Circulating JNK pathway-associated phosphatase level correlates with decreased risk, activity, inflammation level and reduced clinical response to tumor necrosis factor-α inhibitor in Crohn disease patients

Xue Shi, MMa, Wei Yang, MMb, Nian Wang, PhDc, Junyi Zhu, PhDb,∗

Abstract
Objective: This study aimed to investigate the correlation of serum Jun-amino-terminal kinase (JNK) pathway-associated phosphatase (JKAP) level with disease risk, severity, inflammation, and treatment response to tumor necrosis factor (TNF)-α inhibitor in Crohn disease (CD) patients.

Method: Ninety-six active CD patients and 90 healthy controls (HCs) were consecutively enrolled. Serum JKAP level of participants was determined via enzyme-linked immunosorbent assay (ELISA). In CD patients, C-reactive protein (CRP), erythrocyte sedimentation rate, Crohn disease activity index (CDAI), and inflammatory cytokine levels (determined by ELISA) were recorded. All CD patients underwent infliximab (IFX) treatment for 12 weeks, then treatment response (defined as decrement of CDAI ≥70) was assessed at week 12 (W12).

Results: Serum JKAP level in CD patients was lower compared to HCs, and it disclosed a good predictive value for decreased CD risk; meanwhile, it was negatively correlated with CRP level, CDAI score, TNF-α, interleukin (IL)-6, and IL-17 levels in CD patients. Sixty-eight (70.8%) patients achieved treatment response to IFX at W12, and JKAP level was increased at W12 compared to baseline. Interestingly, baseline JKAP level in response patients was decreased compared to nonresponse patients, and it exhibited a good predictive value for decreased treatment response to IFX, multivariate logistic regression revealed that JKAP was an independent factor for predicting reduced IFX response.

Conclusion: Circulating JKAP expression correlates with decreased disease risk, activity, and inflammation level, and it could be served as a novel biomarker for predicting reduced clinical response to TNF-α inhibitor in CD patients.

Abbreviations: AUC = area under curve; CD = Crohn disease; CDAI = Crohn disease activity index; CI = confidence interval; CRP = C-reactive protein; DUSPs = dual-specificity phosphatases; EAE = experimental autoimmune encephalomyelitis; ELISA = enzyme-linked immunosorbent assay; ESR = erythrocyte sedimentation rate; HCs = health controls; IBD = inflammatory bowel disease; IFN = interferon; IFX = infliximab; IL = interleukin; JKAP = JNK pathway-associated phosphatase; JNK = Jun-amino-terminal kinase; MAPK/ERK = mitogen-activated protein kinase / extracellular signal-regulated kinase; ROC = receiver operating characteristic; SAPK = stress-activated protein kinase; SLE = systemic lupus erythematosus; SLEDAI = systemic lupus erythematosus activity index; STAT3 = signal transducer and activator of transcription 3; TNF = tumor necrosis factor; W12 = 12 weeks.

Keywords: Crohn disease, JNK pathway-associated phosphatase, serum, treatment response, tumor necrosis factor-α

1. Introduction
Crohn disease (CD), a chronic inflammatory disease which mainly affects the gastrointestinal tract, is becoming more and more popular around the world. According to a previous study, the annual overall incidence of CD in China is highest among Asia countries, with more than 1 per 100,000 individuals, and the
number is still increasing.[1,2] Owing to the elevated morbidity and the high recurrence rate, the CD has grown into one of the most burdensome chronic diseases worldwide.[2,3] Among various therapeutic agents, tumor necrosis factor (TNF-α) inhibitors are commonly applied for inducing and maintaining therapies in severe CD patients, especially for patients who lose response or become intolerant to conventional treatment drugs such as mercaptopurine and methotrexate.[4] Despite of the superb efficacy of TNF-α inhibitors in treating CD, the high cost and the potential nonresponse bother both families and doctors, thus, exploring novel markers that could predict treatment response to TNF-α inhibitors in CD patients is pivotal.[5,6]

