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Original article

The immune response mediator genes polymorphic variants as predictors of the etanercept efficacy in juvenile idiopathic arthritis

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Abstract: *Objective* — The aim of the study was to investigate the relationship of the alleles and genotypes of the immune response mediator genes polymorphic loci (rs1800629, rs909253, rs16944, rs6822844, rs2104286, rs1800795, rs1800872, rs3087243, rs755622 rs28362491, rs2240336, rs2476601) with the etanercept efficacy in juvenile idiopathic arthritis (JIA) patients.
Material and Methods — The study included 39 JIA patients from Bashkortostan, Russia. Achieving the American College of Rheumatology Pediatric 70 (ACR Pedi 70) response was regarded as the presence of the response to etanercept (otherwise – as the absence), while achieving clinical remission on medication – as the sufficient response (otherwise – as the insufficient). Genotyping was performed using real-time polymerase chain reaction method.
Results — The predictors of an increased risk of the non-response to etanercept were the *IL1B* rs16944*TT (p_{cor}=0.023), *NFKB1* rs28362491*II (p_{cor}=0.042) genotypes, and of the increased risk of the insufficient response to etanercept – the *IL2RA* rs2104286*AA (p_{cor}=0.010), *NFKB1* rs28362491*II (p_{cor}=0.026) genotypes. The markers of the decreased risk of the non-response to etanercept were the *IL1B* rs16944*C (p_{cor}=0.046), *NFKB1* rs28362491*D (p_{cor}=0.029) alleles, and of the decreased risk of the insufficient response to etanercept – the *IL2RA* rs2104286*AG genotype (p_{cor}=0.049), *IL2RA* rs2104286*G allele (p_{cor}=0.005).
Conclusion — In this study the association of the alleles and genotypes of the *IL1B* rs16944, *IL2RA* rs2104286, *NFKB1* rs28362491 polymorphic loci with the etanercept efficacy in JIA patients was established.

Keywords: juvenile idiopathic arthritis, polymorphic loci, etanercept efficacy, predictors.

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Introduction

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in children. The disease has no known cause, develops before the 16th birthday and is characterized by persistent joint inflammation (longer than 6 weeks) [1-3].

It was shown, that JIA can lead to severe disability and is accompanied by a significant impairment in the quality of life of patients [1, 4]. The important role in the preventing of JIA progression and patients disability is given to the timely appointment of an adequate therapy [5-8].

The main therapeutic agents for the JIA treatment include nonbiologic and biologic disease-modifying antirheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticosteroids, but their effectiveness is different in patients [7, 8]. Therefore, it is an essential problem to find the predictors of the corresponding drugs efficacy, primarily for the DMARDs.

According to the American College of Rheumatology (ACR) recommendations for the treatment of JIA (2011), three tumor necrosis factor alpha (TNFα) inhibitors (etanercept, adalimumab and infliximab) are recommended for the patients with an active arthritis and an insufficient response to the previous therapy [7].

Etanercept is a fully humanized soluble TNF receptor, which binds to TNFα and attenuates its effects [1]. Many cytokines, including TNFα, lymphotoxin alpha (LTα), macrophage migration inhibitory factor (MIF), interleukins (ILs), and other regulatory molecules (such as cytotoxic T-lymphocyte associated protein 4 (CTLA4), nuclear factor kappa B subunit 1 (NF-κB1), protein tyrosine phosphatase, non-receptor type 22 (PTPN22)), as well as their genes polymorphisms are believed to play an important role in JIA pathogenesis and the disease progression [9-11]. The complex interaction of immune cells and mediators determines the specific clinical manifestations of JIA [12]. Thus, it can be assumed, that the changes in the regulatory molecules production and underlying genetic factors also affect the treatment effectiveness in JIA.

The aim of the study was to investigate the relationship of the alleles and genotypes of the immune response mediator genes polymorphic loci (*TNFA* rs1800629 (-308G>A), *LTA* rs909253

(252A>G), *IL1B* rs16944 (-511C>T), *IL2RA* rs2104286, *IL6* rs1800795 (-174G>C), *IL10* rs1800872 (-592C>A), *CTLA4* rs3087243, *MIF* rs755622 (-173G>C), *NFKB1* rs28362491 (-94I>D), *PADI4* rs2240336, *PTPN22* rs2476601 (1858G>A)) and the intergenic region locus (*IL2-IL21* rs6822844) with the etanercept efficacy in JIA patients.

