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JNK Pathway-Associated Phosphatase as a Serum Marker for Disease Activity and Treatment Outcome of Juvenile Idiopathic Arthritis

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Juvenile idiopathic arthritis (JIA) is a heterogeneous autoimmune disease characterized by arthritis of unknown etiology. JNK pathway-associated phosphatase (JKAP) is reported to be a negative regulator of T-cell activation, but its clinical role in JIA is unknown. This study aimed to investigate the correlation of JKAP with disease activity and treatment response to a tumor necrosis factor (TNF) inhibitor, etanercept (ETN), in JIA patients. Totally, 104 JIA patients (6.9 ± 2.7 years old) and 100 age- and sex-matched healthy controls (HCs) (7.2 ± 2.4 years old) were enrolled, and their serum samples were collected for measuring JKAP by enzyme-linked immunoassay. In JIA patients, after 24-week ETN treatment, clinical response was assessed based on the American College of Rheumatology pediatric criteria (ACRpedi) 50 criteria. Results showed that JKAP levels were significantly lower in JIA patients compared with HCs, and of good value in differentiating JIA patients from HCs. Among JIA patients, higher JKAP levels were associated with lower disease activity indexes, including C-reactive protein, number of joints with active arthritis, physician's global assessment of disease activity, and the present history of disease-modifying antirheumatic drugs; higher baseline JKAP levels were correlated with worse ACRpedi 50 response to ETN at week 24, and was also an independent predictive factor for worse ACRpedi 50 response to ETN. Thus, it may be inappropriate to use ETN for JIA patients with higher JKAP levels. In conclusion, serum JKAP is a potential biomarker for JIA activity and treatment response to a TNF inhibitor.

Keywords: disease activity; enzyme-linked immunoassay; JNK pathway-associated phosphatase; juvenile idiopathic arthritis; tumor necrosis factor inhibitor
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Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous autoimmune disease characterized by arthritis of unknown etiology that predominantly presents with peripheral arthritis (Prakken et al. 2011; Barut et al. 2017). Notably, JIA has been a critical health challenge affecting a large population of children and adolescents globally, with the incidence ranging from 1.6 to 23 per 100,000 annually and the prevalence varying from 3.8 to 400 per 100,000 (Thierry et al. 2014; Barut et al. 2017). The clinical symptoms of JIA involve the limited functional ability and less productivity in daily life caused by chronic inflammation of the joints, and JIA patients also suffer from several common complications, including uveitis, blindness, life-threatening macrophage activation syndrome. (Prakken et al. 2011; Crayne and Beukelman 2018; Lee and Schneider 2018). Introduction of tumor necrosis factor (TNF) inhibitor eases the disease activity level and improves the life quality to a large extent, contributing to the promoted clinical outcomes in JIA patients, but there is still a proportion of JIA patients with poor treatment response to TNF inhibitors

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(Kasapçopur and Barut 2015; Giancane et al. 2016). It is therefore essential to identify novel biomarkers for monitoring disease activity and predicting treatment efficacy to TNF inhibitor in JIA management.

JNK pathway-associated phosphatase (JKAP), also known as dual specificity phosphatase (DUSP) 22, locates in the actin filament-enriched region and specifically activates c-Jun N-terminal kinase signaling pathway (Chen et al. 2002). In addition, JKAP presents regulatory effect on various substrates in other signaling cascades, such as interleukin (IL)-6-induced activation of signal transducer and activator of transcription 3 (STAT3) and estrogen-mediated signaling, and displays modulatory role in focal adhesion kinase phosphorylation as well as cell motility (Li et al. 2010, 2014). Furthermore, several recent studies reveal the inactivating effect of JKAP in inflammatory and immune response, and also indicate that downregulation of JKAP is correlated with higher risk of exacerbated inflammation and autoimmunity-related disease (Alonso et al. 2002; Li et al. 2014; Mélard et al. 2016; Zhou et al. 2017). Mechanically, JKAP suppresses T-cell-mediated immune responses via regulating T-cell receptor signaling (Li et al. 2014), and mice with JKAP-knockdown T cells exhibit enhanced cytokine production and exacerbated symptoms of autoimmune diseases (Li et al. 2014). Clinically, one study indicates that JKAP is downregulated in patients with systemic lupus erythematosus (SLE) compared with healthy controls (HCs), and higher JKAP levels are correlated with lower SLE disease activity in SLE patients (Chuang et al. 2016); meanwhile, for patients with active inflammatory bowel disease (IBD), JKAP expression is inversely associated with disease activity and systematic inflammation (Chuang et al. 2016; Zhou et al. 2017). Moreover, higher baseline JKAP levels display good value for predicting worse clinical response to TNF inhibitor in patients with Crohn's disease (Shi et al. 2019). Given the evidence mentioned above, we have hypothesized that JKAP might be of clinical implication in JIA management as well. Furthermore, etanercept (ETN), as an inhibitor of TNF-α, reduces TNF receptor-mediated signaling via binding to soluble TNF-α, inhibiting proinflammatory cytokine-induced inflammatory responses and leading to low disease activity (Walters et al. 2016; Mori et al. 2018). Based on numerous previous evidence, ETN is efficacious and safe in large JIA patient population (Shepherd et al. 2016; Alexeeva et al. 2017; Mori et al. 2018; Foeldvari et al. 2019). However, still some patients present poor treatment response to ETN, and there are limited researches indicating biomarkers for predicting treatment response to ETN in JIA patients. Hence, we performed the present study to investigate the JKAP levels in JIA patients and HCs, and further explored the correlation of JKAP with disease activity and the clinical response to 24-week TNF inhibitor treatment in JIA patients.

