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HUMAN GENETICS

Association between Allelic Variants of the Genes Involved in Glucocorticoids Metabolism and Asthma

Yu. Yu. Fedorova[a, *], A. S. Karunas[a, c, **], R. R. Murzina[c], O. N. Savelieva[b], G. F. Gimalova[a], R. F. Gatiyatullin[c], E. I. Etkina[c], and E. K. Khusnutdinova[a, c]

[a] *Institute of Biochemistry and Genetics, Ufa Federal Research Centre, Russian Academy of Sciences, Ufa, 450054 Russia*
[b] *Bashkir State University, Ufa, 450076 Russia*
[c] *Bashkir State Medical University, Ufa, 450000 Russia*
**e-mail: fedorova-y@yandex.ru*
***e-mail: carunas@list.ru*

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Abstract—Bronchial asthma (BA) is a common severe and disabling multifactorial disease. Up to 50–60% of differences in sensitivity to therapy in patients with BA is due to genetic variability. We studied polymorphic variants of four genes involved in the metabolism of glucocorticosteroids in patients with asthma and healthy individuals of Russian, Tatar and Bashkir ethnic origin: rs37973 of the glucocorticoid-induced transcript 1 gene (*GLCCI1*), rs2305089 of the transcription factor T gene (*TBXT*), rs10044254 of the F-box and leucine-rich repeat protein gene (*FBXL7*), rs11123610 of the allantoicase gene (*ALLC*). It has been established that, in Tatars, the rs37973 *G* allele of the *GLCCI1* gene is a marker of an increased risk of developing BA with an uncontrolled course, while a decrease in spirography is observed in patients with the rs37973 *AG* and rs37973 *GG* genotypes of the *GLCCI1* gene compared with children with rs37973 *AA* genotypes. In Bashkirs, a marker of an increased risk of developing the disease is the rs2305089 *T* allele of the *TBXT* gene polymorphic variant.

Keywords: bronchial asthma, polymorphic variant, genes involved in the regulation of glucocorticoids metabolism, association

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INTRODUCTION

Bronchial asthma (BA) is one of the most common severe and disabling multifactorial disease developing as a result of genetic and environmental interaction. Glucocorticosteroids (GC) appear to be an effective therapeutic agent used for the treatment of chronic allergic inflammation of the respiratory tract in patients with BA [1]. Despite the achievements in clinical pharmacology, the disease in 10–20% of patients, especially with severe BA, cannot be controlled [1, 2]. The response to GC therapy represents the basis for both the definition of uncontrolled BA and the classifications of therapeutic resistance. The study of the individual response of BA patients to GC therapy demonstrated that up to 50–60% of differences in sensitivity to therapy are caused by genetic factors; hence, the contribution of genetic component to the efficacy of GC therapy is actively being studied worldwide [1–4].

According to the published data and the results of our previous studies, the changes in the efficacy of GC therapy in BA patients were associated with polymorphic variants in the glucocorticoid receptor (*NR3C1*) and corticotropin-releasing hormone receptor (*CRHR1*) genes [2–4]. Several studies established the role of polymorphic variants in the *TBX21*, *WDR21A*, *eNOS*, *ORMDL3*, *HDAC1*, and *ADRB2* genes in BA development and treatment efficacy [2, 4]. Significant progress in the study of BA pathogenesis and the genetic basis of therapy sensitivity in BA patients was achieved owing to the use of genome-wide association studies (GWAS) and the examination of large samples (>100000 individuals) within the framework of international consortia. Since 2007 more than 90 GWAS on bronchial asthma have been conducted in different populations worldwide, which succeeded in identifying more than 1100 polymorphic variants associated with BA, including SNPs located in the genes involved in inflammation (*IL13*, *IL6R*, *DENND1B*, *LRRC32*, *IL2RB*, and *IL1RL1*), the barrier function of the epithelium (*IL33*, *IL1RL1*, *C11orf30*, *TSLP*, and *CDHR3*), the contraction of smooth muscle of the respiratory tract (*PDE4D*), apoptosis and cell differentiation (*GSDMB*), etc. (http://www.ebi.ac.uk/gwas/) [5–7]. As a result of GWAS, novel genes associated with modified lung function in BA patients in response to glucocorticoid therapy (*GLCCI1*, *TBXT*, *FBLX7*, and *ALLC*), to b2-agonist therapy (*SPATS2L*,