Material and Methods

Study design

A case-control study was conducted. The study was approved by Local ethical committee of Bashkir State Medical University (Ufa, Russia). The parents of all patients signed the voluntary informed consent.

JIA patients' characteristics

Initially the whole JIA group included 330 children, who underwent examination and treatment in the Republican Children's Clinical Hospital (Ufa, Russia) in 2012-2017 years. The JIA diagnosis was established according to the International League of Associations for Rheumatology (ILAR) criteria [3].

The inclusion criteria to the JIA group were:

i) the presence of arthritis;
ii) the duration of arthritis more than 6 weeks;
iii) the patient's age less than 18 years;
iv) the onset of the disease at the age less than 16 years;
v) the absence of other diseases accompanied by arthritis;
vi) the signing of the voluntary informed consent by the patient's parents.

The exclusion criteria were:

i) the duration of arthritis less than 6 weeks;
ii) the patient's age 18 years and over;
iii) the onset of the disease at the age 16 years and over;
iv) the established diagnosis of other diseases accompanied by inflammation in the joints;
v) the refusal to participate in the study by the patient or his parents.

The etanercept therapy (in a combination with methotrexate) was prescribed to 48 patients. The efficacy of the therapy was assessed in 39 JIA patients aged 1.9 to 16.7 years. The mean age of 39 examined JIA patients was 8.4±3.7 years, girls/boys ratio – 1.8/1.0.

According to the ILAR criteria, the following JIA subtypes were presented: systemic arthritis (n=3), rheumatoid factor positive polyarthritis (n=3), rheumatoid factor negative polyarthritis (n=16), persistent oligoarthritis (n=1), extended oligoarthritis (n=9), enthesitis related arthritis (n=5), psoriatic arthritis (n=1), undifferentiated arthritis (n=1). The duration of the etanercept treatment was from 8 months to 7 years. Achieving the ACR Pediatric 70 (ACR Pedi 70) response was regarded as the presence of the response to etanercept (otherwise – as the absence), while achieving clinical remission on medication (Wallace et al., 2011) was regarded as the sufficient response to etanercept (otherwise – as the insufficient) [1, 6, 13-15]. The presence of the response to etanercept was observed in 27 patients (69.23%), while the sufficient response – in 21 patients (53.85%).

Experimental methods

Deoxyribonucleic acid (DNA) was isolated from the lymphocytes of the whole blood samples using standard phenol-chloroform method [16].

Twelve polymorphic loci (*TNFA* rs1800629 (-308G>A), *LTA* rs909253 (252A>G), *IL1B* rs16944 (-511C>T), *IL2-IL21* rs6822844, *IL2RA* rs2104286, *IL6* rs1800795 (-174G>C), *IL10* rs1800872 (-592C>A), *CTLA4* rs3087243, *MIF* rs755622 (-173G>C), *NFKB1* rs28362491 (-94I>D), *PADI4* rs2240336, *PTPN22* rs2476601 (1858G>A)) were examined. The genotyping was performed by real-time polymerase chain reaction (PCR) method using StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA) and commercial kits of sequence-specific primers and allele-specific probes (DNK-syntez, Russia).

Statistical analysis

The differences between the frequencies of the polymorphic loci alleles and genotypes in the studied groups were assessed using two-tailed Fisher's exact test in Microsoft Excel software. The odds ratio (OR) with 95% Baptista-Pike confidence interval (CI) were calculated for the identified markers in Microsoft Excel and R v.3.4.2 software [17].

The models of inheritance (co-dominant, dominant, recessive, over-dominant and log-additive) were studied by applying logistic regression in the SNPStats package [18]. The best model was the one with the lowest value of the Akaike information criterion (AIC). For the multiple comparison correction the permutation test with 10,000 permutes was performed in PowerMarker v.3.25 package (p_{cor}) [19, 20].

In all the cases the results considered statistically significant at $p<0.05$.

