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Original Article

The potential of TNF and TNFRSF1B gene polymorphism in predicting the clinical response of anti-TNF therapy in patients with juvenile idiopathic arthritis

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Abstract: Juvenile idiopathic arthritis (JIA) is the most frequent rheumatic disease in children and could lead to severe disability. The aim of this study was to determine the role of gene polymorphisms in predicting the clinical response of anti-TNF therapy in JIA patients. 68 patients with JIA and 20 healthy controls (HC) were included in the study, and patients with JIA received anti-TNF treatment for 24 weeks. Genomic DNA was extracted from peripheral blood of the subjects and five potentially functional SNPs were selected for genotyping (TNF-308 (rs1800629), TNF-238 (rs361525), TNFRSF1B (rs1061622), TRAF1/C5 (rs3761847), and PTPN22 (rs2488457)) by PCR methods. No significant genotype frequency differences of TNF-308 (rs1800629), TNF-238 (rs361525), TNFRSF1B (rs1061622), TRAF1/C5 (rs3761847), and PTPN22 (rs2488457) were found between JIA patients and HC. Genotype of TNF-308 GG (rs1800629) and TNFRSF1B TT (rs1061622) were observed to be increased in responders group compared with non-responders group (P=0.034, P=0.048, respectively). Furthermore, univariable logistic regression revealed that TNF-308 A allele (rs1800629) and TNFRSF1B G allele (rs1061622) were risk factors for clinical response (OR: 0.328, 95% CI: 0.117-0.914, P=0.033; OR: 0.387, 95% CI: 0.142-1.055, P=0.063, respectively). However, only TNF-308 A allele (rs1800629) was independent risks for clinical response by multivariable logistic regression (OR: 0.354, 95% CI: 0.142-0.885, P=0.026), while TNFRSF1B G allele (rs1061622) might be potential risks (OR: 0.418, 95% CI: 0.155-1.130, P=0.086). TNF-308 (rs1800629) and TNFRSF1B (rs1061622) variants were potential biomarkers of clinical response in JIA patients treated by anti-TNF drugs.

Keywords: Juvenile idiopathic arthritis, anti-TNF therapy, TNF-308, TNFRSF1B

Introduction

Juvenile idiopathic arthritis (JIA) is a chronically inflammatory autoimmune disease, which is usually affecting children below 16 years old and characterized by chronic inflammation of one or even more joints [1-3]. The main clinical presentations of JIA often are dominant by joint pain, stiffness and swelling, which persist for more than 6 weeks [4]. And the pathological features of JIA are chronic synovial inflammation with high levels of pro-inflammatory cytokines in peripheral blood and synovial fluid [5]. The prevalence of JIA varies in different geographical locations, ethnic and populations, however, the exact etiology of JIA is still obscure [6]. Previous study has demonstrated that the etiology is associated with genetic and environmental factor [7].

Studies have reported that response to treatment in patients with JIA can be affected by genetic and biological factors [6]. Genome-wide association studies (GWAS) has revealed numerous regions along the genome are associated with drug response to different treatments in JIA. Moreover, single nucleotide polymorphisms (SNP), which locate in gene coding or no-coding region, are the variation in a single nucleotide. Each variation is present within a population to some appreciable extent [8]. Data have been reported that SNP is related with disease severity and response to treatment [9]. Recent years some studies begin to define the potential role of polymorphisms in JIA, and aim to guide proper treatment of JIA, and to identify correlation of polymorphisms with therapeutic response [10]. Previous research has found that gene polymorphisms of ABCB1 and ABCC3

in patients with JIA are correlated with response to methotrexate [11], and some SNPs of the TNF-α gene have been identified to be related with disease phenotype and or clinical outcome [12]. However, the role of SNPs in response to anti-TNF treatment is stile obscure.

In this current study, we aimed to determine the phenotype of five SNPs in JIA, and analyze the influence of SNPs on response to anti-TNF treatment in patients with JIA.

