

# Association Between Alleles of Cytokine Genes with Rheumatoid Arthritis in Russian Population

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## Abstract

**Background:** Rheumatoid arthritis (RA) is a multi-factor disease with a key role of genetic component in its genesis. Development of osteoporosis and articular destruction is attributed with overexpression of pro-inflammatory cytokines and reduced production of anti-inflammatory ones. In this work probable associations of IFN- $\gamma$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-18 and TNF $\alpha$  alleles with rheumatoid arthritis were studied among Russian ethnic group.

**Methods:** A total of 69 Russian ethnical group patients with RA (cases) and 133 healthy control individuals (controls) were genotyped for variants in 8 cytokines genes (IFN- $\gamma$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-18 and TNF $\alpha$ ). Individual genotype and haplotype frequencies were compared between cases and controls. Odds ratios were calculated with asymptotic 95% confidence intervals and P values less than 0.05 were considered statistically significant.

**Results:** The distribution of studied alleles of cytokine genes in Russian population is similar to other European populations. Genotype, allele and haplotype frequencies were equally distributed between RA cases and controls for IFN- $\gamma$  (rs2430561), IL-1 $\alpha$  (rs1800587), (rs17561), IL-1 $\beta$  (rs1143627), (rs16944), (rs1143634), IL-10 (rs1800871), IL-18 (rs1946518) and TNF $\alpha$  (rs361525). Meanwhile, significant associations ( $p < 0.05$ ) were found between RA cases and controls for IL-4 (rs2243250) T/T, IL-6 (rs1800795) G/G, IL-10 (rs1800872) A/A, IL-10 (rs1800896) G/A, IL-18 (rs187238) G/C and TNF $\alpha$  (rs1800629) G/A.

**Conclusions:** These results indicate that common variants of the IL-4, IL-6, IL-10, IL-18 and TNF $\alpha$  may significantly contribute to RA susceptibility.

**Abbreviations:** CI: Confidence Interval; CIC: Circulatory Immune Complexes; DNA: Deoxyribonucleic Acid; HLA: Human Leukocyte Antigen; IFN: Interferon; Ig: Immunoglobulin; IL: Interleukin; OR: Odds Ratio; PCR: Polymerase Chain Reaction; RA: Rheumatoid Arthritis; RR: Relative Risk; SNP: Single Nucleotide Polymorphism; TNF: Tumor Necrosis Factor

## Background

Rheumatoid arthritis (RA) is a progressive systemic chronic inflammatory autoimmune disease: which is characterised by destruction of joints and sometimes abarticular onsets with prevalence of 1% worldwide. Etiology of A is still unknown: but it is considered to have both a genetic and an environmental basis [1]. Genetic component of A susceptibility was established by data from twin and family studies [2]. And recent twin analysis estimates RA heritability is about 60% [3].

The number of molecular markers of RA increases rapidly [4,5]. Pro-inflammatory cytokines such as TNF- $\alpha$  and L-1 are involved in an inflammatory process: which is partly counterbalanced by anti-inflammatory mediators: such as IL-10 [6]. Polymorphisms in cytokine genes could cause high expression of pro-inflammatory or low expression of anti-inflammatory ones. SNPs of regulator sites or introns influence on transcription by transformation of ranscriptional factor binding sites or enhancer structures. Such positive and negative regulation of cytokine expression plays an important role in development of local inflammations, and is one of RA etiological factors. Main effects of studied cytokines are indicated in Table 1. According to previously published data, distribution and role of FN- $\gamma$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-18 and TNF $\alpha$  in RA development differ from one ethnic or population group to the other [15-19], but no studies have been done in Russian ethnic group. So, the purpose of the research was he studies of frequencies of alleles of above-mentioned cytokines in patients with RA in comparison with healthy

individuals of Russian ethnic group and the search for possible associations between these alleles and RA.

## Methods

### Clinical samples

A total of 69 venous blood samples (obtained from 11 white Caucasian men (Russians) and 58 women at the age of 17-70: average duration of illness is 9 years) with RA and 133 samples from healthy unrelated individuals were used in this study. From all participants an informed consent was obtained. RA was diagnosed according to diagnostic criteria's of the Institute of Rheumatology of Russian Academy of Medical Sciences (RAMS). For genetic analysis 1 ml of venous blood was used.