Materials and Methods

Participants

This study consecutively enrolled 104 JIA patients in our hospitals from January 2016 to December 2018. The inclusion criteria consisted of: (i) met the definition of JIA in International League of Associations for Rheumatology (ILAR) (Petty et al. 2004); (ii) about to receive at least 24 weeks of ETN treatment; (iii) age $\leq$ 16 years old; (iv) able to be followed up regularly. The exclusion criteria were: (i) complicated with infections; (ii) Hepatitis B positive; (iii) abnormality of hepatic and renal function; (iv) severe deformation of joint; (v) history of malignancies or immunodeficiency diseases; (vi) having contraindications to ETN. Meanwhile, between October 2018 and March 2019, 100 healthy children were recruited as HCs. All HCs were recruited from the children who underwent healthy examination in our hospital. After confirmation of their health status by physical examination, they were enrolled in the study for collection of blood samples, with the informed consents from themselves (who were above age of 10) and all their patients. The HCs including 51 boys and 49 girls had mean age of 7.2 $\pm$ 2.4 years. There was no significant difference in the sex and age between JIA patients and HCs. This study was approved by the Institutional Review Board of Maternal and Child Health Hospital of Hubei Province. The written informed consents were acquired from the guardians of all the participants before enrollment.

JIA diagnosis and data collection

The diagnosis of JIA was based on clinical features and the exclusions of other causes of chronic arthritis predominantly. Initially, medial history examination and physical examination were performed to identify the clinical manifestations and exclude other causes of chronic arthritis. Meanwhile, imaging examinations, such as ultrasound (US), magnetic resonance imaging (MRI), X-ray, computed tomography (CT), scintigraphy, and positron emission tomography, were conducted to identify joint inflammation. In the meantime, routine laboratory tests, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor, HLA-B27 antigen and antinuclear antibody, were also carried out for auxiliary diagnosis. A final diagnosis of JIA was established based on the above examinations in accordance with the criteria of ILAR (Petty et al. 2004). Characteristics of JIA patients were recorded, which included: age, sex, height, weight, disease duration, disease subtype, CRP level, ESR, number of joints with active arthritis, number of joints with limited range of motion, the physician's global assessment of disease activity, parent/patient global assessment of overall well-being, childhood health assessment questionnaire (CHAQ), and history of treatments. For the HCs, the demographics (including age, sex, height and weight) were documented at enrollment.

Sample collection

Peripheral blood (PB) of the JIA patients was collected before initiation of ETN treatment, and the PB of HCs was collected from the blood routine examination during the physical examination. After collection, serum samples were isolated from the PB samples by centrifugalizing and stored at −70°C for the following detection.

JKAP detection

The levels of JKAP in serum samples were measured by enzyme-linked immunosorbent assay (ELISA) using the commercial human JKAP ELISA Kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). All procedures were carried out according to the manufacturer's protocol. In brief, reagents in the ELISA kit were well mixed before use, and then PB samples were diluted with diluent at a same size ratio. After that, 50 μl diluent sample, 50 μl diluted standards as well as 50 μl biotin-labeled antibody were added into the reaction well, followed by covering the membrane plate, gently shaking, mixing, and incubating at 37°C for 1 hour. After washing, 80 μl streptavidin-HRP was added to each well, which was then gently shaken, mixed, and incubated at 37°C for 30 minutes. Subsequently, the substrates were added into each well after washing, which was shaken gently and incubated for 10 minutes at 37°C in darkness. After taking out from the ELISA plate, 50 μl termination solution was quickly added, which was immediately followed by the determination. Finally, the optical density (OD) value of each well was measured at 450-nm wavelength. The JKAP levels in the sample were obtained according to the standard curve.

Treatment and assessment

According to the disease status and clinical needs, all JIA patients received ETN treatment (0.4 mg/kg, subcutaneous injection, twice weekly) for 24 weeks. Besides, the combinations of other disease-related drugs (such as methotrexate (MTX), leflunomide (LEF) and other disease-modifying antirheumatic drugs (DMARDs)) were allowed, and the related information was documented. After 24 weeks of ETN treatment, the clinical response was assessed referring to the American College of Rheumatology (ACR) Pediatric (pedi) 50 criteria: 50% improvement from baseline in at least 3 of any 6 measures of the JIA core sets without more than 1 measure worsening by over 30% (Consolaro et al. 2009; Zhou and Gu 2019). The 6 measures were erythrocyte sedimentation rate (ESR), joints with active arthritis, joints with limited range of motion, physician's global assessment of disease activity, parent/patient global assessment of overall well-being and CHAQ scores. In terms of the JIA patients (7 patients) who lost follow-up, based on the intention-to-treat (ITT) principle, the clinical response to ETN was analyzed with the last observation carried forward (LOCF) method for the missing data.