Table 1. Characteristics of BA patients and control group

Parameter	Sample		
	Russians	Tatars	Bashkirs
BA patients			
Sample size	84	108	44
Age, years (M ± S.E.)	10.45 ± 0.39	10.72 ± 0.31	10.34 ± 0.54
Age at BA onset, years (M ± S.E.)	3.85 ± 0.34	3.48 ± 0.29	3.73 ± 0.47
Women, number (%)	24 (28.57)	37 (34.26)	9 (20.45)
Level of total IgE, MU/mL (M ± S.E.)	432.15 ± 46.15	431.67 ± 38.86	425.30 ± 58.0
FEV1, % of normal (M ± S.E.)	62.51 ± 3.59	73.27 ± 4.64	81.22 ± 8.30
Daily norm of GC, mg (M ± S.E.)	275.41 ± 23.39	275.81 ± 13.49	289.58 ± 23.50
Control group			
Sample size	75	83	36
Age, years (M ± S.E.)	11.49 ± 0.43	13.54 ± 0.42	14.19 ± 0.58
Women, number (%)	43 (57.33)	58 (69.88)	18 (50.0)

M—mean, S.E.—standard error of mean.

THRB, and *SLC22A15*), and to antileukotriene therapy (*MRPP3*) were identified [2, 4, 8–11]. Considering the existence of ethnic differences and the necessity to replicate GWAS results in different populations to identify common and ethnicity-specific disease markers, it seems relevant to study the genetic markers of the efficacy of GC therapy in patients with bronchial asthma of different ethnicity from the Republic of Bashkortostan.

The present study aimed to estimate the role of polymorphic markers of the glucocorticoid-induced transcript 1 gene (*GLCCI1*, rs37973), transcription factor T gene (*TBXT*, rs2305089), F-box and leucine-rich repeat protein gene (*FBXL7*, rs10044254), and allantoicase gene (*ALLC*, rs11123610) in the development of bronchial asthma and the efficacy of therapy response in the group of patients treated with glucocorticosteroids.

MATERIALS AND METHODS

DNA samples of 430 unrelated individuals from the Republic of Bashkortostan aged 2–17 years were used in the present study (Table 1). The group of patients consisted of 236 patients with bronchial asthma (70 girls, 166 boys) of different ethnic origin (84 Russians, 108 Tatars, 44 Bashkirs). All examined individuals were patients at the children's clinic at Bashkir State Medical University of the Ministry of Health of Russia (Ufa, Russia) and the Allergology Department of the Republican Children's Clinical Hospital (Ufa, Russia). The criteria for inclusion of children in the main observation group included the previously established diagnosis of "bronchial asthma" and therapy with inhaled glucocorticosteroids (ICS) for at least three months. BA diagnosis was established in accordance with GINA (Global Initiative for Asthma) criteria and the criteria of the Russian programme documents on BA diagnosis, treatment, and prevention [1, 12]. The evaluation of respiratory function was performed using a computer spirometer (Erich Jaeger, Germany) with flow–volume curve analysis. The following parameters were assessed (in percent of the expected value present in the computer database of the spirometer): vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), forced expiratory flow between 25 and 75% of forced vital capacity (FEF25, FEF50, FEF75, respectively). The normal range and reduction in parameters of spirogram (in percent of the standard value) for children under 18 years were assessed according to Klement and Zilber (Table 2) [13]. A group of apparently healthy children without bronchopulmonary, allergic, and autoimmune diseases and any familial history of allergic diseases with low levels of total immunoglobulin (IgE) (0–150 MU/mL) consisting of 194 individuals (119 girls, 75 boys) of the corresponding ethnicity (75 Russians, 83 Tatars, 36 Bashkirs) served as a control. An informed consent to participate in the study was obtained from all the children over 15 years and parents of children under 15 years participating in the study. The study protocol was approved by the local Bioethical Committees at the Bashkir State Medical University (Protocol no. 28 dated October 29, 2012) and the Institute of Biochemistry and Genetics of the Ufa Federal Research Centre of the Russian Academy of Sciences (Protocol no. 4 dated November 15, 2012).

