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GENES AND DISEASE

The association of CAT-262C/T polymorphism with catalase activity and treatment response in juvenile idiopathic arthritis

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Abstract

Oxidative stress is believed to be of great importance for both the etiology and the persistence of juvenile idiopathic arthritis (JIA). The aim of this study was to investigate the association of -262C/T polymorphism of the catalase (CAT) gene with JIA, as well as to evaluate whether this polymorphism can influence plasma CAT activity and outcome in JIA patients treated with etanercept. A total of 154 subjects (60 JIA patients and 94 healthy volunteers) were screened for CAT-262C/T gene polymorphism using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. Plasma CAT activity was determined using the spectrophotometric method according to Goth, prior to and 12 months after anti-TNF (etanercept) therapy. Clinical outcome was assessed using the JIA ACR (American College of Rheumatology) response criteria. The genotype and allele frequency distributions of CAT-262C/T polymorphism in the patients were significantly different from those of the controls ($p=0.014$, $p=0.006$). The TT genotype (polymorphic homozygous) was associated with a 4.36-fold higher likelihood of having JIA (95% CI 1.545–12.323, $p=0.005$) as compared to the CC genotype (wild-type). At month 12 of treatment, JIA patients, carriers of the CC genotype, showed significantly higher plasma CAT activity ($p=0.004$) and achieved the JIA ACR 70 response more often ($p=0.003$) than the patients, carriers of the CT/TT genotype. This is the first study implying the possible association of CAT-262C/T polymorphism with JIA. The results suggest the potential protective effect of the CC genotype, with regard to CAT activity and treatment outcome.

Keywords Catalase · CAT-262C/T polymorphism · Etanercept · Juvenile idiopathic arthritis

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Introduction

Juvenile idiopathic arthritis (JIA) is the most common chronic inflammatory disease in childhood that can lead to severe disability. According to the International League of Associations for Rheumatology (ILAR) classification, JIA is divided into seven distinct categories [1]. JIA is characterized by inflammatory reaction, in which antigen-activated $CD4^+$T cells stimulate macrophages and synovial fibroblasts to the extreme production of proinflammatory cytokines: tumor necrosis factor (TNF-α), interleukin-1 (IL-1), IL-6, and chemokines (IL-8) [2]. During the antigen presentation, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme that represents the major source of reactive oxygen species (ROS) in this disease, is activated. The reaction catalyzed by this enzyme primarily generates the superoxide anion radical which produces hydrogen peroxide (H_2O_2), further leading to the formation of the most potent $OH^{\bullet}$ radical. Increased levels of ROS and/or damage of antioxidant defense system lead to oxidative modification of lipids, proteins and nucleic acids with the consequent disturbance of the cell function [3]. It is shown that ROS formed in polymorphonuclear cells can cause erosive destruction of joints in arthritis [4]. ROS have been proven to stimulate chondrocytes and synovial fibroblasts to produce matrix metalloproteinase, finally leading to the degradation of the extracellular matrix. Furthermore, H_2O_2 inhibits proteoglycan synthesis and stimulates bone resorption by osteoclasts. As one of the "key" molecules in JIA, TNF-α has a crucial role in oxidative stress development. This cytokine has long been known to activate phagocytic NADPH oxidase and stimulate the production of ROS, but also the production of H_2O_2 in fibroblasts, chondrocytes, and endothelial cells [5].

To prevent damage, cells contain antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) which neutralize superoxide anions, H_2O_2 and lipid peroxide. Catalase (EC 1.11.1.6) (CAT), is an oxidoreductase, a tetramer of four identical subunits, each containing a polypeptide chain of 527 amino acids and a heme molecule. CAT is one of the most active and most widespread enzymes (liver, kidneys), localized in either peroxisomes or the cytosol (red blood cells—RBC). It catalyzes the decomposition reaction of H_2O_2 to water and molecular oxygen [6]. Although numerous studies point to the increased production of ROS in JIA, the results of studies describing antioxidant enzymes activities in this disease are contradictory [7–9].