Statistical analyses

All patients were included in the statistical analyses, and in terms of the JIA patients (7 patients) who lost follow-up, based on the intention-to-treat (ITT) principle, the clinical response to ETN was analyzed with the last observation carried forward (LOCF) method for the missing data. All statistical analyses were performed using SPSS version 22.0 (IBM, Chicago, USA), and figures were plotted using GraphPad Prism version 7.00 (GraphPad Software, La Jolla, USA). Kolmogorov-Smirnov test was performed to determine the normality of continuous data. Normally or approximately normally distributed continuous data were presented as mean ± standard deviation (SD), and skewed distributed continuous data were presented as median with interquartile range (IQR). Categorized data were presented as count (percentage). The comparison of JKAP levels between JIA patients and HCs was determined using Wilcoxon rank-sum test. Receiver operating characteristic (ROC) curve, area under the curve (AUC), sensitivity and specificity at the best cut-off point were used to assess the performance of JKAP in distinguishing JIA patients from HCs. To analyze the correlation of JKAP levels with JIA patients' clinical features and clinical response to ETN, we further classified JKAP expression of JIA patients as four grades: quantile 1 (0-25% percentile, n = 26), quantile 2 (26-50% percentile, n = 26), quantile 3 (51-75% percentile, n = 26) and quantile 4 (76-100% percentile, n = 26). The correlation of JKAP with JIA patients' clinical features and clinical response to ETN were determined by Pearson's correlation test or Linear-by-Linear Association. All potential predictive factors for 24-week ACRpedi 50 response to ETN treatment were included in univariate and multivariate logistic regression model analysis, and forward stepwise regression method was used in the multivariate logistic regression model analysis to screen out the independent predictive factors for 24-week ACRpedi 50 response. *P* value < 0.05 was considered to be significant.

Results

Clinical characteristics of JIA patients

The mean age of JIA patients was 6.9 ± 2.7 years (Table 1). There were 46 (44.2%) males and 58 (55.8%) females among JIA patients. As for JIA subtypes, there were 29 (27.9%) patients with oligoarthritis, 35 (33.7%) patients with rheumatoid factor (RF) negative polyarthritis, 12 (11.5%) patients with RF positive polyarthritis, 15 (14.4%) patients with systemic subtype, 10 (9.6%) patients with enthesitis-related arthritis and 3 (2.9%) patients with psoriatic subtype. Furthermore, the average CRP was 40.4 ± 23.1 mg/L, and the average ESR was 23.3 ± 17.7 mm/h. The mean number of joints with active arthritis and joints with limited range of motion were 5.6 ± 2.8 and 3.5 ± 2.1, respectively. The score of physician's global assessment of disease activity, parent/patient global assessment of overall well-being and CHAQ were 5.8 ± 1.4, 5.6 ± 1.7 and 1.8 ± 0.5 in average, respectively. Other detailed information

Table 1. Characteristics of JIA patients.

Items	JIA patients (n = 104)
Age (years), mean ± SD	6.9 ± 2.7
Sex, No. (%)	
Male	46 (44.2)
Female	58 (55.8)
Height (cm), mean ± SD	119.6 ± 17.2
Weight (kg), mean ± SD	24.3 ± 8.4
Disease duration (years), mean ± SD	2.8 ± 1.6
JIA Subtype, No. (%)	
Oligoarthritis	29 (27.9)
RF negative polyarthritis	35 (33.7)
RF positive polyarthritis	12 (11.5)
Systemic	15 (14.4)
Enthesitis-related arthritis	10 (9.6)
Psoriatic	3 (2.9)
CRP (mg/L), mean ± SD	40.4 ± 23.1
ESR (mm/h), mean ± SD	23.3 ± 17.7
Joints with active arthritis (number), mean ± SD	5.6 ± 2.8
Joints with limited range of motion (number), mean ± SD	3.5 ± 2.1
Physician's global assessment of disease activity, mean ± SD	5.8 ± 1.4
Parent/patient global assessment of overall well-being, mean ± SD	5.6 ± 1.7
CHAQ, mean ± SD	1.8 ± 0.5
History of DMARDs, No. (%)	77 (74.0)
History of biologics, No. (%)	15 (14.4)
Combination of MTX, No. (%)	50 (48.1)
Combination of LEF, No. (%)	26 (25.0)
Combination of other DMARDs, No. (%)	19 (18.3)

JIA, juvenile idiopathic arthritis; SD, standard deviation; RF, rheumatoid factor; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CHAQ, childhood health assessment questionnaire; DMARDs, disease-modifying antirheumatic drugs; MTX, methotrexate; LEF, leflunomide.

about the clinical characteristics in JIA patients were shown in Table 1.