### SNP selection and genotyping

Functional SNPs in IFN- $\gamma$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-18 and TNF $\alpha$  genes were selected using genotyping data available from the International Hapmap project. Blood samples were collected in EDTA-anticoagulated tubes. DNA was extracted using standard methods.

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Cytokine name	Effects	References
IL-1	Activation of osteoclasts Activation of T-cells Activation of matrix metalloproteinases, destroying cartilage	[7, 8]
TNF- $\alpha$	Activation of osteoclasts, activation of matrix metalloproteinases Increase expression of HLA II molecules by antigen presenting cells Increase expression of intercellular adhesion molecules	[9-11]
IL-6	Activation of osteoclasts Support of B cells differentiation and antibody production	[12]
IL-4	Decrease of pro-inflammatory cytokines production Support of B cells differentiation and antibody production	[13]
IL-10	Decrease of pro-inflammatory cytokines synthesis Support of B cells differentiation and antibody production	[12]
IFN- $\gamma$	Increase expression of HLA II molecules by antigen presenting cells Increase expression of intercellular adhesion molecules Enhancement of T-cell proliferation and Th1 cytokine production	[14]
IL-18	Increase of IFN- $\gamma$ T-cells production	[9-11]

**Table 1:** Key effects of studied cytokines.

Gene (SNP)	Genotype	RA cases, no (%)	Controls, no (%)
<i>IFN-<math>\gamma</math></i> (+874, rs2430561)	A/A	20 (29%)	40 (30%)
	A/T	37 (54%)	64 (48%)
	T/T	12 (17%)	29 (22%)
<i>IL-1<math>\alpha</math></i> (-899, rs1800587)	C/C	36 (52%)	68 (51%)
	T/C	27 (39%)	53 (40%)
	T/T	6 (9%)	12 (9%)
<i>IL-1<math>\alpha</math></i> (+4845, rs17561)	G/G	29 (42%)	66 (50%)
	G/T	32 (46%)	55 (41%)
	T/T	8 (11%)	12 (9%)
<i>IL-1<math>\beta</math></i> (-31, rs1143627)	T/T	22 (32%)	48 (36%)
	T/C	43 (62%)	71 (53%)
	C/C	4 (6%)	14 (11%)
<i>IL-1<math>\beta</math></i> (-511, rs16944)	C/C	17 (25%)	49 (37%)
	T/C	44 (63%)	70 (53%)
	T/T	8 (12%)	14 (10%)
<i>IL-1<math>\beta</math></i> (+3953, rs1143634)	C/C	38 (55%)	84 (63%)
	T/C	28 (41%)	39 (29%)
	T/T	3 (4%)	10 (8%)
<i>IL-4</i> (-590, rs2243250)	C/C	20 (29%)	81 (61%)
	C/T	45 (65%)	48 (36%)
	T/T	4 (6%)	4 (3%)
<i>IL-6</i> (-174, rs1800795)	G/G	31 (45%)	37 (28%)
	G/C	29 (42%)	68 (51%)
	C/C	9 (13%)	28 (21%)
<i>IL-10</i> (-592, rs1800872)	C/C	28 (41%)	84 (63%)
	C/A	33 (48%)	44 (33%)
	A/A	8 (12%)	5 (4%)
<i>IL-10</i> (-819, rs1800871)	C/C	37 (54%)	85 (64%)
	C/T	24 (35%)	43 (32%)
	T/T	8 (11%)	5 (4%)
<i>IL-10</i> (-1082, rs1800896)	G/G	12 (17%)	28 (21%)
	G/A	49 (71%)	70 (53%)
	A/A	8 (12%)	35 (26%)
<i>IL-18</i> (-137, rs187238)	G/G	26 (38%)	70 (53%)
	G/C	43 (62%)	52 (39%)
	C/C	0	11 (8%)
<i>IL-18</i> (-607, rs1946518)	C/C	28 (42%)	45 (34%)
	C/A	29 (41%)	64 (48%)
	A/A	12 (17%)	24 (18%)
<i>TNF<math>\alpha</math></i> (-238, rs361525)	G/G	63 (91%)	129 (97%)
	G/A	6 (9%)	4 (3%)
	A/A	0	0
<i>TNF<math>\alpha</math></i> (-308, rs1800629)	G/G	37 (54%)	99 (74%)
	G/A	32 (46%)	34 (26%)
	A/A	0	0

**Table 2:** Distribution of genotypes among patients with RA and healthy control group.

All SNPs were genotyped in duplicates using SNIptest PCR kits (DNA-Technology JSC, Moscow, Russia). Multiple positive and negative controls were included in all genotyping plates to ensure genotyping data. 10% of genotypes were also confirmed by sequencing. Thermal cycling and genotyping were performed in 384-well plates on DT-384 Real-Time PCR Cycler (DNA-Technology JSC, Moscow: Russia).