The CAT gene is located on chromosome 11p13 and it has 13 exons [10]. A large number of genetic variations in the CAT gene results in disturbed gene expression and the change of its activity in RBC and plasma [6]. Genetic variation of CAT-262C/T (rs1001179) is located in the promoter region of the CAT gene, and could affect its transcriptional activity. Although the results of different studies have showed the correlation of this polymorphism with the risk of developing diabetes mellitus, inflammatory bowel disease, cancer, especially breast- and hepatocellular cancer [11–16], its functional significance in JIA has not yet been established.

TNF-α inhibitors, such as etanercept, have proven their clinical efficacy in chronic inflammatory arthritides [17]. Given the destructive nature of JIA, the risk of adverse effects, and considerable costs for anti-TNF-α treatment, there is a strong need to identify predictors of response prior to the start of the treatment. The aim of this study was to investigate the association of CAT-262C/T polymorphism with JIA, as well as to evaluate the influence of this polymorphism on plasma CAT activity and on the outcome in JIA patients treated with etanercept.

Patients and methods

Subjects

This was a prospective open label study, which enrolled 60 JIA patients with polyarticular disease course, diagnosed using ILAR criteria [1], included in the national biologics registry, and 94 healthy subjects, without previous history of JIA or other diseases. Only JIA patients with active disease, despite the usage of methotrexate (MTX) in maximal tolerated dose of 10–20 mg/m^2/week, during past 6 months who needed to start etanercept therapy (0,8 mg/kg/week subcutaneously) were included in the study. To be eligible for enrollment, patients have had to stop steroids usage at least 3 months prior to entering the study and had to maintain MTX treatment. Polyarticular disease was defined by the presence of five or more joints with active arthritis. Joints were defined as active by the presence of swelling or, if no swelling was present, by the limitation of motion accompanied by pain, tenderness, or both. By definition, patients with rheumatoid factor (RF) positive polyarthritis must have two positive tests for IgM RF with 3 month-interval during the first 6 months of disease [1]. Paired blood samples were collected (before starting etanercept therapy and 12 months after the continuous therapy). Disease activity was evaluated at study entry and after 12 months of etanercept therapy. Treatment response was measured according to the JIA ACR (American College of Rheumatology) criteria, using six core variables: (1) the physician global assessment of disease activity, on a 10-cm visual analog scale (phVAS; 0–100, where 0 means no activity and 10 means maximum activity); (2) the parent/patient global assessment of overall wellbeing,

on a 10-cm visual analog scale (pVAS; 0–10, where 0 means very well and 10 means very poor); (3) the Childhood Health Assessment Questionnaire (CHAQ; 0–3, where 0 means no difficulty and 3 unable to perform); (4) the number of joints with a limited range of motions (LOM); (5) the number of joints with active arthritis (AA); and (6) the erythrocyte sedimentation rate (ESR). The JIA ACR 30/50/70 response was defined as: three or more JIA criteria improved by at least 30%/50%/70% with respect to baseline and no more than one core set criterion worsened by 30% [18].

The study was performed in compliance with the Declaration of Helsinki and the Ethical Committee of the Medical Faculty University of Niš approved the study protocol. All participants voluntarily agreed to participate in the study and informed consent was signed by the parents or by the patients if they were aged ≥ 12 years. The research was conducted in the Clinic of Pediatrics, Clinical Centre Niš, Serbia, Institute of Rheumatology, Belgrade, Serbia, and the Laboratory for Functional Genomics and Proteomics, at the Faculty of Medicine, University of Niš, Serbia.

Blood sample preparation and genotyping

Blood samples were taken in the morning, after fasting. From all the blood samples (with ethylenediaminetetraacetic acid (EDTA) as the anticoagulant), we separated 200 µL of blood, which was used for DNA isolation. The blood samples were then centrifuged at 3500 rpm for 10 min at + 4 °C, after which the plasma was separated and frozen at − 80 °C.