Materials and methods

Patients

68 patients with JIA (25 males and 43 females) aged 8.9-15.0 years at the Department of Paediatrics, Central Hospital of Wuhan (Wuhan, China) from 2013/11/01 to 2015/10/31, were enrolled in this study according to the International League of Associations for Rheumatology (ILAR) criteria [13]. And a total of 20 healthy volunteers (7 males and 13 females) aged 9.1-15.0 years with sex and age matched were also recruited in this study and served as a control group. The study was approved by the Institutional Review Board for Clinical Research Central Hospital of Wuhan. Written informed consent was also obtained from all subjects before initiating the study protocol.

SNPs selection and genotyping

Five potentially functional SNPs (TNF-308 (rs1800629), TNF-238 (rs361525), TNFRSF1B (rs1061622), TRAF1/C5 (rs3761847), and PTPN22 (rs2488457)) were selected for genotyping in DNA according to previous studies, which have reported that these SNPs were associated with the diagnosis and/or prognosis of patients with JIA or other inflammation diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and osteoarthritis (OA) [14-17]. EDTA-anticoagulant peripheral blood samples were obtained from patients with JIA and HC. Genomic DNA was extracted from whole blood samples with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and stored at -80°C until used. PCR analysis was performed in 25 μl reaction volume containing 50 ng DNA, using KAPA Taq DNA polymerase (94°C for 9 min, 94°C for 30 s with 30 cycles, 63°C for 30 s, 72°C for 8 min). The amplification products were analyzed with the ABI Prism 7900HT detection system using the SDS 2.4 software.

Response to treatment

68 subjects with JIA were received treatment of etanercept (administered by subcutaneous injection twice weekly at the dose of 0.4 mg/kg for 24 weeks), which is a biologic agent (TNF inhibitor). After 24 weeks of treatment of TNF inhibitor, the effect of treatment was evaluated according to the ACR pediatric response criteria, and the JIA patients were divided into responders and non-responders according to the ACRpedi 50 improvement criteria [18]. The definition of ACRpedi 50 was depending on 50% improvement from baseline in at least 3 of any 6 variables of the JIA core set, with no more than 1 variable worsening by more than 30% [19]. The 6 variables were as follows: physician's and parent's evaluation, childhood health assessment questionnaire (CHAQ), number of joints with active arthritis and number of joints with limited range of motion (LOM), ESR. Moreover, other characteristics (e.g., disease duration, CRP, physician's global assessment of disease activity, Parents' global assessment of overall well-being, and patient (parent) evaluation of pain), concomitant medications, JIA subtype were also evaluated and included in this study.

Statistically analysis

Statistical analysis was performed using SPSS V.20.0 (SPSS, Chicago, Illinois, USA). Data are presented as median (25^{th} and 75^{th} IQR) or counts (percentages), and significance of the comparison was determined by the Mann-Whitney test and the Chi-square test. Univariable logistic regression with additive model was performed to evaluate whether the TNF-308 A (rs1800629) and TNFRSF1B G allele (rs1061622) could predict the clinical response, while multivariable logistic regression was completed to identify whether they were independent factors to predict the clinical response. A p value <0.05 was considered statistically significant and p value <0.01 as highly significant.

Results

Association of SNPs with risk of JIA

The basic Characteristics of 68 patients with JIA and 20 age and gender matched healthy controls were demonstrated in **Table 1**. All

Table 1. Characteristics of juvenile idiopathic arthritis (JIA) patients and health controls

Parameters	JIA patients (n=68)	Health Controls (n=20)	*p* Value
Age (years)	11.8 (8.9-15.0)	12.1 (9.1-15.0)	0.776
Female (%)	43 (63%)	13 (65%)	0.885
Disease Duration (months)	41 (25-67)	-	-
CRP (mg/l)	29.5 (21.7-38.2)	3.9 (3.1-5.4)	<0.001
ESR (mm/h)	25.1 (18.9-39.1)	7.8 (5.5-9.2)	<0.001

Data are presented as median (25^{th} and 75^{th} IQR) or counts (percentages). A *p* Value <0.05 was considered statistically significant and *p* Value <0.01 as highly significant. Significance of the comparison is determined by the Mann-Whitney test and the Chi-square test. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