### Statistical analysis

$\chi^2$  test, Fisher's exact test with Bonferroni adjustment: odds ratio with confidence interval of 95% (CI=95%) and relative risk (RR) analysis were used. For all statistical analysis Statistica 8.0 (StatSoft, USA) was used.

## Results and Discussions

### Frequencies of some SNPs of cytokine genes in Russian ethnic group

We characterised frequencies of the listed above polymorphisms for the group of patients with RA and healthy individuals of Russian ethnic group (Table 2). The distribution of studied alleles of the control group corresponds to ones in European. The control group is in Hardy-Weinberg proportions due to Pearson's chi-squared test for deviation ( $\chi^2$  values in all alleles are less than 5% significance level).

### SNPs not associated with RA

No significant differences were found between healthy individuals and patients with RA on the following SNPs, A+874T (*IFN- $\gamma$* ), C-899T, G+4845T (*IL-1 $\alpha$* ) and T-31C, C-511T, C+3953T (*IL-1 $\beta$* ), C-819T (*IL-*

*10*), C-607A (*IL-18*), G-238A (*TNF $\alpha$* ). These results corresponds to Tulusso et al., who also showed no associations between RA and T-31C, C-511T (*IL-1 $\beta$* ) [20]. On the other hand: Arman et al. showed association between RA among Turkish population and C+3953T (*IL-1 $\beta$* ) in absence of any with T-31C [15]. Finally, similar to our data, Moreno et al. showed association between RA and C-819T (*IL-10*) for Colombian population [16]. Our data differs from Huang et al., who indicated association between RA and C-607A (*IL-6*) for Chinese population [17]. This difference may be caused by both interethnic and interpopulational differences and shows necessity of similar studies of different populations.

### SNPs associated with RA: *IL-4*

All SNPs associated with RA are indicated in table 3. Among patients with RA frequency of C-590T (*IL-4*) C/C homozygotic variant is two times less than homozygotic T/T and heterozygotic C/T (the

Gene	SNP	P-value	Allele associated with susceptibility (Allele Frequency)	Genotype associated with susceptibility	Odds Ratio (OR) (95% CI)	Relative Risk (RR)	Effect: $\times$ risk for RA
IFN- $\gamma$	A/T (rs2430561)	>0.05	–	–	–	–	–
IL-1 $\alpha$	C/T (rs1800587)	>0.05	–	–	–	–	–
	G/T (rs17561)	>0.05	–	–	–	–	–
IL-1 $\beta$	C/T (rs1143627)	>0.05	–	–	–	–	–
	C/T (rs16944)	>0.05	–	–	–	–	–
	C/T (rs1143634)	>0.05	–	–	–	–	–
IL-4	C/T (rs2243250)	0.0001	T (0.164)	T/T C/T	1.98 (0.4808 to 8.1912) 3.32 (1.8062 to 6.1036)	1.93 1.81	$\times 2.0$ $\times 3.2$
IL-6	G/C (rs1800795)	0.0147	G (0.479)	G/G	2.12 (1.1531 to 3.8852)	1.62	$\times 2.1$
IL-10	C/A (rs1800872)	0.0022	A (0.239)	A/A C/A	3.36 (1.0544 to 10.6909) 1.85 (1.0231 to 3.3605)	3.08 1.45	$\times 3.4$ $\times 1.9$
	C/T (rs1800871)	>0.05	–	–	–	–	–
	G/A (rs1800896)	0.0118	G (0.533)	G/G G/A	2.72 (1.1851 to 6.2574) 2.21 (1.1843 to 4.1053)	1.35 1.19	$\times 2.7$ $\times 2.2$
IL-18	G/C (rs187238)	0.0017	C (0.292)	G/C	2.58 (1.4157 to 4.6878)	1.59	$\times 2.6$
	C/A (rs1946518)	>0.05	–	–	–	–	–
TNF $\alpha$	G/A (rs361525)	>0.05	–	–	–	–	–
	G/A (rs1800629)	0.0028	A (0.217)	G/A	2.52 (1.3643 to 4.6483)	1.81	$\times 2.5$

Table 3: Association between rheumatoid arthritis and investigated SNPs.