JKAP levels in JIA patients and HCs

JKAP levels were lower in JIA patients (34.730 pg/mL; 20.125 pg/mL-55.192 pg/mL) compared to HCs (102.200 pg/mL; 53.465 pg/mL-157.256 pg/mL) ($P < 0.001$) (Fig. 1A). Furthermore, ROC curve illuminated that JKAP was of good value in differentiating JIA patients from HCs (AUC: 0.848, 95% CI: 0.796-0.900), and the sensitivity as well as specificity at the best cut-off point (the point where the largest sum of sensitivity and specificity occurred) were 94.2% and 65.0%, respectively. Meanwhile, the cut-off value of JKAP level was 75.2 pg/mL at this point (Fig. 1B).

Correlation of JKAP with disease activity indexes in JIA patients

According to the baseline JKAP level, all JIA patients were classified as four grades: quantile 1 (0-25% percentile, median: 16.985 (range: 9.637-20.059), n = 26), quantile 2 (26-50% percentile, median: 27.981 (range: 20.323-34.526), n = 26), quantile 3 (51-75% percentile, median: 46.944 (range: 34.934-55.172), n = 26) and quantile 4 (76-100% percentile, median: 65.054 (range: 55.199-152.241), n = 26). Higher baseline JKAP grades were associated with decreased median value of CRP level ($r = -0.374$, $P < 0.001$) (Fig. 2A), reduced median number of joints with active arthritis ($r = -0.317$, $P = 0.001$) (Fig. 2C), decreased median number of joints with limited range of motion ($r = -0.314$, $P = 0.001$) (Fig. 2D), reduced median score of physician's global assessment of disease activity ($r = -0.251$, $P = 0.010$) (Fig. 2E); however, there was no correlation of baseline JKAP with ESR ($r = -0.131$, $P = 0.184$) (Fig. 2B), parent/patient global assessment of overall well-being ($r = -0.107$, $P = 0.279$) (Fig. 2F) or CHAQ ($r = -0.129$, $P = 0.191$) (Fig. 2G).

Correlation of JKAP with clinical features apart from disease activity indexes in JIA patients

Higher baseline JKAP grades were associated with present history of DMARDs ($P = 0.002$); however, there

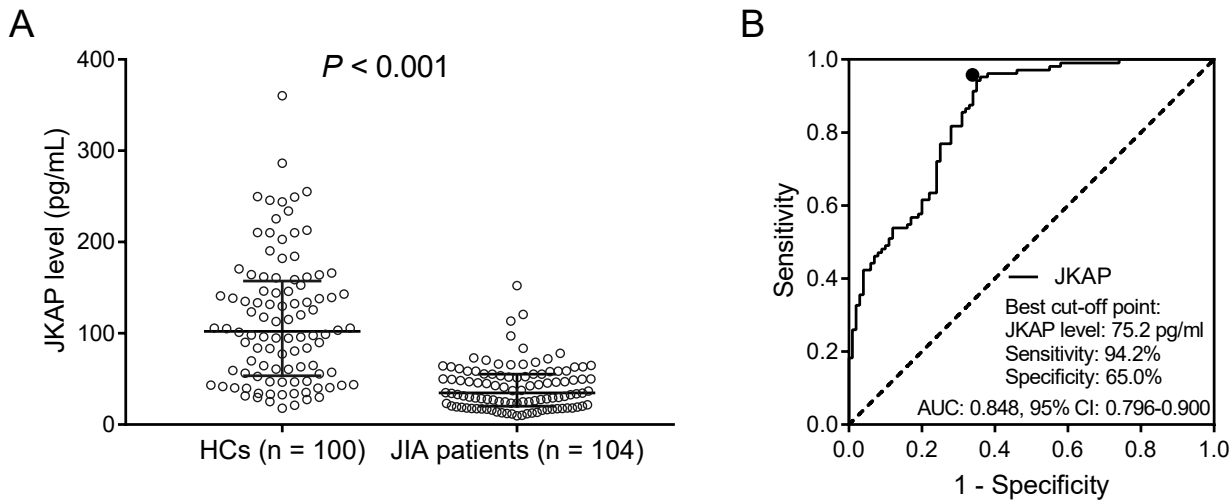

Fig. 1. Correlation of JKAP with JIA risk.
Comparison of JKAP level between JIA patients and HCs (**A**). The performance of JKAP in distinguishing JIA patients from HCs (**B**). The closed circle on the ROC curve represented the best cut-off point where the largest sum of sensitivity and specificity occurred.
JKAP, JNK pathway-associated phosphatase; JIA, juvenile idiopathic arthritis; HCs, healthy controls; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.

was no association of baseline JKAP with age (P = 0.987), sex (P = 0.114), height (P = 0.877), weight (P = 0.788), disease duration (P = 0.574), JIA subtypes (all P > 0.05) or history of biologics (P = 0.106) (Table 2).

Correlation of JKAP with clinical response to ETN in JIA patients

After 24-week ETN treatment, clinical response was assessed according to ACRpedi 50 criteria (Fig. 3). There were 23 (88.5%) patients with JKAP quantile 1, 20 (76.9%) patients with JKAP quantile 2, 16 (61.5%) patients with JKAP quantile 3 and 13 (50.0%) patients with JKAP quantile 4 who achieved ACRpedi 50 response to ETN treatment at week 24, suggesting that higher baseline JKAP grades appeared to be associated with worse ACRpedi 50 response to ETN treatment at week 24 in JIA patients (P = 0.001).