Table 2. Genotypic frequencies of five studied single-nucleotide polymorphisms (SNP) in juvenile idiopathic arthritis (JIA) patients and health controls

Gene_SNP	JIA patients (n=68)	Health Controls (n=20)	*p* Value
TNF-308 (rs1800629)			
GG (%)	46 (68%)	16 (80%)	0.490
GA (%)	20 (29%)	4 (20%)	
AA (%)	2 (3%)	0 (0%)	
HWE	-	0.540	
TNF-238 (rs361525)			
GG (%)	58 (85%)	18 (90%)	0.590
GA (%)	10 (15%)	2 (10%)	
AA (%)	0 (0%)	0 (0%)	
HWE	-	0.871	
TNFRSF1B (rs1061622)			
TT (%)	45 (66%)	12 (60%)	0.833
TG (%)	21 (31%)	7 (35%)	
GG (%)	2 (3%)	1 (5%)	
HWE	-	0.999	
TRAF1/C5 (rs3761847)			
AA (%)	28 (41%)	6 (30%)	0.641
AG (%)	30 (44%)	10 (50%)	
GG (%)	10 (15%)	4 (20%)	
HWE	-	0.995	
PTPN22 (rs2488457)			
GG (%)	33 (48%)	13 (65%)	0.426
GC (%)	29 (43%)	6 (30%)	
CC (%)	6 (9%)	1 (5%)	
HWE	-	0.995	

Data are presented as counts (percentages). A *p* Value <0.05 was considered statistically significant and *p* Value <0.01 as highly significant. Significance of the comparison is determined by the Chi-square test. HWP, Hardy-Weinberg equilibrium.

SNPs were in Hardy-Weinberg equilibrium. No differences of the phenotype frequencies in five studied SNPs (TNF-308 (rs1800629), TNF-238 (rs361525), TNFRSF1B (rs1061622), TRAF1/C5 (rs3761847), and PTPN22 (rs2488457)) were found between JIA patients and Health controls presented in **Table 2** (P>0.05), which indicates that the five SNPs don't correlates with the risk of JIA.

Baseline characteristics of final analyzed JIA patients

Among enrolled 68 JIA patients, 8 cases didn't complete a 24-weeks observation period (2 cases for poor therapeutic effects and were included into final analysis as non-responders, while 4 cases for infection and 2 cases for other reason which exclude from the final response analysis). Therefore, 62 subjects (aged 8.8-14.7 years, 21 males and 41 females, 25-67 months' mean disease duration) were included into the final analysis for clinical response of TNF inhibitor treatment. Mean levels of CRP, ESR, and CHAQ score were 29.5 mg/l, 25.1 mm/h, and 1.6. The mean numbers of joints with active arthritis, joints with limited range of motion were 10 and 6. Levels of physician's global assessment of disease activity, overall well-being, and patient (parent) evaluation of pain were 42-72 (mm), 41-78 (mm), and 29-67 (mm), respectively. Among 62 subjects who received anti-TNF treatment, 24 cases meanwhile received MTA treatment, 23 cases received LFF treatment, and 15 cases received other medications at the same time. Moreover, about JIA Subtype, 10% of 62 subjects were systemic, 35% oligoarthritis, 10% RF positive polyarthritis, 32% RF negative polyarthritis, 8% enthesitis related arthritis and 5% psoriatic arthritis, respectively.

Frequencies of studied genes phenotype in responders and non-responders

After 24 weeks, 45 patients with JIA achieved ACRpedi 50 and were divided into the responders group, other 17 cases were included in non-responders group. The baseline characteristics of responders and non-responders of JIA patients treated by TNF inhibitor were present in **Table 3**. And no significant differences in

Table 3. Baseline characteristics of responders and non-responders of juvenile idiopathic arthritis (JIA) patients treated by TNF inhibitors