Gene	Genotype	RA patients, no. (%)	Controls, no. (%)	Alleles associated with susceptibility (P-value)
-590 (IL-4) and -137 (IL-18)	CC GG	9 (13%)	46 (35%)	590T and 137C (0.01955)
	TT GG	3 (4%)	1 (1%)	
	CT GG	14 (20%)	23 (17%)	
	CC GC	11 (16%)	31 (23%)	
	TT GC	1 (1%)	3 (2%)	
	CT GC	31 (45%)	18 (14%)	
	CC CC	0	4 (3%)	
	TT CC	0	0	
	CT CC	0	7 (5%)	
-819 (IL-10) and -592 (IL-10)	CC CC	28 (41%)	84 (63%)	819C and 592A (0.00754)
	TT CA	0	0	
	CT CA	24 (35%)	43 (32%)	
	CC CA	9 (13%)	1 (1%)	
	CC AA	0	0	
	TT CC	0	0	
	TT AA	8 (11%)	5 (4%)	
	CT AA	0	0	
	CT CC	0	0	
-238 (TNF $\alpha$ ) and -590 (IL-4)	GG CC	17 (25%)	79 (59%)	238A and 590T (0.0311)
	GA CC	3 (4%)	2 (1%)	
	GG CT	42 (61%)	46 (34%)	
	GA CT	3 (4%)	2 (1%)	
	GG TT	4 (6%)	4 (3%)	
	GATT	0	0	
-308 (TNF $\alpha$ ) and -590 (IL-4)	GG CC	12 (17%)	61 (45%)	308A and 590T (0.0361)
	GA CC	8 (12%)	20 (15%)	
	GG CT	23 (33%)	36 (27%)	
	GA CT	22 (32%)	12 (9%)	
	GG TT	2 (3%)	2 (2%)	
	GATT	2 (3%)	2 (2%)	
-308 (TNF $\alpha$ ) and -592 (IL-10)	GG CC	19 (27%)	56 (42%)	308A and 592A (0.00471)
	GA CC	9 (13%)	28 (21%)	
	GG CA	14 (20%)	38 (29%)	
	GA CA	19 (27%)	6 (4%)	
	GA CA	4 (6%)	5 (4%)	
	GAAA	4 (6%)	0	
-308 (TNF $\alpha$ ) and -137 (IL-18)	GG GC	20 (29%)	42 (32%)	308A and 137C (0.0294)
	GA GC	23 (33%)	10 (7%)	
	GG GG	17 (25%)	51 (38%)	
	GA GG	9 (13%)	19 (14%)	
	GG CC	0	6 (5%)	
	GA CC	0	5 (4%)	

**Table 4:** Presence of combined genotypes among patients with RA and healthy individuals, and their significant association with RA ( $p < 0.05$ ).

most significant association,  $p < 0.0001$ ). At the same time, T/T and C/T genotypes are high risk factors. Allele T is associated with higher expression rate of *IL-4* in comparison with allele C [21]. *IL-4* provides activation of antigen-detection cell properties by increasing the level of HLA-DR antigens and intensive production of different immunoglobulines, sometimes resulting hypergammaglobulinemia. Our results on association between RA and allele T corresponds to findings of Moreno et al. in Columbian ethnic group [22].

#### SNPs associated with RA: *IL-6*

For polymorphism G-174C (*IL-6*) a significant ( $p = 0.0147$ ) association was discovered for G/G genotype. Presence of G allele is associated with overexpression of *IL-6* [23]. *IL-6* is a pro-inflammatory cytokine, and its overexpression may be one of etiological factors of RA. A capacity of *IL-6* to regulate B-cells differentiation may cause overexpression of rheumatoid factors (and subsequent hypergammaglobulinemia). These autoantibodies (Rheumatoid factors and Ig) can form circulating immune complexes (CIC) that sediment in synovial membranes of joints and provoke pathological process. Moreover, *IL-6*, equally with *IL-1*, takes part in osteoporosis development and its overexpression cause destruction of bones. So, G allele in G-174C polymorphism (*IL-6*) may be an RA risk factor.

