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HUMAN  
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## Polymorphism of the Interleukin- and Interleukin Receptor Genes: Population Distribution and Association with Atopic Asthma

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**Abstract**—Population distribution and pathogenetic significance for bronchial asthma (BA) of the eight polymorphic variants of six interleukin- (IL) and interleukin receptor genes, C-589T, G/C 3'-UTR *IL4*, C-703T *IL5*, T113M *IL9*, Q551R, 150V *IL4RA*, G-80A *IL5RA*, and G1972A *IL5RB*, was examined. In the population samples of Russians, Tajiks, Buryats, and Tuvinians racial and ethnic specificity of these polymorphisms was established. These specific features were manifested as population-specific “genetic portraits” in respect of polymorphic allele frequencies. Analysis of the BA patients and their relatives from Tomsk by use of transmission/disequilibrium test (TDT) revealed the presence of a statistically significant association between the C-703 *IL5* allele and the disease ( $P = 0.005$ ). This is the first evidence of an association between the *IL5* gene polymorphism and BA.

### INTRODUCTION

Atopic bronchial asthma (BA) is a common and severe complex disorder (CD) characterized by reversible episodes of dyspnea with normal breathing between them [1]. The disease is a consequence of an allergic reaction and results from complex interaction between immunocompetent cells, mediators, and bronchial cells and tissues, which leads to acute bronchial constriction, edema of bronchial wall, hypersecretion, and rearrangement of the bronchial tree [2]. Elucidation of etiology and pathogenesis of BA led to the understanding that a group of related interleukin (IL) molecules, responsible for the induction and maintenance of inflammation, play an important role in these processes [3]. The main role in pathogenesis of BA is played by *IL4*, *IL5*, *IL9*, and *IL13*, since it is these interleukins that initiate cellular and molecular events leading to the ejection of mediators and the migration of effector cells to the shock organ (bronchi, nasopharynx, skin, etc.), where the inflammatory reaction takes place.

Considering the genetic bases of asthma, it is worth mentioning that the genes encoding these BA-important cytokines are located tandemly in one cluster on chromosome 5q31–33 [4]. Linkage between BA and the disease-related traits, on the one hand, and this locus, on the other, has been established [5, 6]. For these reasons, ILs-coding genes located in the region 5q31–33 seem promising as candidate genes for asthma. Genes for the IL receptors, promoting their functioning, are also worth studying in this respect.

The data on the association of these polymorphisms with asthma and its risk factors reported in a number of studies are controversial and do not provide unambigu-

ous evidence on their involvement in the asthma development [7–10]. In Russia polymorphism of the IL genes in the populations and among the BA patients is scarcely studied.

In this study the distribution of eight dinucleotide polymorphic variants of the genes coding for IL-4 (*IL4*, C-589T, and G/C 3'-UTR), IL-5 (*IL5* and C-703T), IL-9 (*IL9* and T113M),  $\alpha$ -chain of the IL-4 receptor (*IL4RA*, Q551R, and I59V),  $\alpha$ -chain of the IL-5 receptor (*IL5RA* and G-80A), and  $\beta$ -chain of the IL-5 receptor (*IL5RB* and G1972A) was examined in the populations of different racial and ethnic composition. The association between these polymorphisms and atopic asthma in Russian patients from Tomsk was also analyzed.

### MATERIALS AND METHODS

Polymorphism of the IL genes and receptors was studied in four population samples and one sample comprised of atopic BA patients and their relatives.

Population samples including Russians ( $n = 66$ ), Tajiks ( $n = 33$ ), Buryats ( $n = 60$ ), and Tuvinians ( $n = 59$ ) were formed based on the data from the DNA bank of the Laboratory of population genetics, Institute of Medical Genetics, Russian Academy of Medical Sciences, Tomsk, Russia. Only unrelated subject were selected for the analysis.

