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## Polymorphisms of Tuberculosis Susceptibility Candidate Genes in the Slavonic Population of Siberia: A Pilot Study

V. P. Puzyrev<sup>1,2</sup>, M. B. Freidin<sup>1</sup>, A. A. Rudko<sup>1</sup>, A. K. Strelis<sup>2</sup>, and O. V. Kolokolova<sup>2</sup>

<sup>1</sup> Institute of Medical Genetics, Tomsk Research Center, Russian Academy of Medical Sciences, Tomsk, 634050 Russia;  
E-mail: valery@img.tsu.ru

<sup>2</sup> Siberian State Medical University, Tomsk, 634050 Russia

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**Abstract**—The 469+14 G/C (INT4), 1465-85 G/A, and C274T polymorphisms of *NRAMP1* and the A/C polymorphism of *IL12* 3'UTR were analyzed in ethnic Russians with ( $N = 58$ ) or without ( $N = 127$ ) tuberculosis (TB) from Tomsk. On evidence of allele and genotype frequencies, none of the polymorphisms was associated with TB. In the healthy controls, the three *NRAMP1* polymorphisms were in linkage disequilibrium with each other ( $p < 0.001$ ) but not with the *IL12* polymorphism. Still the four polymorphisms are potentially informative as concerns their association with TB.

**Key words:** tuberculosis, susceptibility, candidate gene, polymorphism, *NRAMP1*, *IL12* p40

### INTRODUCTION

Genetic predisposition to tuberculosis (TB) is beyond question now, being supported by the data on animal strains resistant or susceptible to mycobacterial infection [1] and the results of epidemiological [2, 3] and twin [4, 5] studies in humans. Recent molecular genetic analysis has associated TB susceptibility with polymorphisms of several genes [6]. Of these, the best-studied human gene is *NRAMP1*, a homolog of mouse *NRAMP1* coding for a protein responsible for membrane transport of metal ions in macrophage phagolysosomes [7]. A hypothesis has been advanced that susceptibility or resistance to mycobacterial infection are determined by the phagolysosomal ion concentration depending on *NRAMP1* [8]. TB has been linked to chromosome 2q35 containing *NRAMP1* [9]. At present, data are accumulated on the association of *NRAMP1* polymorphisms with TB.

By functional properties, *IL12* is another candidate gene for TB susceptibility. This gene codes for subunit p40 of interleukin 12, a pro-inflammatory cytokine which induces production of  $\alpha$ -interferon to enhance cell immunity against mycobacteria, viruses, and other agents [10]. Rare mutations in the genes for IL12 p40 and for the  $\beta$ 1 subunit of the IL12 receptor are known to cause severe monogenic mycobacterial infection in humans [11]. However, *IL12* has not been tested for association with TB yet. The gene is in chromosome 5q31–33. Transversion from A to C in its 3'-untranslated region (3'UTR) is frequent in various

populations [12]. Hence it is possible to evaluate the contribution of the *IL12* polymorphism to polygenic susceptibility to TB.

In this work, the INT4, 1465-85 G/A, and C274T polymorphisms of *NRAMP1* and the A/C polymorphism of *IL12* 3'UTR were analyzed in TB patients and healthy residents of Tomsk in order to check their informativeness in TB association studies.

### EXPERIMENTAL

**Material.** DNA specimens were obtained from 58 ethnic Russians with active TB (mostly primary TB of intrathoracic lymph nodes or secondary TB of the lungs). Mean age ( $\pm$ SD) of the patients was  $19.3 \pm 15.2$ . The control group included older ethnic Russians without TB record ( $N = 127$ , mean age  $39.1 \pm 13.4$ ). The subjects were unrelated to each other in at least two generations back.

**Genotyping** with respect to 469+14 G/C (INT4), 1465-85 G/A, and C274T polymorphisms of *NRAMP1* and the A/C polymorphism of *IL12* 3'UTR followed published protocols [12, 13].

**Statistical analysis.** Allele and genotype frequencies were checked for deviation from the Hardy–Weinberg equilibrium and compared for the two samples by the Fisher–Irwin exact test [14, 15]. Pairwise linkage disequilibrium analysis was carried out as in [16].

**Table 1.** Polymorphisms of *NRAMP1* and *IL12* in TB patients and healthy controls from Tomsk

Gene	Polymorphism		Patients	Controls	<i>p</i>
<i>NRAMP1</i>	INT4 (469+14 G/C)	GG	36 (62.1)	62 (59.6)	0.820
		GC	22 (37.9)	40 (38.5)	
		CC	0 (0.0)	2 (1.9)	
	1465-85 G/A	G	0.810 ± 0.036	0.788 ± 0.028	0.669
		GG	23 (41.1)	62 (48.8)	0.574
		GA	28 (50.0)	57 (44.9)	
		AA	5 (8.9)	8 (6.3)	
	C274T	G	0.661 ± 0.045	0.713 ± 0.028	0.356
		CC	31 (56.4)	69 (57.0)	0.391
		CT	24 (43.6)	47 (38.9)	
		TT	0 (0.0)	5 (4.1)	
		C	0.782 ± 0.039	0.764 ± 0.027	0.785
<i>IL12</i>	A/C 3'-UTR	AA	36 (64.3)	69 (60.0)	0.547
		AC	16 (28.6)	41 (35.6)	
		CC	4 (7.1)	5 (4.4)	
		A	0.786 ± 0.039	0.778 ± 0.027	0.999

Note: Frequencies of the genotypes and the most common alleles (percentage in parentheses) are given; *p* is the significance level for frequency comparisons between the patients and controls.