The isolation of DNA was performed using a commercial kit (QIAamp DNA Blood Mini Kit, Quiagen GmbH, Hilden, Germany). We examined the polymorphism CAT-262C/T, using the polymerase chain reaction–restriction fragment length polymorphism method (PCR–RFLP) [19]. The fragment of 185 base pairs (bp) was amplified using a forward (5′-AGA GCC CGC TCG CCC CGG ACC G-3′) and a reverse primer (5′-TAA CTG GAG AGA CAT AAG AGC T-3′). The PCR reaction mixture in a volume of 25 µL contained: 12.5 µL of KAPA 2G Fast HS Ready-Mix PCR kit solution (KAPA Biosystems, Germany), 0.5 µL of primer (10 pmol/µL) (Fermentas GmbH, St. Leon-Rot, Germany) and 20 ng of DNA. The PCR conditions were: the initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 63 °C for 15 s, elongation at 72 °C for 15 s, and termination at 72 °C for 30 s. The amplified PCR products were visualized under UV light after agarose gel (2%) electrophoresis. PCR products were cut into smaller fragments by SmaI restriction enzyme (Fermentas GmbH, St.Leon-Rot, Germany) at 37 °C overnight and analyzed by a vertical polyacrylamide gel (8%) electrophoresis. Homozygous for the C allele (wild type) was detected as two fragments of 155 and 30 bp (genotype CC), while the polymorphic homozygous (TT) was shown as one fragment (185 bp). Heterozygous (CT) was confirmed by the presence of three fragments on the gel (185, 155 and 30 bp).

Catalase activity measurement

Plasma CAT activity was determined by Goth's spectrophotometric method [20], based on the ability of CAT to decompose the substrate (H_2O_2), whereby the enzymatic reaction was stopped by the addition of ammonium molybdate, and the resulting yellow complex of H_2O_2 and molybdate was measured at 405 nm against the reagent blank. The enzyme activity was expressed in kU/L.

Statistical analysis

For testing the normality of the parameters' distribution, the Kolmogorov–Smirnov test was used. Numerical variables with normal distribution are expressed as mean (M) ± standard deviation (SD), while variables which deviate from normal distribution are shown as median with data range (minimum–maximum). The statistically significant differences in variables with normal distribution between the groups were determined by the t test for two independent samples, while the significant differences in numerical variables that deviate from normal distribution between the groups were determined by the Mann–Whitney U test or by the Wilcoxon test. Spearman's correlation analysis was performed to examine associations between variables of interest. The frequency of alleles and genotypes in the patients and controls was analyzed and compared using the χ^2 test. The possible deviation from the expected values of the Hardy–Weinberg equilibrium was determined. We performed the Fisher's exact test to compare JIA ACR 30/50/70 response between different genotypes. Univariate binary logistic regression was used to analyze the association between genetic polymorphism and JIA. Multivariate binary logistic regression was performed to measure the influence of multiple factors on the probability of high response achievement in JIA patients. The model contained 5 independent variables (CAT-262C/T polymorphism, RF positivity, age at disease onset, disease duration, and gender). The ACR70 response rate was the dependent variable. The Hosmer–Lemeshow goodness-of-fit test was performed. Odds ratios with 95% confidence interval (CI) were calculated from estimates of the logistic regression models. $P < 0.05$ value was considered statistically significant. The statistical analysis was conducted using the SPSS software package version 20.0 (SPSS Inc., Chicago, IL, USA).

Table 1 Demographic and clinical characteristics of JIA patients at baseline

Parameter	JIA patients Baseline ($N=60$)
Gender	
Female	39 (65%)
Male	21 (35%)
Age at study entry (M ± SD)	14.18 ± 3.18
Age at disease onset (M ± SD)	9.16 ± 2.53
Disease duration (years) (M ± SD)	5.01 ± 1.84
JIA subtypes	
Extended-oligoarthritis	5 (8.3%)
RF − polyarthritis	30 (50%)
RF + polyarthritis	13 (21.7%)
Psoriatic arthritis	2 (3.3%)
Enthesitis-related arthritis	10 (16.7%)
Concomitant treatment at enrolment (MTX)	
Duration of MTX treatment (months)	
Median (range)	51.5 (6–181)
Dose (mg/m^2/week)	
Median (range)	15 (10–20)

N number of subjects, *RF* Rheumatoid factor, *MTX* methotrexate

Results

Demographic and clinical characteristics of patients are shown in Table 1. The control group consisted of 94 healthy subjects, whose gender (44 males and 50 females) and age (14.77 ± 2.27 years) corresponded to those of the JIA patients ($p=0.179$, $p=0.181$ respectively).