The family sample studied was ascertained through the children probands for asthma, which were followed up in clinics of Tomsk in 1997 through 2000. A total of 66 families ( $n = 183$ ) were examined, including 51 complete (two parents and affected child) and 15 incomplete families (lacking one of the parents). The proband

age varied from 1.5 to 15 years (the mean age constituted  $8.4 \pm 3.4$  years). Probands were mostly boys ( $n = 46$ ). The mean age of the probands was similar for both sexes ( $8.1 \pm 3.6$  years for boys and  $8.9 \pm 2.8$  years for girls, respectively;  $P > 0.100$ ). Atopic asthma in all probands was diagnosed by clinical examination according to the WHO criteria [11].

We examined eight polymorphic variants of six genes coding for the ILs and their receptors: *IL4*, *IL5*, and *IL9* (IL-4, IL-5, and IL-9), *IL4RA* ( $\alpha$ -chain of the IL-4 receptor), *IL5RA* and *IL5RB* ( $\alpha$ - and  $\beta$ -chains of the IL-5 receptor) (Table 1). Four of the mutations examined are located in the regulatory gene regions, including three mutations in the promoters (C-589T, G-80A, and C-703T) and one mutation in the 3'-untranslated region (G/C 3'-UTR). The remaining polymorphisms are represented by three missense mutations (Q551R, I50V, and T113M) and one conservative substitution (G1972A).

Individuals were genotyped by RFLP analysis of the PCR-amplified specific genome regions. For these purposes DNA samples from the bank of the Institute of Medical Genetics, Russian Academy of Medical Sciences, Tomsk (population sample), as well as the DNA samples extracted from the whole venous blood by a non-enzymatic method [14] (family sample) were used. In most cases the PCR primers and the reaction conditions used were those described in literature (Table 1). The primer structure and the annealing temperature for the analysis of the *IL5RA* G-80A polymorphism were selected experimentally on the basis of the reported gene sequence (GeneBank AJ002523). Primer sequences for the analysis of the *IL5* C-703T and *IL5RB* G1972A polymorphisms were kindly provided by T. Shirakawa (Experimental Medicine Unit, University of Wales, Swansea, United Kingdom).

A PCR mixture included 2.5 pmol of each specific primer; 1.2 to 2.0  $\mu$ l of  $10 \times$  PRC buffer; 0.5 units of *Taq* DNA polymerase (Sibenzim, Novosibirsk); and 100 to 200 ng of genomic DNA. The amplification program included denaturation at  $94^\circ\text{C}$  for 5 min followed by 30 cycles of annealing at the temperature specific for each primer (1 min); chain elongation at  $72^\circ\text{C}$  (45 s); and denaturation at  $94^\circ\text{C}$  (45 s) followed by a final elongation at  $72^\circ\text{C}$  for 3 min.

Amplification products were digested with appropriate restriction enzymes (Table 1) at optimal temperatures for 12–24 h. The reaction mixture included 5 to 7  $\mu$ l of PCR solution; 1.0 to 1.2  $\mu$ l of  $10 \times$  restriction buffer (Sibenzim, Novosibirsk, or New England Biolabs, United States); and 1 to 5 units of the restriction enzyme. The reaction products were separated on 2–3% agarose gels with ethidium bromide at 120–130 V for 30–45 min and visualized in the UV light.

Testing of genotype distribution for conformity to the Hardy–Weinberg equilibrium (HWE) and analysis of allele frequencies in the populations were carried out using Fisher's exact test [15]. Expected heterozygosity

was calculated according to Nei. Specificity of the samples studies with respect to the whole assemblage of the IL genes polymorphic variants was estimated by use of the multidimensional scaling. Using this procedure, the closeness/remoteness of the populations relative to one another is estimated on the basis of their positions in the space of latent variables. Multidimensional scaling was performed based on the matrix of Euclidian distances between the populations obtained from allele frequencies using the UPGA algorithm.

Association of the examined polymorphisms with BA was studied by TDT [16]. The significance level for TDT was estimated using the Monte Carlo algorithm realized in the N $\times$ 23 software program designed by Yu.S. Aul'chenko (Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk) and available at the Internet site <http://mga.bionet.nsc.ru>.