Genomic DNA was isolated from peripheral blood lymphocytes by phenol-chloroform extraction [14]. Analysis of polymorphic variants of four genes involved in glucocorticosteroid metabolism (rs37973

Table 2. The normal range and deviations of the main parameters of spirogram in % from the estimated value for children under 18 years [13]

Parameter	Normal range	Gradations of decrease		
		mild	moderate	severe
VC	79.3–112.6	66.8	60.6	54.4
FVC	78.1–113.3	67.6	62.4	57.2
FEV1	78.1–113.6	67.3	61.9	56.5
FEF25	71.7–117.2	54.7	46.2	37.7
FEF50	71.5–117.3	51.1	40.9	30.7
FEF75	61.2–123.6	44.5	36.1	27.8

Shown values indicate the lower border of decrease.

(c.-1106G>A) of the glucocorticoid-induced transcript 1 gene (*GLCCI1*), rs2305089 (c.530C>T) of the transcription factor T gene (*TBXT*, *TFT*, *T*), rs10044254 (c.128-144403A>G) of the F-box and leucine-rich repeat protein gene (*FBXL7*), rs11123610 (c.84+1311G>A) of the allantoicase gene (*ALLC*)) in BA patients and healthy individuals was carried out using DNA amplification by the polymerase chain reaction (PCR) with fluorescent detection (FLASH/RTAS) (Testgen, Moscow) according to the manufacturer's protocol using the CFX96 real-time PCR detection system (Bio-Rad, United States). We conducted the association analysis of examined loci with BA development and clinical and functional parameters of BA (disease severity, age of manifestation, level of total IgE, spirometry parameters) in individuals of Russian, Tatar, and Bashkir ethnic origin. Selection of genes was carried out on the basis of published genome-wide association studies (GWAS) and the suggested role of identified genes in BA development.

The χ^2 criterion was used to verify the correspondence of the observed distribution of genotype frequencies to the expected one according to the Hardy–Weinberg equilibrium. A pairwise comparison of allele and genotype frequencies between the patients and controls was based on the χ^2 criterion for 2 × 2 contingency tables with Yates correction. In the case of significant differences in the studied samples, the odds ratio (OR) and the boundaries of 95% confidence interval (95%CI) were estimated. Statistical analysis of quantitative data was performed using parametric and nonparametric tests depending on the scales and the distribution of variables via SPSS v. 23 (SPSS Inc.). The distribution of quantitative data was assessed according to the Kolmogorov–Smirnov criterion. The equality of general variances was assessed using Levene's test. In the case of normality of the data distribution and equality of variances in the compared samples, the Student's *t*-criterion was used to compare two groups, and univariate analysis of variance was used to compare more than three samples (individuals with different genotypes or diagnoses). Nonparametric tests (Mann–Whitney *T* criterion and Kruskal–Wallis *H* criterion) were used in similar comparisons in the case of abnormal distribution or failed equality of variances. The association analysis of polymorphic variants with BA risk was conducted under different models considering categorical variables (sex and ethnicity) entered into the regression model as independent variables via series of logistic regression using SPSS v. 23 and SNPStats (https://www.snpstats.net/start.htm) [15].