Genotype and allele frequencies of CAT-262C/T polymorphism

Genotype frequencies of CAT-262C/T polymorphism did not deviate from the normal distribution of the Hardy–Weinberg equilibrium in JIA patients and control group ($p>0.05$). The distribution of genotypes of the CAT-262C/T polymorphism in JIA patients showed a statistically significant difference compared to the control group ($\chi^2=8.468$, $df=2$, $p=0.014$). JIA patients had a higher frequency of the -262T allele in comparison to the group of healthy subjects ($\chi^2=7.474$, $df=1$, $p=0.006$). Additionally, univariate logistic regression showed that the TT genotype was associated with a 4.36-fold higher likelihood of having JIA as compared to the CC genotype (95% CI 1.545–12.323, $p=0.005$), while the presence of the T allele was not associated with a higher likelihood of having JIA compared to the C allele ($p=0.081$; Table 2).

There were no differences in age at disease onset and disease duration between CC and CT/TT genotype carriers (8.63 ± 2.40 vs. 9.47 ± 2.59 $p=0.221$; 5.22 ± 1.84 vs. 4.89 ± 1.85, $p=0.506$, respectively).

The influence of CAT-262C/T polymorphism on plasma CAT activity and disease activity

During 12 months of etanercept and MTX treatment, there was a significant improvement in all evaluated outcome variables and 95% (57/60) of JIA patients achieved the ACR 30, 88.3% (53/60) of patients achieved the ACR 50 and 55% (33/60) of JIA patients achieved the ACR 70 or more improvement (details presented in Table 3).

The median plasma CAT activity in JIA patients at baseline was significantly lower compared to healthy subjects (21.15 kU/L (4.82–52.16); $z=-8.342$, $p<0.001$). Introduction of etanercept treatment was associated with the increase of plasma CAT activity ($z=-3.938$, $p<0.001$) compared to the values before etanercept introduction, but these values were still lower compared to healthy controls ($z=-6.925$, $p<0.001$). Baseline CAT activity did not correlate with disease duration ($p=0.700$), age at disease onset ($p=0.651$), pre-treatment ESR ($p=0.940$), AA ($p=0.052$), LOM ($p=0.255$), phVAS ($p=0.951$), pVAS ($p=0.294$) and CHAQ ($p=0.926$). By correlation analysis, we did not find an association between post-treatment CAT activity and any of the post-treatment clinical variables (ESR $p=0.728$,

Table 2 Genotype and allele frequencies of CAT-262C/T polymorphism in JIA patients and healthy controls

CAT-262C/T polymorphism	Control $N=94$	JIA $N=60$	p value (χ^2 test)	OR (95% CI)	p value
Genotype					
CC	48 (51.1%)	22 (36.7%)	0.014	1	
CT	39 (41.5%)	24 (40.0%)		1.343 (0.656–2.748)	0.420
TT	7 (7.4%)	14 (23.3%)		4.364 (1.545–12.323)	0.005
Allele					
C	135 (71.8%)	68 (56.7%)	0.006	1	
T	53 (28.2%)	52 (43.3%)		1.802 (0.929–3.497)	0.081

N number of subjects, *OR* odds ratio, *95% CI* 95% confidence interval

Table 3 Plasma CAT activity and clinical variables in JIA patients at baseline and 12 months after treatment

	Baseline Median (range)	After 12 months Median (range)	*p* value
CAT activity (kU/L)	4.71 (0.58–55.51)	10.02 (0.63–54.04)	< 0.001
ESR (mm/h)	28 (2–100)	12 (2–78)	< 0.001
No. of joints with AA	6.5 (2–39)	1 (0–6)	< 0.001
No. of joints with LOM	8 (0–45)	3 (0–40)	< 0.001
phVAS	45 (15–82)	10 (0–50)	< 0.001
pVAS	41 (0–100)	10 (0–60)	< 0.001
CHAQ	0.68 (0–2.25)	0.25 (0–2.25)	< 0.001

CAT catalase, *ESR* erythrocyte sedimentation rate, *N* number, *AA* active arthritis, *LOM* limitation of motion, *ph* physician, *p* parent/patient, *VAS* visual analog scale, *CHAQ* Childhood Health Assessment Questionnaire

AA $p = 0.067$, LOM $p = 0.069$, phVAS $p = 0.181$, pVAS $p = 0.063$, CHAQ $p = 0.370$).