## RESULTS AND DISCUSSION

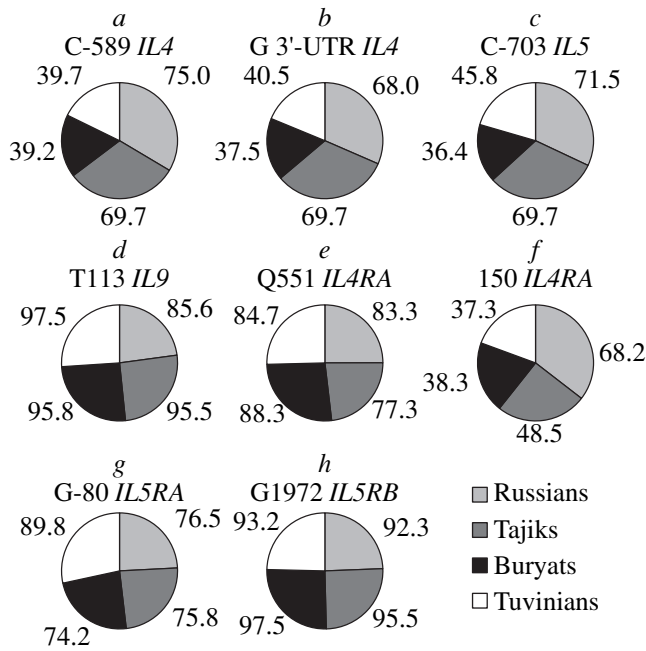
### *Population Distribution of the IL Alleles*

According to the modern concepts of human genetics, susceptibility of the individuals to common complex diseases is determined by the association of "normal" widely distributed alleles, the individual contributions of which to the pathology can be rather small [17]. In the process of ethnogenesis allele frequencies acquire ethnic specificity, which is supposed to be the basis of different susceptibility to CD in different populations. In this connection, studying variation of the candidate genes for CD is important.

Eight polymorphisms of the genes encoding for the ILs and their receptors were studied in the four population groups of different racial and ethnic attribution: Russians, Tajiks, Buryats, and Tuvinians. Russians and Tajiks belong to Eastern Slavic (in this case) and Pamir–Iranian subgroups of the large Caucasoid race, respectively. At the same time, Buryats and Tuvinians belong to Mongoloids of the Central Asian type.

The genotype distribution in the populations studied was consistent with that expected under HWE (Table 2). The *IL9* T113M and *IL5RB* G1972A polymorphisms were characterized by extremely low allelic diversity: heterozygosity for both loci in Buryats was 0.050, while in Russians these values constituted 0.288 and 0.154, respectively. Nevertheless, the frequencies of rare alleles of these genetic variants in the populations studied were above 2%, indicating that the variability observed is the result of polymorphism rather than mutation (Fig. 1). Maximum locus diversity estimated based on the values of observed heterozygosity was observed for C-589T, G/C 3'-UTR, and C-703T in all samples studied.

A specific feature of the C-589T, G/C 3'-UTR *IL4* and C-703T *IL5* polymorphisms established was that in respect of their allele frequencies the populations examined fell into two groups. In the first group comprised of Caucasoid populations (Russians and Tajiks),



**Fig. 1.** Allele frequencies of the genes encoding interleukins and their receptors in the populations (in %). Statistically significant differences were established ( $0.05 < P < 0.001$ ): a, b, and c, Russians, Tajiks–Buryats, Tuvinians; d, Russians–Buryats, Tuvinians; f, Russians–other populations; g, Tuvinians–other populations.