Results

Genetic predictors of the non-response to etanercept

As a result of the comparative analysis it was shown, that the *IL1B* rs16944*TT genotype was significantly more common and the *IL1B* rs16944*C allele – significantly less common in JIA patients with the absence of the response to etanercept, than in those with its presence (*TT: p=0.025, p_{cor}=0.023, OR=13.00, 95% CI 1.57-163.39; *C: p=0.044, p_{cor}=0.046, OR=0.33, 95% CI 0.13-0.89) (*Table* 1). The best inheritance model was the recessive (TT vs. CC+CT, p=0.014, OR=13.0, 95% CI 1.26-133.64). Due to the small sample size, the stratification by sex was not carried out.

The *NFKB1* rs28362491 polymorphism analysis showed that the frequency of the *NFKB1* rs28362491*II genotype was significantly higher, and the frequency of the *NFKB1* rs28362491*D allele was significantly lower in etanercept non-responders, than in responders (*II: p=0.043, p_{cor}=0.042, OR=5.75, 95% CI 1.28-22.26; *D: p=0.028, p_{cor}=0.029, OR=0.31, 95% CI 0.12-0.85) (*Table* 1). The log-additive model of inheritance was the best (2DD+ID vs. II, p=0.016, OR=0.27, 95% CI 0.08-0.87).

For the other single nucleotide polymorphisms (SNPs) the differences were not significant ($p_{cor}>0.05$). There was only a trend towards a lower frequency of the *IL2RA* rs2104286*G allele in JIA patients who did not respond to etanercept therapy in comparison with the responders (p=0.092, p_{cor}=0.093).

Table 1. The distribution of the genotypes and alleles of the studied polymorphic loci in relation to the response to etanercept in JIA patients