Dual-specificity phosphatases (DUSPs) that dephosphorylate both tyrosine and serine/threonine residues are involved in numerous biological activities.[7,8] As a member of the DUSPs family, Jun-amino-terminal kinase (JNK) pathway-associated phosphatase (JKAP, also named DUSP 22) is a 184-residue protein tyrosine phosphatase that is widely expressed in diverse human tissues.[9,10] A few studies discover that JKAP is involved in T-cell-mediated signaling pathways, and the decreased JKAP expression in peripheral blood T cells positively correlates with disease risk, activity, and inflammation level of systemic lupus erythematosus (SLE).[11,12] Meanwhile, JKAP expression in intestinal mucosa is reported to be associated with higher disease risk, activity, and inflammation level of inflammatory bowel disease (IBD). However, JKAP level in intestinal mucosa is not feasible to obtain and the sample size is small, while the correlation of circulating JKAP level with disease risk, activity, and inflammation level of CD as well as clinical response to TNF-α inhibitors is still unknown.[13] Therefore, the present study aimed to investigate the association of serum JKAP expression with disease risk, activity, and inflammation cytokine levels of CD, and more importantly, to explore whether JKAP level could be served as a novel biomarker for predicting treatment response to TNF-α inhibitor in CD patients.

2. Materials and methods

2.1. Participants

Ninety-six active CD patients underwent infliximab (IFX) treatment, at Tongji Hospital between January 2014 and September 2017 were consecutively enrolled in this study. Patients with the following conditions were included: diagnosed as CD according to clinical characteristics, radiological findings, endoscopic examination, and histological confirmation,[14]; age above 18 years; active disease condition which was defined as Crohn disease activity index (CDAI) equal or above 150; and about to undergo IFX treatment. While patients with the following conditions were excluded: complicated with other severe intestinal tract diseases; moderate to severe renal, hepatic, or heart diseases; history of intestinal surgery, solid tumor, or hematological malignancies; underwent biologics treatment within 6 months or underwent glucocorticoid treatment within 1 month; unable to be followed up for 12 weeks after the treatment; pregnancies; or lactation. In addition, 90 healthy controls (HCs) with age and gender matching to CD patients were also recruited to investigate the value of JKAP expression for predicting CD risk. This study was approved by the Ethics Committee of Tongji Hospital and conducted in accordance to the declaration of Helsinki; besides, each participant signed informed consent.

2.2. Baseline data collection

Age, gender, disease duration, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) of CD patients were recorded, and CDAI was evaluated.

2.3. Sample collection

Peripheral blood was obtained from CD patients before treatment and at week 12 (W12) after treatment, and from HCs after enrollment. The blood sample was then centrifuged at 4000 revolutions per minute for 6 minutes, and the serum sample was subsequently collected.

2.4. Enzyme-linked immunosorbent assay

Serum JKAP, tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-6, IL-10, IL-17 and IL-23 expressions were determined by enzyme-linked immunosorbent assay (ELISA) with the application of commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co, Ltd, China).

2.5. Treatment response assessment

Treatment response to IFX was assessed at W12, which was defined as a decrease of CDAI score equal or above 70 after IFX treatment, and according to whether treatment response was achieved at W12, CD patients were divided into response group and nonresponse group.

2.6. Statistics

Statistical analyses were performed using SPSS 19.0 Software (IBM, New York), and graphs were drawn using GraphPad Prism 6.01 Software (GraphPad, California). Data were displayed as mean ± standard deviation, median (25th–75th quantiles) or count (with or without percentage). Comparison between 2 groups was determined by Wilcoxon rank sum test, comparison between each visit in the same group was determined by Wilcoxon signed-rank sum test. Correlation of JKAP level with continuous data was determined by Spearman test. Receiver operating characteristic curves were made to assess the predictive value of JKAP expression for CD risk and IFX treatment response. In addition, logistic regression analysis was performed to detect the factors affecting IFX treatment response. P < .05 was considered as significant.

3. Results

3.1. Study flow

One hundred sixty-five CD patients were invited in the present study, among whom 29 patients missed the invitation, while 12 patients declined to attend prescreening procedure (Fig. 1). Thus, a total of 124 CD patients were screened for eligibility; during this process, 21 patients were ineligible, 7 patients disagreed to sign informed consents. Subsequently, the remaining 96 CD patients were enrolled and no patient quit during the study. Therefore, all the 96 CD patients completed our study and were included in analysis.