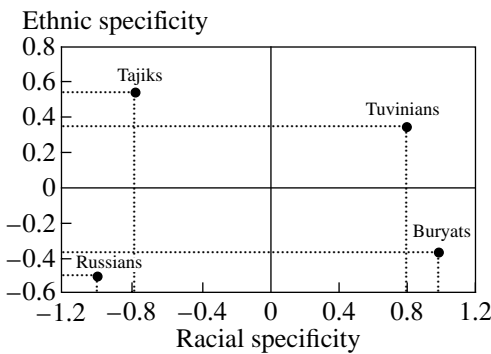
the C-589, G3'-UTR, and C-703 alleles prevailed, while in Mongoloids (Buryats and Tuvinians) alternative alleles were more frequent (Fig. 1). This finding characterizes the polymorphisms examined as race-specific. It can be hypothesized that the C-589T, G/C 3'-UTR and C-703T substitutions in the *IL4* and *IL5* genes appeared long before the subdivision of the mankind into the main races. Later processes of racial divergence resulted in the accumulation of different gene variants in the Caucasoids and Mongoloids. High heterozygosity

observed for the C-589T, G/C 3'-UTR and C-703T polymorphisms in all populations examined, which was close to maximum for a biallelic marker, confirmed the possible ancient origin of these gene variants.

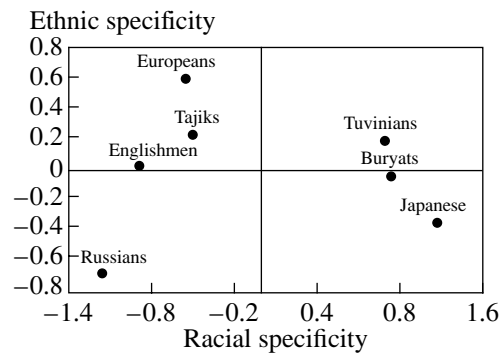
Comparison of the allele frequencies for other polymorphisms revealed statistically significant differences between Russians and Mongoloids in T113M *IL9*, between Russians and other populations in 150V *IL4RA*, and between Tuvinians and other populations in G-80A *IL5RA* (Fig. 1). In these cases an effect of inbreeding and genetic drift associated with the isolated positions of these populations cannot be excluded. These data suggest the existence of racial and ethnic specificities in the frequency distributions of the IL and their receptor encoding polymorphic alleles.

The latter observation is confirmed by the data on multidimensional scaling performed for the populations of Russians, Tajiks, Tuvinians, and Buryats based on the matrices of the Euclidian distances constructed from the allele frequencies of the examined genes. The populations studied were placed in the space of two variables, one of which in this context can be characterized as racial specificity (abscissa), and the other one, as ethnic specificity (ordinate). In this space, Mongoloid populations are located in the field of positive values of the "racial" variable, approximately on one projection to abscissa, while Caucasoids are located in the field of the negative variable values (Fig. 2). Projection onto the ordinate shows substantial dissociation of the populations within racial groups, demonstrating the postulated ethnic specificity of the polymorphisms examined.

To compare the data on the markers examined with those for other world populations, the results of genotyping of a sample of 150 unrelated healthy Britons kindly provided by J.M. Hopkin (Experimental Medicine Unit, University of Wales, Swansea, United Kingdom), as well as the data on the corresponding allele frequencies among healthy Europeans (Dutch, French-



**Fig. 2.** Allocation of the populations studied in the space of variables reflecting their closeness/remoteness relative one another according to the data on polymorphism of the genes encoding interleukins and their receptors. Projections onto axes of coordinates are designated by dashed lines.



**Fig. 3.** Allocation of the seven populations in the space of variables reflecting their closeness/remoteness relative one another according to the data on polymorphism of the genes encoding interleukins and their receptors. Russians, Tajiks, Buryats, and Tuvinians: the data of the present study; other populations: the data from literature [10, 13, 18–23].