Factors predicting 24-week ACRpedi 50 response to ETN treatment

Univariate logistic regression model revealed that higher JKAP quantile (OR = 0.515, P = 0.002), systemic subtype (OR = 0.232, P = 0.012) and history of biologics (OR = 0.323, P = 0.047) associated with worse 24-week ACRpedi 50 response to ETN treatment; however, CRP (OR = 1.051, P = 0.001) correlated with better 24-week ACRpedi 50 response to ETN treatment in JIA patients (Table 3). Further forward stepwise multivariate logistic regression model displayed that higher JKAP quantile (OR = 0.565, P = 0.021) and systemic subtype (OR = 0.190, P = 0.015) were independent predictive factors for worse 24-week ACRpedi 50 response to ETN treatment in JIA patients, but CRP (OR = 1.037, P = 0.018) was an independent predictive factor for better 24-week ACRpedi 50 response to ETN treatment in JIA patients.

Discussion

In the present study, we found that, first, JKAP levels were reduced in JIA patients compared with HCs, and was of good value in distinguishing JIA patients from HCs. Secondly, higher baseline JKAP grades were associated with lower CRP level, reduced number of joints with active arthritis, joints with limited range of motion, decreased disease activity, and present history of DMARDs in JIA patients. Thirdly, higher baseline JKAP grades were associated with worse ACRpedi 50 response to ETN at week 24, and higher baseline JKAP grade was an independent predictive factor for worse 24-week ACRpedi 50 response to ETN treatment in JIA patients.

DUSPs are a type of protein tyrosine phosphatases, and dephosphorylates threonine as well as tyrosine residues on mitogen-activated protein kinases (MAPKs), modulating MAPK-dependent immune responses in inflammatory disorder (Jeffrey et al. 2007). JKAP, an atypical DUSP family member, is reported to present with inhibitory effect on the T-cell receptor signaling by dephosphorylating and inactivating the tyrosine kinase Lck (Li et al. 2014). Moreover, one previous study discloses that JKAP-knockdown mice exhibit high levels of pro-inflammatory cytokines, including TNF-α, IFN-γ, IL-6, and IL-17A (Chuang and Tan 2019). Existing accumulating evidence indicates that JKAP is involved in the development and progression of autoimmune disease (Chuang et al. 2016; Chuang and Tan 2019; Shi et al. 2019). For example, JKAP protein levels are reduced in peripheral blood T cells of SLE patients compared with HCs, and lower JKAP protein levels are correlated with higher disease activity and the level of anti-