Parameters	Analyzed patients (n=62)	Responders ACRpedi 50 (n=45)	Non-Responders Not ACRpedi 50 (n=17)	*p* Value
Demographic characteristics				
Age (years)	11.7 (8.8-14.7)	11.4 (8.5-14.2)	11.9 (8.9-14.9)	0.809
Female (%)	41 (66%)	30 (67%)	11 (65%)	0.884
Disease Duration (months)	41 (25-67)	37 (20-59)	43 (26-69)	0.351
Clinical characteristics				
CRP (mg/l)	29.5 (21.7-38.2)	33.7 (23.0-41.8)	25.9 (17.8-35.9)	0.196
ESR (mm/h)	25.1 (18.9-39.1)	28.4 (23.2-44.1)	22.5 (15.1-34.6)	0.085
CHAQ score (0-3)	1.6 (0.9-2.1)	1.4 (0.9-2.0)	1.8 (0.9-2.2)	0.253
Joints with active arthritis (No.)	10 (7-21)	9 (6-20)	11 (8-22)	0.214
Joints with limited range of motion (No.)	6 (3-11)	6 (2-11)	7 (4-12)	0.328
Physician's global assessment of disease activity (mm)	58 (42-72)	60 (43-71)	56 (41-72)	0.362
Parents' global assessment of overall well-being (mm)	57 (41-78)	59 (42-79)	54 (39-77)	0.253
Patient (parent) evaluation of pain (mm)	47 (29-67)	50 (28-67)	46 (30-68)	0.317
Concomitant Medications				
MTX (%)	24 (39%)	18 (40%)	6 (35%)	0.734
LEF (%)	23 (37%)	17 (38%)	6 (35%)	0.857
Other DMARDs (%)	15 (24%)	10 (22%)	5 (30%)	0.555
JIA Subtype				
Systemic (%)	6 (10%)	3 (7%)	3 (18%)	0.192
Oligoarthritis (%)	22 (35%)	17 (38%)	5 (29%)	0.539
RF positive polyarthritis (%)	6 (10%)	4 (9%)	2 (12%)	0.733
RF negative polyarthritis (%)	20 (32%)	15 (33%)	5 (29%)	0.768
Enthesitis relatedarthritis (%)	5 (8%)	4 (9%)	1 (6%)	0.698
Psoriatic arthritis (%)	3 (5%)	2 (4%)	1 (6%)	0.814

Data are presented as median (25^{th} and 75^{th} IQR) or counts (percentages). A *p* Value <0.05 was considered statistically significant and *p* Value <0.01 as highly significant. Significance of the comparison is determined by the Mann-Whitney test and the Chi-square test. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CHAQ, childhood health assessment questionnaire; MTX, methotrexate; LEF, leflunomide; RF, rheumatoid factors.

demographic characteristics, clinical characteristics, concomitant medications or JIA subtype were observed between responders and non-responders groups (P>0.05).

Frequencies of TNF-308 GG (rs1800629) and TNFRSF1B TT (rs1061622) genotypes were observed to be increased in responders group compared with non-responders group (P=0.034, P=0.048, respectively, **Table 4**). However, there were no obvious significance in TNF-238 (rs361525), TRAF1/C5 (rs3761847) and PTPN22 (rs2488457) between responders and non-responders group (P>0.05, **Table 4**).

TNF-308 (rs1800629) could predict the clinical response in JIA

Given that the frequencies of TNF-308 GG (rs1800629) and TNFRSF1B TT (rs1061622) phenotype were increased in responders group, we next investigate the association of TNF-308 (rs1800629) A and TNFRSF1B (rs1061622) G allele with the prediction for clinical response by additive model with univariable and multivariable logistic regression analysis.