The influence of CAT-262C/T polymorphism on CAT activity and clinical variables are shown in Table 4. In samples taken at enrollment, there were no differences in plasma CAT activity ($p = 0.282$), ESR ($p = 0.272$), AA ($p = 0.994$), LOM ($p = 0.292$), phVAS ($p = 0.939$), pVAS ($p = 0.994$) and CHAQ ($p = 0.427$) between carriers of the CC and CT/TT genotype. After 12 months of treatment, there was significantly lower plasma CAT activity in JIA patients, carriers of the polymorphic T allele compared to the CC genotype carriers ($z = -2.848$, $p = 0.004$). Conversely, ESR ($z = -2.065$, $p = 0.039$), AA ($z = -3.199$, $p = 0.001$), and phVAS ($z = -2.289$, $p = 0.022$) were significantly higher in T allele carriers in comparison to carriers of the wild-type (CC). No differences were observed in LOM ($p = 0.075$), pVAS ($p = 0.325$) and CHAQ ($p = 0.387$), when CC and CT/TT genotype carriers were compared.

The results of CAT-262C/T polymorphism influence on the JIA ACR 30/50/70 response in JIA patients at month 12

Table 4 The influence of CAT-262C/T polymorphism on plasma CAT activity and clinical variables in JIA patients

	Baseline			After 12 months		
	CC ($N = 22$) Median (range)	CT/TT ($N = 38$) Median (range)	*p* value	CC ($N = 22$) Median (range)	CT/TT ($N = 38$) Median (range)	*p* value
CAT activity (kU/L)	5.50 (1.26–43.78)	3.89 (0.58–55.51)	0.282	15.18 (2.09–21.28)	8.29 (0.63–54.04)	0.004
ESR (mm/h)	22.5 (2–57)	28 (6–100)	0.272	10 (2–78)	13 (4–62)	0.039
No. of joints with AA	7 (2–39)	6.5 (2–36)	0.994	0 (0–6)	2 (0–6)	0.001
No. of joints with LOM	6 (0–34)	10 (2–45)	0.292	2 (0–34)	4 (0–40)	0.075
phVAS	45 (15–82)	43.5 (20–75)	0.939	3.5 (0–50)	14 (0–35)	0.022
pVAS	42.5 (0–100)	39 (0–100)	0.994	8.5 (0–55)	17.5 (0–60)	0.325
CHAQ	0.94 (0–2.25)	0.56 (0–2)	0.427	0 (0–2.25)	0.25 (0–1)	0.387

CAT catalase, *ESR* erythrocyte sedimentation rate, *N* number, *AA* active arthritis, *LOM* limitation of motion, *ph* physician, *p* parent/patient, *VAS* visual analog scale, *CHAQ* Childhood Health Assessment Questionnaire

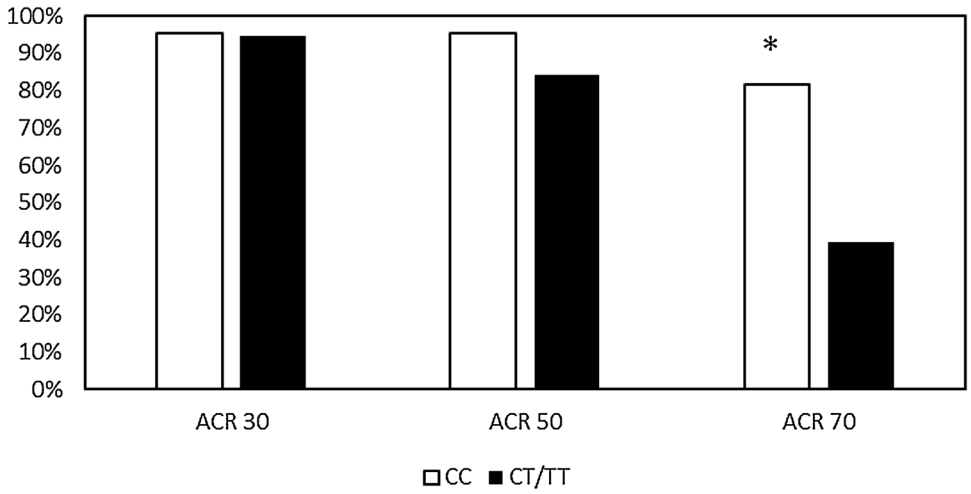

Fig. 1 The influence of CAT-262C/T polymorphism on the JIA ACR 30/50/70 response at month 12. *$p = 0.003$ vs. CT/TT (ACR70)