RESULTS

The distribution of genotype frequencies corresponded to the Hardy–Weinberg equilibrium ($p > 0.05$) in all examined polymorphisms. Allele and genotype frequencies of *GLCCI1* gene rs37973 polymorphism in the BA patients and control group are presented in Table 3. The frequency of the *G* allele at rs37973 was 50.67% in control group of Russians, 41.57% in the group of Tatars, and 44.44% in the group of Bashkirs. The association of the rs37973 *G* allele with bronchial asthma development was observed in Tatars ($p = 0.03$; OR = 1.57; 95%CI 1.04–2.36) (Table 3). The *AA* genotype and *A* allele of the rs37973 appeared to be the markers of decreased risk of BA development in Tatars ($p = 0.02$; OR = 0.45; 95%CI 0.23–0.87 and $p = 0.03$; OR = 0.64; 95%CI 0.42–0.96, respectively). The rs37973 *G* allele was also associated with uncontrolled course of BA in Tatars ($p = 0.02$; OR = 1.74; 95%CI 1.07–2.83). The frequency of the *A* allele at rs37973 was significantly lower (44.64%) in the sample of uncontrolled BA patients of Tatar ethnic origin compared with the control group ($p = 0.02$; OR = 0.57; 95%CI 0.35–0.93).

The analysis of variations in the quantitative parameters of spirometry depending on the genotypes of examined locus in the *GLCCI1* gene conducted in the present study demonstrated reduced FEF75 in individuals of Tatar ethnic origin with the *AG* genotype (M ± S.E. = 74.24 ± 5.02) and the *GG* genotype at rs37973 (81.11 ± 7.21) compared with BA patients bearing the *AA* genotype at rs37973 (103.8 ± 9.53; $p = 0.013$). As a result of pairwise comparison of groups, a statistically significant decrease in FEF75 was

Table 3. Distribution of genotype and allele frequencies of *GLCCI1* rs37973, *TBXT* rs2305089, *FBXL7* rs10044254, and *ALLC* rs11123610 gene polymorphisms in BA patients and control group

		Genotypes			Alleles		
		n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*N*
rs37973		*AA*	*AG*	*GG*	*A*	*G*	
BA patients	Russians	26 (30.95)	42 (50.0)	16 (19.05)	94 (55.95)	74 (44.05)	84
	Tatars	20 (18.52) $p = 0.02$; OR = 0.45 (0.23–0.87)	62 (57.41)	26 (24.07)	102 (47.22) $p = 0.03$; OR = 0.64 (0.42–0.96)	114 (52.78) $p = 0.03$; OR = 1.57 (1.04–2.36)	108
	Bashkirs	15 (34.09)	20 (45.45)	9 (20.45)	50 (56.82)	38 (43.18)	44
Control	Russians	20 (26.67)	34 (45.33)	21 (28.0)	74 (49.33)	76 (50.67)	75
	Tatars	28 (33.73)	41 (49.40)	14 (16.87)	97 (58.43)	69 (41.57)	83
	Bashkirs	12 (33.33)	16 (44.44)	8 (22.22)	40 (55.56)	32 (44.44)	36
rs2305089		*CC*	*CT*	*TT*	*C*	*T*	
BA patients	Russians	27 (32.14)	39 (46.43)	18 (21.43)	93 (55.36)	75 (44.64)	84
	Tatars	29 (26.85)	62 (57.41)	17 (15.74)	120 (55.56)	96 (44.44)	108
	Bashkirs	10 (22.73) $p = 0.04$; OR = 0.37 (0.14–0.96)	21 (47.73)	13 (29.55)	41 (46.59) $p = 0.02$; OR = 0.46 (0.24–0.88)	47 (53.41) $p = 0.02$; OR = 2.16 (1.14–4.09)	44
Control	Russians	22 (29.33)	38 (50.67)	15 (20.0)	82 (54.67)	68 (45.33)	75
	Tatars	19 (23.17)	41 (50.00)	22 (26.83)	79 (48.17)	85 (51.83)	82
	Bashkirs	16 (44.44)	15 (41.67)	5 (13.89)	47 (65.28)	25 (34.72)	36
rs10044254		*AA*	*AG*	*GG*	*A*	*G*	
BA patients	Russians	51 (61.45)	26 (31.33)	6 (7.23)	128 (77.11)	38 (22.89)	83
	Tatars	67 (62.04)	36 (33.33)	5 (4.63)	170 (78.70)	46 (21.30)	108
	Bashkirs	27 (62.79)	12 (27.91)	4 (9.30)	66 (76.74)	20 (23.26)	43
Control	Russians	36 (50.0)	32 (44.44)	4 (5.56)	104 (72.22)	40 (27.78)	72
	Tatars	45 (57.69)	24 (30.77)	9 (11.54)	114 (73.08)	42 (26.92)	78
	Bashkirs	24 (66.67)	10 (27.78)	2 (5.56)	58 (80.56)	14 (19.44)	36
rs11123610		*AA*	*AG*	*GG*	*A*	*G*	
BA patients	Russians	27 (32.93)	39 (47.56)	16 (19.51)	93 (56.71)	71 (43.29)	82
	Tatars	42 (39.25)	53 (49.53)	12 (11.21)	137 (64.02)	77 (35.98)	107
	Bashkirs	22 (50.0)	15 (34.09)	7 (15.91)	59 (67.05)	29 (32.95)	44
Control	Russians	26 (35.14)	39 (52.7)	9 (12.16)	91 (61.49)	57 (38.51)	74
	Tatars	27 (33.33)	44 (54.32)	10 (12.35)	98 (60.49)	64 (39.51)	81
	Bashkirs	16 (45.71)	17 (48.57)	2 (5.71)	49 (70.0)	21 (30.0)	35