**Table 1.** Characteristics of the polymorphisms examined and the genotyping methods

Gene	Polymorphism	Location in the gene	Primer structures, 5' → 3'	Primer annealing temperature, °C	Restriction endonuclease	Restriction products		Authors of genotyping method
						wild-type allele	mutant allele	
<i>IL4</i>	C-589T	5'-UTR	F: act-agg-cct-cac-ctg-ata-cg R: gtt-gta-atg-cag-tcc-tcc-tg	57	<i>BsmFI</i>	192; 60	252	[8]
	G/C	3'-UTR	F: ctc-agt-aca-cca-tat-ggc-t R: cca-gtg-act-ata-att-ata-att-cc	60	<i>VneI</i>	332; 269	601	Shirakawa, personal communication
<i>IL5</i>	C-703T	5'-UTR	F: cag-gga-gag-cca-ata-agg R: atg-atg-tcc-aga-ctc-cag-gat-ct	60	<i>AlwNI</i>	160; 18	178	Shirakawa, personal communication
<i>IL9</i>	T113M	Exon 5	F: act-ctg-gct-ctt-ggc-agg-ta R: cct-atg-agg-ctg-agg-gtc-tg	57	<i>Bsp19I</i>	462	321; 141	[12]
<i>IL4RA</i>	Q551R	Exon 12	F: gtc-tcg-gcc-acc-acc-acc-ggc-tat-c R: acc-caa-gcc-cac-cac-cgc-act	59	<i>Bsc4I</i>	87	64; 23	[10]
	150V	Exon 3	F: ggc-agg-tgt-gag-gag-cat-cc R: gcc-tcc-gtt-gtt-ctc-agg-ta	60	<i>RsaI</i>	273	254; 19	[13]
<i>IL5RA</i>	G-80A	5'-UTR	F: aat-ggc-tat-ctg-gac-gag-ag R: tta-gag-gcg-gtt-ctt-cac-tc	57	<i>AcsI</i>	206	154; 52	–
<i>IL5RB</i>	G1972A	Exon 5	F: agc-cag-acg-tcc-cca-cct-cc R: act-agg-gag-aca-gac-gag-gcc	59	<i>MspI</i>	182; 95; 67	182; 162	Shirakawa, personal communication

Note: The wild-type allele is given as first in the Polymorphism column.

**Table 2.** Polymorphism of the genes encoding for interleukins and their receptors

Gene	Polymorphism	Population	<i>n</i>	Genotype distribution, %			$H_o$	$H_e$	<i>P</i>
				<i>CC</i>	<i>CT</i>	<i>TT</i>			
<i>IL4</i>	C-589T	Russians	64	59.4	31.3	9.4	0.313	0.375	0.185
		Tajiks	33	42.4	54.5	3.0	0.545	0.422	0.203
		Buryats	60	15.0	48.3	36.7	0.483	0.477	0.994
		Tuvinians	58	12.1	55.2	32.8	0.552	0.479	0.406
	G/C 3'-UTR	Russians	64	46.9	42.2	10.9	0.422	0.435	0.773
		Tajiks	33	42.4	54.5	3.0	0.545	0.422	0.206
		Buryats	56	14.3	46.4	39.3	0.464	0.469	0.993
		Tuvinians	58	17.2	46.6	36.2	0.466	0.482	0.784
<i>IL5</i>	C-703T	Russians	65	52.3	38.5	9.2	0.385	0.407	0.753
		Tajiks	33	51.5	36.4	12.1	0.364	0.422	0.417
		Buryats	59	8.5	55.9	35.6	0.559	0.463	0.161
		Tuvinians	59	22.0	47.5	30.5	0.475	0.496	0.79
<i>IL9</i>	T113M	Russians	66	71.2	28.8	0.0	0.288	0.246	0.319
		Tajiks	33	90.9	9.1	0.0	0.091	0.087	0.931
		Buryats	60	93.3	5.0	1.7	0.050	0.080	0.080
		Tuvinians	59	94.9	5.1	0.0	0.051	0.050	0.931
<i>IL4RA</i>	Q551R	Russians	66	71.2	24.2	4.5	0.242	0.278	0.352
		Tajiks	33	57.6	39.4	3.0	0.394	0.351	0.973
		Buryats	60	76.7	23.3	0.0	0.233	0.206	0.557
		Tuvinians	59	71.2	27.1	1.7	0.271	0.259	0.968
	150V	Russians	66	45.5	45.5	9.1	0.455	0.434	0.778
		Tajiks	33	30.3	36.4	33.3	0.364	0.500	0.159
		Buryats	60	20.0	36.7	43.3	0.367	0.473	0.100
		Tuvinians	59	16.9	40.7	42.4	0.407	0.468	0.399
<i>IL5RA</i>	G-80A	Russians	66	57.6	37.9	4.5	0.379	0.359	0.987
		Tajiks	33	60.6	30.3	9.1	0.303	0.367	0.336
		Buryats	60	55.0	38.3	6.7	0.383	0.383	0.988
		Tuvinians	59	81.4	16.9	1.7	0.169	0.183	0.451
<i>IL5RB</i>	G1972A	Russians	65	84.6	15.4	0.0	0.154	0.142	0.944
		Tajiks	33	90.9	9.1	0.0	0.091	0.087	0.931
		Buryats	60	95.0	5.0	0.0	0.050	0.049	0.931
		Tuvinians	59	86.4	13.6	0.0	0.136	0.126	0.940