RESULTS AND DISCUSSION

Genotyping at the four polymorphisms was inefficient in some cases regardless of the quality of DNA preparations. We failed to establish the genotype at INT4 in 23 controls, at 1465-85 G/A in 2 patients, at C274T in 1 patient and 6 controls, and at 3'UTR A/C in 2 patients and 12 controls (Table 1). Thus, the best results were obtained with 1465-85 G/A. This polymorphism proved to be most informative: the observed heterozygosity (*h* = 0.449) of the control group was close to the maximum possible for a diallelic locus; the heterozygosity in other polymorphisms was lower (0.385 for INT4, 0.388 for C274T, and 0.357 for 3'UTR A/C).

For each polymorphism, genotype frequency distributions in the controls and in the patients obeyed the Hardy–Weinberg equilibrium. No difference in allele or genotype frequencies was observed for the controls and patients, which could be explained by a lack of association between the polymorphisms and TB or by the small sample size (Table 1).

To study the population relationships between the polymorphisms, gametic disequilibrium was computed for pairs of allelic variants in the control sample. The *NRAMP1* polymorphisms proved to be in linkage disequilibrium with each other (Table 2). In all cases, the frequent alleles were in the coupling phase. On the other hand, none of the *NRAMP1* variants was in linkage disequilibrium with the *IL12* polymorphism.

These results are important to minimizing the cost of association studies. Thus, of the three *NRAMP1* polymorphisms, 1465-85 G/A is most informative, best to assay, and thereby most suitable for studying the *NRAMP1* association with TB or other disorders in Siberian Slavs. Since the three polymorphisms are in linkage disequilibrium with each other, analysis of haplotypes, rather than of individual allelic variants, may be more adequate. From this point of view, *IL12* 3'UTR A/C is of importance by itself. The results obtained with Siberian Slavs must be checked with other ethnic groups.

Polymorphism is due to G/C transversion in codon 14 of intron 4 in the case of INT4, a conservative substitution in exon 3 in the case of C274T, and G/A transversion in intron 13 in the case of 1465-85. Along with

**Table 2.** Linkage disequilibrium between each two polymorphisms in healthy Russians from Tomsk

Polymorphism	INT4	1465-85 G/A	C274T	A/C 3'-UTR
INT4	–	0.120	0.150	–0.007
1465-85 G/A	0.000	–	0.112	0.020
C274T	0.000	0.000	–	0.015
A/C 3'-UTR	0.683	0.255	0.374	–

Note: Hill's measure of gametic disequilibrium [16] and the significance level are indicated above and below the central diagonal, respectively.

six other polymorphisms, these ones were revealed in a special study of the *NRAMP1* variation [13]. We chose the polymorphisms that show the highest heterozygosity in Caucasians according to published data.

We could not find any data on the association of C274T or 1465-85 G/A with active TB, while INT4 has been associated with TB in Africans from Gambia [17] and from Guinea [18]. In addition, an association with active TB has been demonstrated for allele 3 of the *NRAMP1* 5'UTR (CT)<sub>n</sub> microsatellite, which is in linkage disequilibrium with INT4 in the Japanese [19]. No association between INT4 and TB has been observed in the Korean population [20]. Since INT4 was not associated with TB in our sample, we think that this polymorphism is not pathogenetically important, and that its association with TB in some populations is due to linkage disequilibrium with other *NRAMP1* polymorphisms, possibly, with 5'UTR (CT)<sub>n</sub>.

The 3'UTR A/C polymorphism of the IL12 p40 gene has recently been studied in healthy people and patients with autoimmune disorders from the United Kingdom, Greece, and Cameroon [12]. Interpopulation variation and a weak association with leukocytosis ( $p = 0.060$ ) have been reported. As far as we know, we were the first to study this polymorphism in TB.

Our results demonstrate that the *NRAMP1* and *IL12* polymorphisms are potentially informative as concerns their association with TB or with pathogenetically important traits. Since the ethnicity is known to play a substantial role in genetic association with complex disorders [21], and TB proved to be associated with INT4 in at least some populations [17–19], our data must be verified with larger samples of Slavs and people of other ethnic groups. Better information might be gained from family studies, e.g., those with the transmission/disequilibrium test [22].

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