N is the number of individuals; *n* is the sample size; allele and genotype frequencies are shown in brackets, %; *p* is the *P*-value and is shown in the case of statistical significance ($p < 0.05$); OR is the odds ratio and 95% confidence interval (in brackets).

observed in patients bearing the rs37973 *AG* genotype compared with individuals with the *AA* genotype ($p = 0.005$).

The results of analysis of the distribution of allele and genotype frequencies of rs2305089 in the transcription factor T gene (*TBXT*) conducted between the BA patients and control group are shown in Table 3. The frequency of the rs2305089 *T* allele was 45.33% in the control group of Russians, 51.83% in the group of Tatars, and 34.72% in the group of Bashkirs. The asso-

ciation of the *T* allele at rs2305089 of *TBXT* and increased bronchial asthma risk was established in Bashkirs ($p = 0.02$; OR = 2.16; 95%CI 1.14–4.09). The *CC* genotype and *C* allele of the rs2305089 appear to be the markers of a decreased risk of BA development in Bashkirs ($p = 0.04$; OR = 0.37; 95%CI 0.14–0.96 and $p = 0.02$; OR = 0.46; 95%CI 0.24–0.88, respectively).

The analysis of the distribution of allele and genotype frequencies of the rs10044254 of F-box and leucine-rich repeat protein gene (*FBXL7*) demonstrated that the *G* allele was less prevalent in control groups of Russians, Tatars, Bashkirs (27.78, 26.92, and 19.44%, respectively) (Table 3). No association of the rs10044254 marker in the *FBXL7* gene and BA development was revealed as a result of comparison between BA patients and healthy donors ($p > 0.05$). The differences in the distribution of genotype frequencies of rs10044254 were detected in the case of division of patients considering the deviations in spirogram parameters from the normal range compared to the control group. The frequency of the rs10044254 *AG* heterozygote (23.33 and 16.67%) in Russian patients with significantly decreased FEV1 and FEF25 was significantly lower compared to the control group ($p = 0.04$; OR = 0.38; 95%CI 0.14–0.99 and $p = 0.008$; OR = 0.25; 95%CI 0.09–0.73, respectively). The frequency of the rs10044254 *AA* homozygote in Russian patients with significantly reduced FEF25 values was significantly higher (73.33%) compared to the control group (50.00%; $p = 0.03$; OR = 2.75; 95%CI 1.08–6.98).