are shown in Fig. 1. JIA patients carriers of the CC genotype achieved the ACR 70 response significantly more often compared to carriers of the polymorphic T allele (18/22 (81.8%) vs. 15/38 (39.5%), $p = 0.003$). No significant difference was observed in ACR 30 response (21/22 (95.5%) vs. 36/38 (94.7%), $p = 0.698$) and ACR 50 response (21/22 (95.5%) vs. 32/38 (84.2%), $p = 0.246$) between wild-type carriers and polymorphic homo- and heterozygous.

Additionally, we performed multivariate binary logistic regression analysis to assess the impact of the predictors on the likelihood of achieving ACR70 response in JIA patients. Our regression model containing five predictors (CAT-262C/T polymorphism, RF positivity, age at disease onset, disease duration and gender) and ACR 70 response rate (as the dependent variable) was statistically significant ($\chi^2 = 12.019$, $df = 5$, $N = 60$, $p = 0.035$). The model successfully differentiated JIA patients with high (ACR70) response ($N = 33$) and JIA patients, who did not achieve ACR70 ($N = 27$). CAT-262C/T polymorphism was shown to be the only statistically significant predictor of high response probability. JIA patients, carriers of the CC genotype had a 7.41-fold higher likelihood of achieving ACR70 response as compared to patients, carriers of the CT/TT genotype (95% CI 1.991–27.560, $p = 0.003$), when holding all the other predictors constant. Patients' gender, age at disease onset, disease duration, RF positivity did not have a significant effect on response to therapy evaluated by ACR score (Table 5).

Discussion

CAT-262C/T polymorphism is one of the most analyzed CAT gene polymorphisms, but there are still no published data in regard to the role of this polymorphism in JIA. To the authors' best knowledge, our study is the first one exploring the possible conection. In this study, significant differences were observed in the distribution of genotypes and alleles of this polymorphism in JIA patients, compared to the control group. Although this is a study with a small number of JIA patients, the obtained results are clearly indicating higher frequency of the -262T allele in JIA patients in comparison to the group of healthy subjects. Additionally, carriers of the TT genotype have a 4.36-fold higher likelihood of having JIA compared to carriers of the CC genotype.

The plasma CAT activity in JIA patients, before and after introduction of etanercept treatment, was found to be significantly lower compared to the control group. Results of studies regarding the CAT activity in JIA patients are contradictory, and are probably dependent on disease activity, treatment being used, the study population, and the type of sample analyzed (RBC or plasma) [21, 22]. It is assumed that in JIA patients oxidative stress occurs not only because of the increased production of ROS [9, 23], but also due to the impaired antioxidant defense system [21, 24].

Bearing in mind the increased production of TNF-α in JIA [25, 26], and its effect on NADPH oxidase activation [5], it is possible that decreased CAT activity in JIA patients at enrollment, found in our study, was the result of the excessive accumulation of ROS in the inflamed joints and oxidative modification of the enzyme. Furthermore, Min et al. [27] showed reduction of CAT gene expression during ROS exposure caused by the hypermethylation of the CpG region in the enzyme gene promoter. This could be one more possible explanation for the lower CAT activity in JIA patients compared to control, found in our study. Introduction of etanercept treatment with consequent anti-inflammatory action, induced outcome improvement and was associated with increase of plasma CAT activity, compared to the values before etanercept introduction. Suppressed inflammation could consequently reduce protein oxidative modification and increase catalase activity. Nevertheless, CAT activity after treatment was still lower compared to healthy controls. Considering the influence of CAT-626C/T polymorphism on CAT activity and on disease outcome, the results of this study showed that, after 12 months of treatment, in wild-type carriers (CC), plasma CAT activity was significantly

Table 5 Multivariate binary logistic regression model of high (ACR 70) response achievement in JIA patients