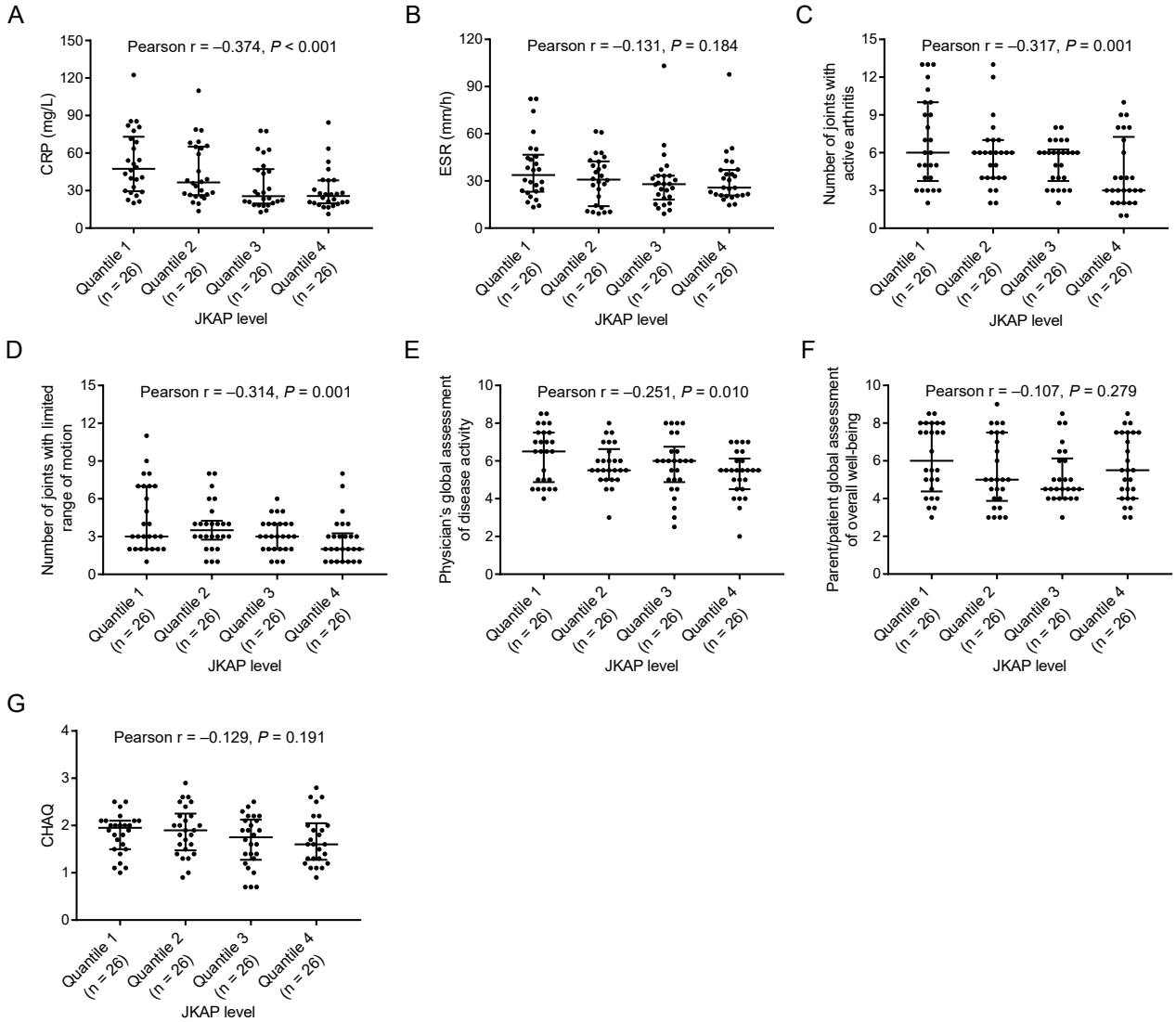

Fig. 2. Comparison of disease activity among JIA patients with different JKAP quantiles.
Comparison of CRP (**A**), ESR (**B**), number of joints with active arthritis (**C**), number of joints with limited range of motion (**D**), physician's global assessment of disease activity (**E**), parent/patient global assessment of overall well-being (**F**) and CHAQ (**G**) among JIA patients with JKAP quantile 1, those with JKAP quantile 2, those with JKAP quantile 3 and those with JKAP quantile 4.
JKAP, JNK pathway-associated phosphatase; JIA, juvenile idiopathic arthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CHAQ, childhood health assessment questionnaire.

dsDNA antibody in SLE patients (Chuang et al. 2016). In addition, JKAP expression is downregulated in inflamed mucosa of patients with activate IBD compared with the mucosa tissues from HCs, and its expression in intestinal mucosa is inversely associated with clinical activity in IBD patients (Zhou et al. 2017). According to the evidence mentioned above, and moreover, considering that JIA was a rheumatic disease accompanied by presence of the inflammation, we hypothesized that JKAP might be of clinical importance in JIA management. In order to verify this hypothesis, we conducted the present study, which observed that serum JKAP levels were lower in JIA patients compared to those in HCs, which is consistent with the previous evidence that JKAP levels were lower in patients with inflammatory diseases, including SLE, IBD and sepsis (Chuang et al. 2016; Zhou et al. 2017; Zhao and Huang 2019). In our study, further analysis indicated that JKAP presented with good value in distinguishing in JIA patients from HCs. The possible reason might include that JKAP might present inhibitory effect on T-cell receptor signaling, further suppressing T-cell activation, and resulting in attenuation of T-cell-mediated autoimmune responses and lower susceptibility to JIA (Chuang and Tan 2019). Following that, the association of JKAP level with clinical features was detected in JIA patients, which exhibited that higher baseline JKAP levels were associated with decreased disease activity (including lower CRP, less number of joints with active arthritis, less number of joints with limited

Table 2. Correlation of JKAP with JIA patients' clinical features apart from disease activity indexes.

Items	JKAP level				*P* value
	Quantile 1 (n = 26)	Quantile 2 (n = 26)	Quantile 3 (n = 26)	Quantile 4 (n = 26)	
Age (years), mean ± SD	7.2 ± 2.5	5.8 ± 2.3	7.8 ± 3.3	6.6 ± 2.1	0.987
Sex, No. (%)					0.114
Male	8 (30.8)	11 (42.3)	14 (53.8)	13 (50.0)	
Female	18 (69.2)	15 (57.7)	12 (46.2)	13 (50.0)	
Height (cm), mean ± SD	122.5 ± 16.9	112.9 ± 14.7	125.6 ± 20.9	117.5 ± 13.4	0.877
Weight (kg), mean ± SD	26.0 ± 8.0	20.4 ± 5.5	27.9 ± 10.9	22.8 ± 6.6	0.788
Disease duration (years), mean ± SD	3.0 ± 2.0	2.2 ± 1.4	2.8 ± 1.4	3.1 ± 1.6	0.574
JIA Subtype, No. (%)					
Oligoarthritis	6 (23.1)	6 (23.1)	5 (19.2)	12 (46.2)	0.098
RF negative polyarthritis	10 (38.5)	8 (30.8)	13 (50.0)	4 (15.4)	0.230
RF positive polyarthritis	3 (11.5)	5 (19.2)	0 (0.0)	4 (15.4)	0.785
Systemic	5 (19.2)	3 (11.5)	4 (15.4)	3 (11.5)	0.535
Enthesitis-related arthritis	0 (0.0)	3 (11.5)	4 (15.4)	3 (11.5)	0.139
Psoriatic	2 (7.7)	1 (3.8)	0 (0.0)	0 (0.0)	0.068
History of DMARDs, No. (%)	14 (53.8)	18 (69.2)	22 (84.6)	23 (88.5)	0.002
History of biologics, No. (%)	3 (11.5)	1 (3.8)	5 (19.2)	6 (23.1)	0.106

Correlation analysis was determined by Pearson's correlation test or Linear-by-Linear Association.
JIA, juvenile idiopathic arthritis; JKAP, JNK pathway-associated phosphatase; SD, standard deviation; RF, rheumatoid factor; DMARDs, disease-modifying antirheumatic drugs.