No statistically significant differences in the distribution of allele and genotype frequencies of rs11123610 of the allantoicase gene (*ALLC*) were detected between the BA patients and control group ($p > 0.05$). The *G* allele at rs11123610 was less prevalent in all examined groups, its frequency was 38.51% in the control group of Russians, 39.51% in the group of Tatars, and 30.0% in the group of Bashkirs.

Together with association analysis of genetic polymorphisms with BA development and sensitivity to GC therapy in distinct ethnic groups, to enhance the study power and to detect the common risk markers, we conducted a regression analysis of the examined gene polymorphisms in combined samples of Russians, Tatars, Bashkirs considering sex and ethnicity as covariates (Table 4). Association with BA was determined on the basis of five possible models of inheritance (codominant, dominant, recessive, overdominant, and log-additive). The data on the models with the lowest value of the Akaike information criterion (AIC) are reported in Table 4 for each polymorphic variant. As a result of the analysis, no statistically significant differences in the rs37973, rs2305089, rs10044254, and rs11123610 were detected between the BA patients and control group in the combined sample ($p > 0.05$).

DISCUSSION

The problem of asthma control requires thorough analysis of the factors responsible for the disease progression and relapse, together with the development of BA targeted therapy taking into account clinical, instrumental, and molecular genetic examination of patients. In recent years, numerous attempts to identify the genes responsible for the metabolism of drugs used for BA treatment were conducted [2, 4, 8–11]. Within these studies, we previously conducted the research of polymorphic variants in the *NR3C1*, *CRHR1*, and *TBX21* genes in BA children treated with GC and healthy individuals from the Republic of Bashkortostan. As a result of this study, the association of polymorphic variants of the *NR3C1* and *CRHR1* genes with the risk of BA development and decreased parameters of respiratory function in BA patients in the period of GC therapy was established [3]. Association analysis of allelic variants of the *GLCCI1*, *TBXT*, *FBLX7*, and *ALLC* genes identified under GWAS and the efficacy of GC therapy have been conducted in BA patients in many populations; however, it was performed for the first time in BA patients of Russian, Tatar, and Bashkir ethnicity from the Republic of Bashkortostan.

The most significant associations with development of BA, including the groups differentiated by disease severity and control, were established for allelic variants of the *GLCCI1* gene (rs37973) encoding glucocorticoid-induced transcript 1. The carriers of the *G* allele at rs37973 of the *GLCCI1* gene of Tatar ethnic origin demonstrated a higher risk of developing an uncontrolled course of BA, while the parameters of spirometry in Tatar patients bearing the *AG* and *GG* genotypes at rs37973 were reduced compared to children bearing the *AA* genotype at rs37973. According to the literature, an association of *GLCCI1* rs37972 being in a close linkage disequilibrium with rs37973 with decreased parameters of lung function in response to corticosteroid therapy was determined in GWAS studies of BA patients of European descent [8]. The *GLCCI1* gene is located on chromosome 7p21.3 and contains eight exons. The functional role of the encoded protein in GC-mediated signaling was primarily described by Chapman et al., who identified several differentially expressed sequences as a response to dexamethasone introduction into GC-sensitive and GC-resistant cell lines. The *GLCCI1* gene is expressed in both lung and immune cells, and its expression is significantly enhanced by GC. Presumably, the protein product of the *GLCCI1* gene represents an early apoptosis marker in GC-treated cells. An impaired regulation of apoptosis plays a key role in the development and maintenance of inflammatory responses accompanying BA [8]. Subsequent studies revealed no statistically significant associations between rs37973 and the response to GC therapy in patients of European origin [16, 17]. BA patients from Japan carrying