Note: *n*, the sample size;  $H_o$  and  $H_e$ , the observed and expected heterozygosities, respectively; *P*, the reached significance level in the test for HWE.

**Table 3.** The number of alleles inherited by atopic asthma probands from their heterozygous parents

Gene	Polymorphism	Number of heterozygous parents	Number of inherited alleles	TDT	<i>p</i>
<i>IL4</i>	C-589T	53	C-26; T-27	0.019	0.999
	G/C 3'-UTR	48	C-21; T-27	0.750	0.476
<i>IL5</i>	C-703T	52	C-34; T-18	4.923	0.005
<i>IL9</i>	T113M	37	T-19; M-18	0.027	1.000
<i>IL4RA</i>	Q551R	36	Q-21; R-15	1.000	0.366
	I50V	60	I-26; V-34	1.067	0.356
<i>IL5RA</i>	G-80A	38	A-22; G-16	0.947	0.386
<i>IL5RB</i>	G1972A	31	G-15; A-16	0.032	0.999

Note: TDT, the value of the transmission/disequilibrium test for the deviation from random allele segregation [16]; *P*, the reached significance level (Monte Carlo procedure, 15000 simulations).

men, and Germans) and Japanese [10, 13, 18–23] were used.

The results of multidimensional scaling performed using the data of the present study and those reported for other populations, confirmed the proposal on racial and ethnic specificity of the loci examined (Fig. 3). This observation deserves special attention, since the distribution patterns of BA and other CD are also characterized by ethnic and racial specificity. This concerns, for instance, such parameters as the disease frequency, the ratio between different diseases, specific features of family accumulation, clinical polymorphism, etc. [24]. This specificity is probably caused by the differences in the frequencies of “pathogenetically significant” alleles of the genes determining the disease predisposition. This assumption is partly confirmed by the data on the linkage between genetic loci and CD in different ethnic populations, showing that in different populations a given disease can be associated with different loci.

#### *Association between the IL Genes Polymorphism and Atopic Asthma*

Analysis of the associations between the polymorphic variants of the candidate genes for CD and the integral clinical phenotypes of the diseases among the most common approaches towards determination of the pathogenic importance of these genes [25]. There are several reasons for the existence of associations between gene polymorphisms and the diseases [26]: functional (pathogenetic) significance of the associated locus, its linkage disequilibrium with other causal genes, and the artifact caused by the effects of the population structure (subdivision, admixture, inbreeding). Today, it is generally accepted that the most promising approach towards detection of susceptibility genes is analysis of the family data, which are insensitive to

the population structure effects, and, therefore, more accurate.