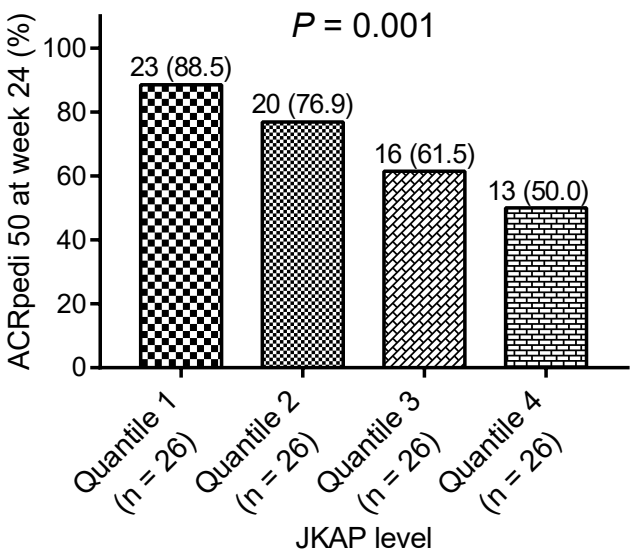

Fig. 3. Comparison of the percentage of patients who achieved ACRpedi response 50 among JIA patients with different JKAP quantiles.
JKAP, JNK pathway-associated phosphatase; JIA, juvenile idiopathic arthritis; ACRpedi, American College of Rheumatology pediatric criteria.

range of motion, physician's global assessment of disease activity), and present history of DMARDs in JIA patients. The possible reasons might include that (1) Considering JKAP was of anti-inflammatory property (Zhou et al. 2017; Chuang and Tan 2019), it possibly inhibited the expressions of pro-inflammatory cytokines, and further inactivated inflammation response in JIA as in IBD and SLE. Therefore, JIA patients with higher JKAP levels might have reduced levels of disease activity. (2) According to the previous evidence, higher serum JKAP levels were correlated with lower serum levels of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-6 and IL-17A), which might lead to increased bone mineral density and further reduced damage of joint cartilage and bone (Twilt et al. 2017; Chuang and Tan 2019). Meanwhile, the results in the current study indicated that in JIA patients, higher JKAP levels were correlated with reduced joints with active arthritis and limited range of motion. Therefore, higher serum JKAP might present tissue protective effect in JIA patients, further reducing JIA disease severity, which needed further investigation. (3) Since there was evidence that anti-TNF-α treatment upregulated JKAP expression in intestinal mucosa from IBD patients (Zhou et al. 2017), DMARDs were speculated to decrease JKAP expression in JIA patients, and higher JKAP levels were therefore associated with present history of DMARDs in JIA patients.

Existing evidence reveals that JKAP functions as a prognostic biomarker in inflammation-related diseases (Chuang et al. 2016; Shi et al. 2019; Zhao and Huang 2019). For instance, in patients with Crohn's disease, baseline JKAP levels are downregulated in patients presented clinical response to TNF inhibitor compared with patients withour clinical response to TNF inhibitor, and higher JKAP levels display predictive value for worse efficacy to TNF inhibitor (Shi et al. 2019). In addition, ETN is widely

Table 3. Factors predicting 24-week ACRpedi 50 response to ETN treatment.

Parameters	Logistic regression model			
	P value	OR	95% CI	
			Lower	Higher
Univariate logistic regression				
Higher JKAP Quantile	0.002	0.515	0.339	0.783
Age	0.912	1.009	0.863	1.179
Sex (Male)	0.430	0.714	0.309	1.649
Height	0.634	1.006	0.982	1.031
Weight	0.717	0.991	0.943	1.041
Disease duration	0.206	0.848	0.657	1.095
Oligoarthritis subtype	0.662	1.235	0.479	3.187
RF negative polyarthritis subtype	0.917	0.955	0.396	2.298
RF positive polyarthritis subtype	0.646	1.381	0.348	5.482
Systemic subtype	0.012	0.232	0.075	0.724
Enthesitis-related arthritis subtype	0.999	–	0.000	–
Psoriatic subtype	0.922	0.886	0.077	10.136
CRP	0.001	1.051	1.020	1.083
ESR	0.731	0.996	0.973	1.019
Joints with active arthritis	0.143	1.127	0.960	1.324
Joints with limited range of motion	0.501	1.074	0.872	1.324
Physician's global assessment of disease activity	0.795	0.960	0.705	1.307
Parent/patient global assessment of overall well-being	0.518	1.084	0.849	1.383
CHAQ	0.797	1.115	0.487	2.552
History of DMARDs	0.737	1.174	0.460	2.997
History of biologics	0.047	0.323	0.106	0.987
Combination of MTX	0.870	1.072	0.466	2.469
Combination of LEF	0.624	1.280	0.476	3.440
Combination of other DMARDs	0.642	1.303	0.426	3.989
Forward stepwise multivariate logistic regression				
Higher JKAP Quantile	0.021	0.565	0.348	0.918
Systemic subtype	0.015	0.190	0.049	0.728
CRP	0.018	1.037	1.006	1.070

Factors predicting 24-week ACRpedi 50 response to ETN treatment were determined by univariate logistic regression analysis, and only factors with *P* value < 0.05 in univariate logistic regression were further included in forward stepwise multivariate logistic regression analysis.