Table 4. Association analysis of *GLCC11* rs37973, *TBXT* rs2305089, *FBXL7* rs10044254, and *ALLC* rs11123610 gene polymorphisms and BA risk in total sample

SNP	Genotype, model	BA patients *n* (%)	Control *n* (%)	p_{adj}	OR_{adj} (95%CI)	AIC	*N*
rs37973	*AA* (*AG* + *GG*), dominant	61 (25.9) 175 (74.1)	60 (30.9) 134 (69.1)	0.47	1.00 1.18 (0.75–1.84)	555.6	430
	AG (*AA* + *GG*), overdominant	124 (52.5) 112 (47.5)	91 (46.9) 103 (53.1)	0.53	1.00 1.14 (0.76–1.71)	555.7	
	Additive	–	–	0.64	1.07 (0.80–1.42)	555.9	
rs2305089	*CC* (*TC* + *TT*), dominant	66 (28.0) 170 (72.0)	57 (29.5) 136 (70.5)	0.41	1.00 1.21 (0.77–1.89)	552.8	429
	TC (*CC* + *TT*), overdominant	122 (51.7) 114 (48.3)	94 (48.7) 99 (51.3)	0.25	1.00 1.27 (0.85–1.91)	552.1	
	Additive	–	–	0.82	1.03 (0.78–1.38)	553.4	
rs10044254	*AA* (*AG* + *GG*), dominant	145 (62.0) 89 (38.0)	107 (56.9) 81 (43.1)	0.34	1.00 0.82 (0.54–1.23)	543.4	422
	GG (*AG* + *AA*), recessive	15 (6.4) 219 (93.6)	15 (8.0) 173 (92.0)	0.40	1.00 0.72 (0.33–1.57)	543.6	
	Additive	–	–	0.27	0.83 (0.60–1.15)	543.1	
rs11123610	*AA* (*AG* + *GG*), dominant	91 (39.1) 142 (60.9)	69 (36.3) 121 (63.7)	0.40	1.00 0.83 (0.55–1.27)	544.9	423
	AG (*AA* + *GG*), overdominant	107 (45.9) 126 (54.1)	100 (52.6) 90 (47.4)	0.15	1.00 0.74 (0.49–1.11)	543.5	
	Additive	–	–	0.89	0.98 (0.72–1.33)	545.6	

N is the number of individuals in regression analysis; *n* is the sample size; genotype frequency is shown in brackets, %; p_{adj} is the *P*-value for the likelihood ratio test of the log-regression model with sex and ethnicity as covariates; OR_{adj} is the odds ratio and 95% confidence interval (in brackets).

the rs37973 *GG* genotype demonstrated a significant decrease in lung functioning during a four-year period compared to patients with other rs37973 genotypes regardless of corticosteroid therapy [18]. Therefore, these studies confirm that patients carrying the minor *G* allele at rs37973 of the *GLCC11* gene may demonstrate a reduced response to GC treatment, however, with a little trait effect.

In the present study we established an association of the *T* allele at rs2305089 of the transcription factor *TBXT* gene with the risk of BA development in children of Bashkir ethnicity. Polymorphic variants in the *TBXT* gene (rs3099266, rs1134481, and rs2305089) associated with variations in parameters of spirometry in patients of European origin treated with GC were established in GWAS [9]. The *TBXT* gene is located on chromosomal region 6q27, contains nine exons, and appears to be a member of an ancient family of genes containing a common motif—T locus. The *TBXT* gene encodes a key mesodermal transcription factor, is expressed at early stages of embryogenesis, and can affect mesoderm development, including the lungs. The role of the *TBXT* gene in BA pathogenesis and GC metabolism was confirmed by computer modeling of potential interactions between the genes encoding transcription factor *TBXT* and glucocorticoid receptor *NR3C1* [9]. The study reported by Keskin et al. [19] revealed no significant associations of polymorphic variants rs3099266 and rs2305089 in the *TBXT* gene with the clinical response to increased doses of ICS in children with bronchial asthma from Turkey. Allelic variants of the *TBXT* gene might represent an important risk factor of BA development and progression; however, additional research with an increased sample size has to be conducted owing to the small sample of children of Bashkir ethnicity.