As presented in **Table 5**, TNF-308 A allele (rs1800629) and TNFRSF1B G allele (rs1061622) were risk factors for clinical response (OR: 0.328, 95% CI: 0.117-0.914, P=0.033; OR: 0.387, 95% CI: 0.142-1.055, P=0.063, respectively) by univariale analysis. However, only TNF-308 A allele (rs1800629) was independent risks for clinical response by multivariable logistic regression (OR: 0.354, 95% CI: 0.142-0.885, P=0.026), while TNFRSF1B G allele (rs1061622) might be potential risks (OR: 0.418, 95% CI: 0.155-1.130, P=0.086), in which analysis of age, gender, disease dura-

Table 4. Genotypic frequencies of five studied single-nucleotide polymorphisms (SNP) in responders and non-responders treated by TNF inhibitor

Gene_SNP	Responders ACRpedi 50 (n=45)	Non-Responders Not ACRpedi 50 (n=17)	*p* Value
TNF-308 (rs1800629)			
GG (%)	34 (76%)	9 (53%)	0.034
GA (%)	11 (24%)	6 (35%)	
AA (%)	0 (0%)	2 (12%)	
TNF-238 (rs361525)			
GG (%)	41 (91%)	14 (82%)	0.331
GA (%)	4 (9%)	3 (18%)	
AA (%)	0 (0%)	0 (0%)	
TNFRSF1B (rs1061622)			
TT (%)	32 (71%)	9 (53%)	0.048
TG (%)	13 (29%)	6 (35%)	
GG (%)	0 (0%)	2 (12%)	
TRAF1/C5 (rs3761847)			
AA (%)	20 (44%)	5 (30%)	0.113
AG (%)	21 (47%)	7 (40%)	
GG (%)	4 (9%)	5 (30%)	
PTPN22 (rs2488457)			
GG (%)	22 (49%)	7 (41%)	0.422
GC (%)	20 (44%)	7 (41%)	
CC (%)	3 (7%)	3 (18%)	

Data are presented as counts (percentages). A p Value <0.05 was considered statistically significant and p Value <0.01 as highly significant. Significance of the comparison is determined by the Chi-square test.

tion, ESR, CRP, CHAQ score, joints with active arthritis, joints with limited range of motion, physician's global assessment of disease activity, parents' global assessment of overall well-being, Patient (parent) evaluation of pain, and concomitant medications as well as JIA subtype were included.

Discussion

JIA, belong to autoimmune disease, is a heterogeneous form of arthritis in children and the result of environmental factors and multiple genes [9]. The aim of modern treatment for JIA is to prevent joint damage, promote normal growth, improve life quality, as well as to achieve these goals with minimal risk of side effects [18]. One of the most common features of JIA is high levels of TNF in peripheral blood and synovial fluid, therefore, biological drugs anti-TNF, including etanercept, infliximab, adalimumab, has been applied to in the treatment of JIA [20]. The application of TNF-blocking agents has greatly reduced inflammation and improved clinic presentations in JIA. However, not all patients respond well to TNF treatment and some even presents non-response [21], the reason of which is still obscure. Due to the expensive coast and non-response of anti-TNF treatment, pursuing some predictive factors for a good response to predict the efficacy of anti-TNF agent and prevent unnecessary biologic therapy is of great value in clinical practice.

Genetic factors are involved in the overall risk, clinical presentations, or even response to treatment in autoimmune diseases, especially JIA [22, 23]. Several studies have demonstrated that SNPs, which located in coding or non-coding regions of the genome, can be considered as biomarkers to predict disease severity and response to treatment [9, 24]. Methotrexate (MTX) is one of a drug that is common used in the treatment for JIA, studies show that SNPs are involved in intracellular MTX accumulation and can affect MTX transport [25], which further influences the effects of treatment. PTPN22 rs2488457 G/C polymorphism and STAT4 rs7574865 G/T polymorphism are also observed to be the risk factors of JIA in Chinese patients with JIA [6]. *PTPN22* polymorphism is found to be significantly associated with JIA risk in America population [17]. Moreover, several studies have found that TNF-related genes (TNFRSF1A, TNFRSF1B, TRAF1-C5, TNFAIP3) may influence the risk of RA, disease severity, or even response to anti-TNF drugs [26, 27]. TRAF1/C5 variant is reported to be potential predictors of response to anti-TNF therapy of Rheumatoid Arthritis [16].