"–", Enthesitis-related arthritis and Psoriatic could not be analyzed due to lack of events.

ETN, etanercept; JKAP, JNK pathway-associated phosphatase; OR, odds ratio; CI, confidence interval; RF, rheumatoid factor; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CHAQ, childhood health assessment questionnaire; DMARDs, disease-modifying antirheumatic drugs; MTX, methotrexate; LEF, leflunomide; ACRpedi, American College of Rheumatology pediatric criteria.

used in JIA patients and is associated with disease quiescence in approximately 50% of ETN-treated patients (Kasapçopur and Barut 2015; Khraishi et al. 2019). Based on these previous evidence, we speculated that JKAP might also be correlated with clinical response to ETN in JIA patients. According to the disease status and clinical needs, all JIA patients received ETN treatment for 24 weeks, and the clinical response was assessed referring to the ACRpedi 50 criteria after 24-week ETN treatment. We found that baseline higher JKAP levels were associated with worse ACRpedi 50 response to ETN treatment at week 24 in JIA patients, and appeared to be an independent predictive factor for worse 24-week ACRpedi 50 response to ETN treatment in JIA patients. As for possible explanations, here were some interpretations of our own: (1) Since higher JKAP levels were associated with reduced levels of disease activity, patients with decreased disease activity might display a smaller gap for improvement of treatment outcomes, and it would be more difficult for them to achieve the treatment response (Kearsley-Fleet et al. 2016). Therefore, higher JKAP levels were associated with worse ACRpedi 50 response to ETN treatment at week 24 in JIA patients.

(2) In addition, according to the observation in the current study, lower JKAP levels were correlated with higher systematic inflammation level, which contributed to better treatment response to anti-inflammatory treatment in JIA patients. (3) Dysregulated JKAP levels might regulate the serum level of TNF-α (Zhou et al. 2017), which affected the drug sensibility of patients to anti-inflammatory biological drugs and formed the drug resistance, resulting in the unfavorable clinical response to ETN treatment in JIA patients. Furthermore, in the present study, further analysis observed that CRP was an independent predictive factor for better 24-week ACRpedi 50 response to ETN treatment in JIA patients, and the possible reasons might include that (1) Patients with higher CRP level presented increased inflammation level, and according to previous evidence that TNF-α inhibitors presented better efficacy in improving treatment response in the treatment of chronic inflammatory immune-mediated diseases (Murdaca et al. 2018), therefore, higher baseline CRP level was correlated with better treatment response to ETN treatment in JIA patients. (2) JIA patients with higher CRP level presented increased disease activity and more gap for relief of disease activity, therefore, it was easier for JIA patients with increased disease activity to achieve 24-week response to ETN, and higher CRP was correlated with better 24-week response to ETN. Furthermore, this was in consistence with the data in previous papers that higher CRP level was correlated with better treatment response to TNF-α inhibitor in treatment of rheumatic disease (Sikorska et al. 2018; Zhang and Jiang 2019). In addition, based on the results of the present study, higher serum level of JKAP could not be simply defined to be good news or bad news. In general, patients with higher serum JKAP level presented reduced severity of JIA, however, according to the results in our study, it was not appropriate for JIA patients with higher serum JKAP level to spend enormous expense on TNF inhibitor considering the relatively poor treatment response. Therefore, for these patients, treatments except for TNF inhibitor (such as: combined DMARDS) might be better.

The present study still existed some limitations as follows: (1) considering that we assessed 24-week ACRpedi 50 response in JIA patients, the long-term effect of JKAP in predicting clinical response to ETN treatment needed to be explored in a longer period. (2) Totally 104 patients were included in the present study, while further investigations with larger sample sizes were needed for validation. (3) Due to that the present study was a clinical study, further cellular studies were needed to explore the function of JKAP in blood, the pathological role of JKAP in JIA, and the underlying mechanism of the interaction between JKAP and ETN treatment in JIA patients. (4) Considering AUC of 0.848, JKAP was of good value in differentiating JIA patients from HCs, but as for diagnostic value, there existed gap between JKAP and some excellent diagnostic biomarkers. (5) Furthermore, JKAP levels of JIA patients and HCs were compared, but the positive control group of JIA (patients with other inflammatory-related arthritis except for JIA) was not set.

Higher serum JKAP levels are associated with lower disease activity state of JIA and are predictive of worse clinical response to TNF inhibitor treatment in JIA patients.

Conflict of Interest

The authors declare no conflict of interest.

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