to reduce Th2 cytokine expression, alter allergen-induced IgG1 antibody responses, and affect mucus production [28,30,31]. The TGF-β/Smad3 signaling axis also exacerbates inflammatory hypersensitivity reactions and disease progression by regulating chemokines and cytokines, facilitating inflammatory cell recruitment, promoting cell proliferation, and modulating antigen-specific antibody responses [31]. In particular, in response to a respiratory chemical sensitizer, Smad3 was found to regulate cytokine and chemokine expression as well as specific antibody production in mice [31]. This experiment may partially explain why the associations of *SMAD3* gene polymorphisms identified in this study were observed exclusively among children living in urban environments, where levels of chemical pollution are significantly higher than in rural areas. It is known that the increased exposure to pollutants in urban settings may contribute to the higher incidence of allergic diseases [32,33]. The deficiency of Smad3 resulted in a pronounced shift toward Th2 differentiation and a significant impairment in IgG production [31]. In particular, Smad3-deficient mice did not form TGF-β1-induced Smad-containing DNA-binding complexes and failed to mediate the effects of TGF-β, as demonstrated in experiments with culture-activated hepatic stellate cells [34]. In exper-

iments involving normal mouse airways, Smad3-deficient (*Smad3-/-*) mice exhibited significantly elevated levels of proinflammatory cytokines and interleukin-4 (IL-4), as well as increased expression of the Th2-associated transcription factor GATA-3 in lung tissue and bronchoalveolar lavage fluid, compared to wild-type mice [30]. Functional studies have also been conducted in patients with atopic dermatitis, a condition pathogenetically related to atypical bronchial asthma [1]. Increased expression of *SMAD3* mRNA in the blood has been associated with heightened sensitization to house dust mites in patients with atopic dermatitis [35]. A functional genetic study of patients with atopic dermatitis conducted by Shafi and colleagues observed a significant decrease in the expression of the *SMAD3* gene in affected skin, accompanied by an upregulation of TGF-β1 mRNA expression [36].

Our study has several limitations. The observed sex-specific associations between *SMAD3* polymorphisms and asthma in our study are likely attributable to the relatively limited sample size, which is considerably smaller compared to the genome-wide association studies performed by large international consortia. Although the phenomenon of sexual dimorphism in the relationship between SNPs and predisposition to bronchial asthma has been identified in various studies [10,37,38], future genetic association studies investigating the *SMAD3* gene should be conducted with substantially larger sample sizes. This will provide more robust and reliable estimates of the gene's association with asthma development, particularly considering the observed sexual dimorphism in disease susceptibility. Since the associations between *SMAD3* polymorphisms and various types of allergies were weak, further studies with larger sample sizes are necessary to confirm these findings. Furthermore, the lack of direct functional experimental data in our study limits the mechanistic explanations of the observed associations between *SMAD3* polymorphisms and asthma or allergy. An interesting finding was that polymorphisms in the *SMAD3* gene are associated with an increased risk of asthma in urban children, suggesting that specific environmental factors—particularly air pollution—may underlie this relationship. Toxicogenomic studies in animal models can help elucidate the underlying mechanisms and assess whether exposure to air pollutants modulates *SMAD3* gene expression, thereby contributing to the development of asthma and allergies.

5. Conclusions

The present study of Russian children confirmed that the rs17293632 and rs2033784 polymorphisms of the *SMAD3* gene are significant genetic markers associated with susceptibility to bronchial asthma. However, the association of *SMAD3* variants with asthma was observed only among girls, marking the first time this finding has been reported. The associations between polymorphisms and asthma, as well as various types of allergies that we identified, confirm the important role of SMAD3 in the development of immunopathological disorders underlying asthma and sensitization to multiple allergens. Based on available genomic and transcriptomic data, there is strong evidence that the asthma-associated loss-of-function alleles rs17293632 and rs2033784 reduce *SMAD3* gene expression, thereby disrupting Smad3-mediated TGF-β signaling. This disruption, particularly in the lungs and airways, may contribute to various immunopathological disorders, including decreased Th2 cytokine levels, altered allergen-induced specific IgG1 responses, and the overproduction of proinflammatory cytokines, as demonstrated by several experimental studies. Further studies are necessary to determine whether the *SMAD3* gene constitutes a viable therapeutic target for treating asthma and allergic diseases.

Abbreviations

BA, bronchial asthma; GWAS, genome-wide association study; Th2, T helper type 2; OR, odds ratio; CI, confidence intervals.

Availability of Data and Materials

The data presented in this study are available upon reasonable request from the corresponding author.

Author Contributions

AS and AP designed the research study. AP conceptualized and supervised the research. AS and AB examined the patients. AS, AP, and MF analyzed the data. AS, OB, and VP performed the laboratory genetic studies. AS drafted the manuscript. AP reviewed and edited the final manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the guidelines of the Declaration of Helsinki. Informed consent for children's participation in the study was obtained from their parents or legal guardians. The study protocol was approved by the regional ethics committee of Kursk State Medical University (protocol No. 4, dated 09.04.2018).

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Conflict of Interest

The authors declare no conflict of interest. Given his role as the Editorial Board member, Alexey V Polonikov had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Gustavo Caetano-Anollés.

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