In this study an association between the polymorphic variants of the genes encoding ILs and their receptors was examined using the transmission disequilibrium test (TDT). The method consists in testing whether the segregation of alleles inherited by the affected children from their heterozygous parents is random [16]. TDT for a biallelic marker locus consists in the comparison of alternative allele frequencies in the affected offspring of the heterozygous parents: if one of the alleles is substantially more frequent in the affected offspring, it is considered to be associated with the disease.

Taking into consideration a rather small size of the sample examined, the Monte Carlo procedure implemented in the Nx23 software program was used for precise estimation of the significance level for TDT. The method implies the following. Based on the assumption on random allele transmission, a large number of pseudosamples of the size analogous to that of the real sample are constructed. For each sample, the TDT value and the significance level are evaluated. The significance levels equal or lower than those in the real sample are used for the calculation of the precise significance level for the sample examined. In this study a total of 15 000 pseudosamples were generated.

Analysis of the inheritance of different genes for ILs and their receptors by the probands with atopic BA carried out using TDT revealed a statistically significant association between the C-703 *IL5* allele and the disease (Table 3). No association with the disease was found for other polymorphisms.

This is the first report of an association between the C-703 *IL5* allele and atopic BA. Since the C-703T transition is located in the promotor region of *IL5*, its phenotypic effect can consist in the modification of the

gene expression, and hence, in the change of the level of its protein product, IL-5. The role of IL-5 in the BA pathogenesis is associated with its ability to activate eosinophiles, which are involved in the development and maintenance of the inflammatory processes in bronchi [3]. In addition, IL-5 stimulates IL-4-induced synthesis of the IgE allergic antibodies [27]. It can be thus hypothesized that IL-5 plays an important role in the development of the inflammatory and allergic components of atopic BA. Since in the sample from Tomsk an association between the C-703 allele and BA was revealed, it can be suggested that this allele provides higher compared to the T-703 allele level of the IL-5 expression. Note that in this respect, the C-703 *IL5* allele is more frequent in Caucasoids compared to Mongoloids (Fig. 1). In other words, the C-703 *IL5* allele is more important as a risk factor for atopic BA in Caucasoid populations.

It is obvious that susceptibility to BA is a polygenic trait, and the *IL5* is only one of the set of the genes, which interact with each other and with the environmental factors. Today, the set of BA candidate genes is well defined and contains at least 100 to 150 different genes [28]. These include the genes encoding for the factors of humoral immune response and inflammation, receptor genes, and also the genes of intracellular signal molecules and transcription factors, as well as the genes for inflammation mediators' metabolism and xenobiotics. Current studies of the genetic bases of BA are characterized by high concordance of the gene mapping data and the association analysis. The results of screening of 10 complete genomes for BA susceptibility were published. Active studies using model animals (mostly mice) are in progress. Moreover, attempts for gene therapy of BA have been reported. For instance, inhalation of the aerosols containing antisense nucleotides against the A<sub>1</sub> adenosine receptor mRNA was shown to substantially reduce airway reactivity in rabbits with asthma-like phenotype [29]. This example of successful gene therapy of BA shows that understanding fine genetic mechanisms that underlie the development of a pathology provides a possibility of addressed and effective treatment of CD.

Nevertheless, the knowledge of the genetic structure of susceptibility to BA is far from complete, since the whole scenario of the interactions between the hereditary and environmental factors leading to the realization of this complex pathological phenotype is not yet clear. Actively developing microassay technologies and bioinformational approaches to the analysis of genetic networks seem promising in this respect [30, 31]. Further studies of the genetic bases of the BA and CD as a whole require a complex approach. Simultaneous analysis of population specificity and pathogenetic value of the groups of hereditary factors characterized by different "areas of competence" and acting at different physiological systems of the organism should be carried out. Determination of the "fields of action" of these gene complexes, their pleiotropic effects for pathological

phenotypes and the risk factors with respect to racial and ethnic attribution of the patients will provide elucidation of the genetic bases of BA. This will also shed light on the mechanisms of the interaction of polygenic systems in the process of realization of hereditary information at the organism level.

#### ACKNOWLEDGMENTS

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