In our study, we analyzed the genotypic frequencies of TNF-308 (rs1800629), TNF-238 (rs361525), TNFRSF1B (rs1061622), TRAF1/C5 (rs3761847), and PTPN22 (rs2488457) in patients with JIA, and found that there was no differences of these SNPs between JIA group and healthy control group. We hypothesized that these SNPs might be correlated with response to anti-TNF treatment; therefore, we analyzed genotypic frequencies of these five

Table 5. Association of TNF-308 (rs1800629) and TNFRSF1B (rs1061622) polymorphisms with the prediction for response by additive model

Gene_SNP	Model	Odds Ratio	95% CI		*p*-Value
			Lower	Higher	
TNF-308 (rs1800629) [A]	Univariable	0.328	0.117	0.914	0.033
	Multivariable	0.354	0.142	0.885	0.026
TNFRSF1B (rs1061622) [G]	Univariable	0.365	0.141	0.946	0.038
	Multivariable	0.418	0.155	1.130	0.086

Data are presented as odds ratio with 95% CI and *p* value. Univariable logistic regression was performed to evaluate whether the TNF-308 (rs1800629) and TNFRSF1B (rs1061622) could predict the clinical response; Multivariable logistic regression was performed to identify whether TNF-308 (rs1800629) and TNFRSF1B (rs1061622) were independent factors to predict the clinical response (age, gender, disease duration, ESR, CRP, CHAQ score, joints with active arthritis, joints with limited range of motion, physician's global assessment of disease activity, parents' global assessment of overall well-being, Patient (parent) evaluation of pain, and concomitant medications as well as JIA subtype were included in the analysis).

SNPs in JIA patients who received the treatment of TNF inhibitors. Frequencies of TNF-308 GG (rs1800629) and TT TNFRSF1B (rs1061622) were found to be increased in responders group compared with non-responders group, which indicating the potential predictive role of TNF-308 (rs1800629) and TNFRSF1B (rs1061622) phenotype in response to TNF-inhibitor treatment in patients with JIA.

Numerous published data has reported that TNFRSF1B gene polymorphisms is associated with response or non-response to anti-TNF therapy in several autoimmune diseases such as RA and Crohn's Disease (CD) [28]. TNFRSF1B has drawn the researchers' attention as several studies have found that TNFRSF1B SNPs may function in regulating RA risk, affecting severity of disease and the response to treatment [26, 29]. Meta-analysis showed that TNFRSF1B rs1061622 T allele predicted a better response compared to patients with the TNFRSF1B rs1061622 G allele in patients with psoriasis and RA receiving the therapy of infliximab, adalimumab and etanercept, and other genotype of TNFRSF1B rs1061622 also had no predictive responses to anti-TNF treatment [20]. TNF-308 gene is reported to be association with response to anti-TNF therapy in several autoimmune diseases, such as sarcoidosis and RA [30, 31]. In patients with JIA, TNF-308 gene is related with increased transcriptional activity, elevated TNF-α protein levels, and more aggressive JIA phenotypes [32]. Jimenez-Morales has reported that the TNF-α 308 A allele was correlated with susceptibility of JIA and RA [33, 34]. In our study, TNF-308 A allele (rs1800629) and TNFRSF1B G allele (rs1061622) predicted poor clinical response in JIA patients receiving TNF inhibitor treatment by univariale analysis with additive model. But only TNF-308 A allele (rs1800629) was independent risky biomarker for clinical response by multivariable logistic regression.

In summary, in our current study, no associations of the five studied gene variance with the risk of JIA were found. Frequencies of TNF-308 (rs1800629) GG and TNFRSF1B (rs1061622) TT phenotypes were evaluated in JIA patients with a better response to anti-TNF therapy, and TNF-308 A allele (rs1800629) was independent risk factors in predicting clinical response, while TNFRSF1B G allele (rs1061622) could predict poor clinical response as well but not independent risky. Our present study provides the first insights of TNF-308 A (rs1800629) and TNFRSF1B G (rs1061622) allele in response to anti-TNF therapy in JIA patients. Therefore, TNF-308 A (rs1800629) and TNFRSF1B G (rs1061622) allele may be used as potential biomarkers in predicting the response of anti-TNF agent in patients with JIA in the future.

Disclosure of conflict of interest

None.

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