Park et al. [10] identified polymorphic locus rs10044254 located in the intron region of the *FBXL7* gene in the GWAS of the efficacy of GC therapy in BA patients of European origin. The authors reported an association of the rs10044254 *G* allele with reduced gene expression in immortalized B cells and worsened well-being in patients in response to ICS treatment. The *FBXL7* gene is located on chromosome 5p15.1 and consists of seven exons and encodes a protein belonging to the F-box protein family and being one of

four subunits of the ubiquitin proteinase complex (SCF, SKP1-cullin-F-box). A possible role of the protein product of the *FBXL7* gene in BA pathogenesis is the degradation of cytokine receptors. For example, FBXL19 degrades the IL33 receptor, thus reducing inflammation in the lungs. Another possible mechanism includes the degradation of hypoxia-induced factor 1 (HIF), which is negatively regulated by the FBXL7-mediated pathway during hypoxia and dyspnea [10]. As a result of the present study, an association of the *AA* genotype at rs10044254 in the *FBXL7* gene with significantly reduced FEF25 value in BA patients of Russian ethnicity was determined; however, the findings obtained at the initial stage of the study did not make it possible to draw an unambiguous conclusion about a significant role of the *FBXL7* gene in the pathogenesis of bronchial asthma and the efficacy of GC therapy.

As a result of genome-wide association analysis of BA conducted in Koreans, polymorphic variants in the allantoicase gene (*ALLC*) associated with variations in FEV1 as a response to ICS were revealed. The *ALLC* gene is located on chromosome 2p25.3 and contains 12 exons. Allantoicase belongs to the class of hydrolases catalyzing the hydrolysis of C–N bonds (nonpeptide) and is involved in metabolism of purines. Despite the absence of *ALLC* gene activity in vertebrates, the *ALLC* gene transcripts have been found in mice and in humans. It was suggested that uric acid levels were significantly higher in patients with allergic asthma without corticosteroid treatment than in patients with corticosteroid therapy [11]. Published findings are scarce and require replication on independent samples of patients. In the present study, no statistically significant associations of the *ALLC* gene polymorphisms and BA development were determined.

To increase the statistical power of the study, we conducted a regression analysis of the association of examined genetic polymorphisms with BA in the combined samples of Russians, Tatars, and Bashkirs, with sex and ethnicity as covariates. No statistically significant differences in rs37973, rs2305089, rs10044254, and rs11123610 were established between BA patients and healthy individuals. The results obtained indicate the presence of ethnic differences in the genetic markers of BA risk and GC sensitivity in examined samples.

Therefore, an association study of polymorphic variants of *GLCCI1*, *TBXT*, *FBXL7*, and *ALLC* genes involved in GC metabolism was carried out in patients with bronchial asthma of different ethnicity and in corresponding control groups from the Republic of Bashkortostan. The *G* allele at rs37973 of the *GLCCI1* gene was established to be a marker of increased risk of developing BA with uncontrolled course in Tatars. A decrease in parameters of spirometry was observed in Tatar patients bearing the *AG* and *GG* genotypes of rs37973 of the *GLCCI1* gene compared to children bearing the *AA* genotype at rs37973. It was revealed that the rs2305089 *T* allele of the *TBXT* gene was the marker of an increased risk of developing BA in Bashkirs. The association of the homozygous rs10044254 *AA* genotype in the *FBXL7* gene with significantly reduced FEF25 value in patients of Russian ethnicity was established.

The results obtained in the present study made it possible to thoroughly understand the molecular basis of BA pathogenesis and to identify genetic markers of GC efficacy in BA patients, which may be relevant for the development of novel approaches of early diagnostics, prognosis of the course of the disease, and personal treatment strategy of BA patients.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of interest. The authors declare no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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