Polymorphic loci		Response to etanercept									
		Absence		Presence		p-level	Insufficient		Sufficient		p-level
Gene, rs	Variants	Abs.	Freq. (%)	Abs.	Freq. (%)		Abs.	Freq. (%)	Abs.	Freq. (%)	
TNFA	GG	12	100.00	23	85.19	0.292	16	88.89	19	90.48	1.000
rs1800629	GA	0	0.00	4	14.81	0.292	2	11.11	2	9.52	1.000
	AA	0	0.00	0	0.00	1.000	0	0.00	0	0.00	1.000
	G	24	100.00	50	92.59	0.306	34	94.44	40	95.24	1.000
	A	0	0.00	4	7.41	0.306	2	5.56	2	4.76	1.000
LTA	AA	6	50.00	11	40.74	0.730	8	44.44	9	42.86	1.000
rs909253	AG	5	41.67	14	51.85	0.731	8	44.44	11	52.38	0.751
	GG	1	8.33	2	7.41	1.000	2	11.11	1	4.76	0.586
	A	17	70.83	36	66.67	0.797	24	66.67	29	69.05	1.000
	G	7	29.17	18	33.33	0.797	12	33.33	13	30.95	1.000
IL1B	CC	2	16.67	11	40.74	0.269	4	22.22	9	42.86	0.307
rs16944	CT	6	50.00	15	55.56	1.000	10	55.56	11	52.38	1.000
	TT	4	33.33	1	3.70	**0.025**	4	22.22	1	4.76	0.162
	C	10	41.67	37	68.52	**0.044**	18	50.00	29	69.05	0.107
	T	14	58.33	17	31.48	**0.044**	18	50.00	13	30.95	0.107
IL2-21	GG	11	91.67	19	70.37	0.228	14	77.78	16	76.19	1.000
rs6822844	GT	1	8.33	8	29.63	0.228	4	22.22	5	23.81	1.000
	TT	0	0.00	0	0.00	1.000	0	0.00	0	0.00	1.000
	G	23	95.83	46	85.19	0.261	32	88.89	37	88.10	1.000
	T	1	4.17	8	14.81	0.261	4	11.11	5	11.90	1.000
IL2RA	**AA**	11	91.67	18	66.67	0.131	17	94.44	12	57.14	**0.011**
rs2104286	**AG**	1	8.33	7	25.93	0.394	1	5.56	7	33.33	**0.049**
	GG	0	0.00	2	7.41	1.000	0	0.00	2	9.52	0.490
	A	23	95.83	43	79.63	0.092	35	97.22	31	73.81	**0.004**
	G	1	4.17	11	20.37	0.092	1	2.78	11	26.19	**0.004**
IL6	GG	7	58.33	12	44.44	0.501	10	55.56	9	42.86	0.527
rs1800795	GC	5	41.67	13	48.15	0.742	8	44.44	10	47.62	1.000
	CC	0	0.00	2	7.41	1.000	0	0.00	2	9.52	0.490
	G	19	79.17	37	68.52	0.420	28	77.78	28	66.67	0.321
	C	5	20.83	17	31.48	0.420	8	22.22	14	33.33	0.321
IL10	CC	5	41.67	14	51.85	0.731	8	44.44	11	52.38	0.751
rs1800872	CA	6	50.00	9	33.33	0.478	9	50.00	6	28.57	0.203
	AA	1	8.33	4	14.81	1.000	1	5.56	4	19.05	0.349
	C	16	66.67	37	68.52	1.000	25	69.44	28	66.67	0.813
	A	8	33.33	17	31.48	1.000	11	30.56	14	33.33	0.813
MIF	GG	6	50.00	15	55.56	1.000	10	55.56	11	52.38	1.000
rs755622	GC	5	41.67	11	40.74	1.000	6	33.33	10	47.62	0.516
	CC	1	8.33	1	3.70	0.526	2	11.11	0	0.00	0.206
	G	17	70.83	41	75.93	0.779	26	72.22	32	76.19	0.796
	C	7	29.17	13	24.07	0.779	10	27.78	10	23.81	0.796
CTLA4	GG	5	41.67	11	40.74	1.000	8	44.44	8	38.10	0.752
rs3087243	GA	6	50.00	16	59.26	0.730	9	50.00	13	61.90	0.528
	AA	1	8.33	0	0.00	0.308	1	5.56	0	0.00	0.462
	G	16	66.67	38	70.37	0.794	25	69.44	29	69.05	1.000
	A	8	33.33	16	29.63	0.794	11	30.56	13	30.95	1.000
NFKB1	**II**	6	50.00	4	14.81	**0.043**	8	44.44	2	9.52	**0.025**
rs28362491	ID	5	41.67	15	55.56	0.501	6	33.33	14	66.67	0.056
	DD	1	8.33	8	29.63	0.228	4	22.22	5	23.81	1.000
	I	17	70.83	23	42.59	**0.028**	22	61.11	18	42.86	0.119
	D	7	29.17	31	57.41	**0.028**	14	38.89	24	57.14	0.119
PADI4	GG	3	25.00	7	25.93	1.000	4	22.22	6	28.57	0.726
rs2240336	GA	7	58.33	17	62.96	1.000	11	61.11	13	61.90	1.000
	AA	2	16.67	3	11.11	0.634	3	16.67	2	9.52	0.647
	G	13	54.17	31	57.41	0.809	19	52.78	25	59.52	0.648
	A	11	45.83	23	42.59	0.809	17	47.22	17	40.48	0.648
PTPN22	GG	10	83.33	19	70.37	0.693	14	77.78	15	71.43	0.726
rs2476601	GA	2	16.67	7	25.93	0.693	4	22.22	5	23.81	1.000
	AA	0	0.00	1	3.70	1.000	0	0.00	1	4.76	1.000
	G	22	91.67	45	83.33	0.487	32	88.89	35	83.33	0.533
	A	2	8.33	9	16.67	0.487	4	11.11	7	16.67	0.533

Statistically significant results are **in bold**. Abs., absolute values; Freq., frequencies.

Genetic predictors of the insufficient response to etanercept

It was shown, that the *IL2RA* rs2104286*AA genotype was significantly more common, while the *IL2RA* rs2104286*AG genotype and the *IL2RA* rs2104286*G allele were significantly less common in JIA patients with the insufficient response to etanercept, than in those with the sufficient response (*AA: p=0.011, p_{cor}=0.010, OR=12.75, 95% CI 1.84-146.67; *AG: p=0.049, p_{cor}=0.049, OR=0.12, 95% CI 0.01-0.88; *G: p=0.004, p_{cor}=0.005, OR=0.08, 95% CI 0.01-0.53) (*Table* 1). The log-additive model described the results better than the others (2GG+AG vs. AA, p=0.0037, OR=0.09, 95% CI 0.01-0.81).