Earlier researches discovered no association between G-174C (*IL-6*) and RA for patients from both Spain and Sweden [24,25]. But according to Huang et al. report, this association is significant for Chinese population [17].

#### SNPs associated with RA: *IL-10*

Differences between frequencies of C-592A and G-1082A (*IL-10*) among patients with RA and the control group are significant ( $p = 0.0022$ ), so these polymorphisms are associated with RA in the studied ethnic group. It should be noticed, that presence of A/A genotype of C-592A (*IL-10*) or A/A genotype of G-1082A (*IL-10*) is high risk factors of the pathology. It has been shown: that C-592A and G-1082A (*IL-10*) impact *IL-10* production. According to published results [26,27], alleles A of both polymorphisms (C-592A and G-1082A) are associated with lower *IL-10* expression than alleles C. *IL-10* is an anti-inflammatory interleukin that decreases production of inflammatory interleukins, increases production of *IL-1* receptor antagonist and decreases adhesion of leucocytes to *IL-1*-activated endothelial cells. It is supposed that insufficient production of *IL-10* may support RA development by low inhibition of production of inflammatory cytokines. So, A alleles of both C-592A and G-1082A (*IL-10*) may be RA risk factors. On the other hand, according to previous

research on Columbian patients with RA [16], no associations were discovered between C-592A and G-1082A polymorphisms and RA.

### SNPs associated with RA: *IL-18*

According to received data: G-137C (*IL-18*) is significantly associated with RA. G allele in G-137C polymorphism is related to a high risk of RA development. G-137C is located in a non-coding region of *IL-18* and is responsible for cytokine expression. G allele is related to low transcription rate of *IL-18* [28,29]. *IL-18* has an anti-inflammatory activity by suppression of macrophage secretion of IL-1, TNF $\alpha$  and IL-6, preventing migration of neutrophils to an inflamed tissue, decreasing adhesion of leucocytes to endothelial cells. Because of these functions low production of *IL-18* must support RA development and G in G-137C site is a high risk factor of the disease. Interestingly, in the study of Chinese population no association of G-137C (*IL-18*) and RA was discovered [17].

### SNPs associated with RA: *TNF $\alpha$*

We discovered a strong association between G-308A (*TNF $\alpha$* ) and RA. G-308A (*TNF $\alpha$* ) is located in a non-coding region and is related to cytokine expression rate. Single nucleotide replacement of A instead of G in -308 position is associated with TNF $\alpha$  overexpression [30-32]. TNF $\alpha$  is a member of pro-inflammatory cytokines and provides the most important functions during the beginning of inflammation; it activates leukocytes, assists adhesion and transmigration of leucocytes to the inflamed tissue. It also stimulates differentiation and proliferation of B-cells: activates transcription of other anti-inflammatory cytokines genes. TNF $\alpha$  overexpression may lead to RA development by initiation and support of inflammation process. The role of TNF $\alpha$  in RA development may also be connected with its ability to induce osteoclasts and chondrocytes activation. According to earlier studies of Holland and French patients with RA, there is no association between G-308A (*TNF $\alpha$* ) and RA [33,34]. Nevertheless: for Ireland patients this association was reported [35].

### Combined genotypes associated with RA

We also conducted an analysis of frequencies of studied cytokines genotypes combinations among patients with RA and the control group (see tables 4). The following combinations of genotypes are significantly associated ( $p < 0.05$ ) with RA, -590 C/T (*IL-4*) & -137 G/C (*IL-18*); -819 C/C (*IL-10*) & -592 C/A (*IL-10*); -238 G/A (*TNF $\alpha$* ) & -590 C/T (*IL-4*); -308 A (*TNF $\alpha$* ) & -592 A (*IL-10*); -308 G/A (*TNF $\alpha$* ) & -137 G/C (*IL-18*); -308 G/A (*TNF $\alpha$* ) & -590 C/T (*IL-4*).

### Conclusions

We conclude that four genotypes and six combinations of genotypes of studied cytokines are associated with RA in Russian ethnic group.

### Competing Interests

The authors declare that they have no competing interests.

### Authors' Contributions

NES carried out the molecular genetic studies, participated in the primer design and drafted the manuscript. SAM participated in the design of the study and performed the statistical analysis. VVI participated in the data analysis and helped to draft the manuscript. DYT participated in the design of the study. DVR conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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