Independent variables	Regression coefficient	Standard error	Wald	*p* value	OR	95% CI	
						Lower	Upper
CAT-262C/T polymorphism (CC)[a]	2.003	0.670	8.925	0.003	7.408	1.991	27.560
RF positivity	−0.618	0.711	0.756	0.385	0.539	0.134	2.172
Age at disease onset	0.037	0.118	0.097	0.756	1.037	0.823	1.308
Disease duration	−0.052	0.159	0.107	0.743	0.949	0.695	1.297
Gender (male)	0.383	0.638	0.360	0.548	1.466	0.420	5.115
Constant	−0.522	1.450	0.130	0.719	0.593		

RF Rheumatoid factor, *OR* odds ratio, *95% CI* 95% confidence interval;

$\chi^2 = 12.019$, $df = 5$, $N = 60$, $p = 0.035$; Nagelkerke $r^2 = 24.3\%$; Hosmer and Lemeshow goodness-of-fit test $p = 0.49$; Classification accuracy = 70%

[a]Analysis was done by grouping heterozygous (CT) with homozygous (TT) patients

higher, while ESR, AA, and phVAS were significantly lower in comparison to polymorphic hetero- and homozygous (CT/TT) patients. The ACR 70 response was reached more frequently in the patients, carriers of the CC genotype, in comparison to carriers of the polymorphic T allele. Additionally, JIA patients, carriers of the wild-type had a 7.41-fold higher likelihood of achieving ACR70 response in comparison to patients, carriers of the CT/TT genotype. Etanercept has proven well established efficacy in almost all JIA subtypes, especially in polyarticular disease course [25, 26, 28–32]. Our previous studies confirmed its anti-inflammatory potential and beneficial effects on matrix-metalloproteinase production [33], lipid profile [34], as well as on the treatment outcome [35]. Our results can support the results of the study by Saify et al. [36], indicating a higher expression of the CAT gene in the CC genotype carriers than in the carriers of the polymorphic T allele. It is possible that in the conditions of suppressed TNF-α effect on ROS formation, the inhibitory effect of ROS on CAT gene expression is missing. Due to suppressed inflammation and oxidative stress, JIA patients, carriers of the CC genotype, could have a higher CAT gene expression after treatment, and increased CAT activity in comparison to the T allele carriers. These results could support the findings that high CAT activity has a protective effect in rheumatism, by lowering the production of ROS, as well as their damaging effects [37]. A study on a larger number of patients is certainly necessary to investigate a possible association of this polymorphism with the risk of JIA and the mutual influence with the variations in other genes associated with this disease. As we showed the potential protective effect of the CAT-262CC genotype, future research should focus on testing the role of this polymorphism in the possible selection of patients for antioxidant therapy and prediction of the treatment outcome.

The limitations of our study are related to a relatively small sample size. Bearing in mind that TT genotype was present in 14 JIA patients, we compared parameters between CC and CT/TT genotype carriers, without analysing the influence of the CT and TT genotype on the tested parameters separately. Considering small number of patients, unfortunately we could not analyze the influence of CAT-262C/T polymorphism on CAT activity and treatment outcome in different JIA subtypes. In the future, it would be very interesting to investigate this in a big sample cochort. Furthermore, we could not evaluate all variables that could affect the disease outcome, such as upper extremity involvement.

Conclusion

This is the first study examining the association of CAT-262C/T polymorphism with JIA, its influence on plasma CAT activity and on disease outcome. The frequency of the -262T allele was significantly higher in JIA patients in comparison to the group of healthy subjects. Carriers of the -262TT genotype had a 4.36-fold higher likelihood of having JIA in comparison with carriers of the CC genotype. At month 12 of treatment, JIA patients, carriers of the CC genotype, had a significantly higher CAT activity, which was accompanied with lower ESR, AA and phVAS score and more frequently reached ACR 70 response in relation to carriers of the polymorphic T allele. Additionally, JIA patients, carriers of the CC genotype had a 7.41-fold higher likelihood of achieving ACR70 response in comparison to polymorphic homo- and heterozygous patients. These findings suggest the potential protective role of the CAT-262CC genotype. However, future research in a larger cohort should focus on testing this polymorphism as a genetic biomarker for the prediction of the treatment outcome.

Author contributions JB, JV, TJS and DP designed the study. JB and JV collected and interpreted data and wrote the initial draft of the manuscript. TJS, MD, TC, DL, GS, VM, MC and DP contributed to the data collection and interpretation and revised the manuscript critically. All authors approved the final version to be submitted for publication and agree to be accountable for all aspects of the work.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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