Analysis of the *NFKB1* rs28362491 polymorphism revealed a significant increase of the *NFKB1* rs28362491*II genotype proportion, and a trend towards a decrease of the *NFKB1* rs28362491*ID genotype proportion in JIA patients who did not achieve clinical remission on medication (on etanercept), compared with those who achieved (*II: p=0.025, p_{cor}=0.026, OR=7.60, 95% CI 1.53-38.68 and *ID: p=0.056, p_{cor}=0.054) (*Table* 1). The best inheritance model was the dominant (ID+DD vs. II, p=0.011, OR=0.13, 95% CI 0.02-0.74).

At the same time, for the other SNPs no significant differences were observed (p_{cor}>0,05). Only testing the inheritance models revealed a trend towards the presence of an effect, that increases the risk of the insufficient response to etanercept, in the *IL1B* rs16944*T allele (log-additive model, 2TT+CT vs. CC, p=0.063) and the *MIF* rs755622*CC genotype (recessive model, CC vs. GG+GC, p=0.073).

Discussion

The analysis of the association between the polymorphic variants of the immune response mediator genes and the efficacy of the etanercept therapy in JIA patients was performed in this study. The predictors of the increased risk of the non-response to etanercept were the *IL1B* rs16944*TT (p_{cor}=0.023), *NFKB1* rs28362491*II (p_{cor}=0.042) genotypes, and of the increased risk of the insufficient response to etanercept – the *IL2RA* rs2104286*AA (p_{cor}=0.010), *NFKB1* rs28362491*II (p_{cor}=0.026) genotypes. The markers of the decreased risk of the non-response to etanercept were the *IL1B* rs16944*C (p_{cor}=0.046), *NFKB1* rs28362491*D (p_{cor}=0.029) alleles, and of the decreased risk of the insufficient response to etanercept – the *IL2RA* rs2104286*AG genotype (p_{cor}=0.049), *IL2RA* rs2104286*G allele (p_{cor}=0.005).

According to the literature, the association of only the *TNFA* rs1800629 locus polymorphic variants was previously investigated with the etanercept efficacy in JIA. Schmeling H. and Horneff G. (2007) showed that the *TNFA* rs1800629*GG genotype serves as a protective marker in relation to non-achieving the ACR Pedi 30 response to etanercept in patients with rheumatoid factor negative polyarticular JIA, but not in the entire JIA group [21]. According to Basic J. et al. (2010), the ACR Pedi 50 response in a year after the etanercept initiation was observed significantly more frequent in polyarticular JIA course patients with the *TNFA* rs1800629*GG genotype, than in those with the *TNFA* rs1800629*AA genotype, but not with the *TNFA* rs1800629*A allele generally [22]. Cimaz R. et al. (2007) did not find the relationship of the *TNFA* rs1800629 locus polymorphic variants with achieving the ACR Pedi 30 response to TNFα inhibitors as a whole (infliximab, etanercept, adalimumab) in JIA patients [23]. These data are consistent with the results of the present work, where no association of the *TNFA* rs1800629 locus polymorphic variants with the response to the etanercept therapy in the entire JIA group was found. Nevertheless, Hong Y. and Wang R. (2016) showed, that the frequency of the *TNFA* rs1800629*GG genotype was significantly increased in Chinese JIA patients achieved the ACR Pedi 50 response with the etanercept therapy [24].

It should be noted, that according to Sode J. et al. (2014), the *NFKB1* rs28362491*D allele serves as a protective marker in relation to the non-response (European League Against Rheumatism (EULAR) criteria) to etanercept in seropositive rheumatoid arthritis patients from Denmark [25]. In addition, Gębura K. et al. (2017) showed, that the presence of the homozygous genotype *NFKB1* rs28362491*II was associated with the increased risk of the non-response (EULAR criteria) to TNFα inhibitors (as a whole), whereas the presence of the *NFKB1* rs28362491*D allele, and in particular the *NFKB1* rs28362491*ID genotype, – with a higher efficacy of this treatment in rheumatoid arthritis patients from Poland [26]. The results of the current work also indicate, that the *NFKB1* rs28362491*D allele reduces the risk of non-achieving the ACR Pedi 70 response to etanercept in JIA patients.

Conclusion

In this study the association of the alleles and genotypes of the *IL1B* rs16944, *IL2RA* rs2104286, *NFKB1* rs28362491 polymorphic loci with the etanercept efficacy in JIA patients was established.

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Conflict of interest: none declared.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the standards of the Local ethical committee of Bashkir State Medical University (Ufa, Russia) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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