3.2. Baseline characteristics of CD patients and HCs

In CD patients, mean age was 34.5 ± 10.4 years, the numbers of females and males were 59 and 37, respectively. Meanwhile,
3.3. Predictive value of serum JKAP level for CD risk

Serum JKAP expression in CD patients was decreased compared to HCs \( P < .001 \), Fig. 2A) and it disclosed a good predictive value for CD risk with an area under curve (AUC) of 0.931 (95% confidence interval [CI]: 0.896–0.966). Meanwhile, the sensitivity and specificity were 87.5% and 88.9% at best cut-off point, at which the value of sensitivity plus specificity was largest (Fig. 2B).

3.4. Association of serum JKAP expression with characteristics of CD patients

JKAP level in serum was negatively associated with CRP \( P = .006 \), Fig. 4A) level and CDAI score \( P = .002 \), Fig. 3B), while no correlation of JKAP level with disease duration \( P = .569 \), Fig. 3C), age \( P = .107 \), Fig. 3D), ESR level \( P = .582 \), Fig. 3E), or gender \( P = .967 \), Fig. 3F) was discovered.

3.5. Association of serum JKAP expression with inflammatory cytokine levels in CD patients

Serum JKAP expression was negatively associated with TNF-\( \alpha \) \( P = .006 \), Fig. 4A), IL-17 \( P = .036 \), Fig. 4B), and IL-6 \( P = .013 \), Fig. 4C).

Table 1

| Characteristics                          | CD patients (N = 96) |
|-----------------------------------------|----------------------|
| Age (years)                             | 34.5 ± 10.4          |
| Gender (female/male)                    | 59/37                |
| Disease duration (years)                | 1.0 (0.0–5.0)        |
| Disease type                            |                      |
| Inflammatory                            | 67 (69.8)            |
| Structuring                              | 16 (16.7)            |
| Penetrating                              | 13 (13.5)            |
| CRP (mg/L)                              | 36.2 (23.8–49.3)     |
| ESR (mm/H)                              | 42.7 (32.6–53.1)     |
| CDAI                                     | 210.4 ± 43.9         |
| TNF-\( \alpha \) (pg/mL)                | 105.4 (55.0–172.4)   |
| IL-1\( \beta \) (pg/mL)                 | 11.0 (7.1–21.3)      |
| IL-6 (pg/mL)                            | 62.8 (37.5–103.6)    |
| IL-10 (pg/mL)                           | 35.4 (17.5–56.4)     |
| IL-17 (pg/mL)                           | 81.8 (44.9–171.6)    |
| IL-23 (pg/mL)                           | 205.7 (148.8–245.5)  |

Data were presented as mean value ± standard deviation, count, count (percentage) or median (25th–75th quantiles).

CD = Crohn disease, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, CDAI = Crohn disease activity index, TNF-\( \alpha \) = tumor necrosis factor-\( \alpha \), IL-1\( \beta \) = interleukin-1\( \beta \), IL-6 = interleukin-6, IL-10 = interleukin-10, IL-17 = interleukin-17, IL-23 = interleukin-23.
Fig. 4C) levels, whereas no correlation was observed between JKAP expression and IL-10 ($P = .965$, Fig. 4D), IL-1β ($P = .468$, Fig. 4E), or IL-23 ($P = .273$, Fig. 4F) level.

3.6. Treatment response rate and comparison of serum JKAP expression before and after IFX treatment in CD patients

A total of 68 (70.8%) CD patients achieved treatment response while the rest of 28 (29.2%) CD patients failed to achieve response after IFX treatment (W12) (Fig. 5A). After IFX treatment, serum JKAP expression in all CD patients was increased ($P < .001$, Fig. 5B), including response patients ($P < .001$, Fig. 5C) and nonresponse patients ($P < .001$, Fig. 5D), and response patients seemed to present with higher change of JKAP expression after IFX treatment compared to nonresponse patients.

3.7. Predictive value of serum JKAP level at baseline for treatment response of IFX

As shown in Figure 6A, serum JKAP expression at baseline in response patients was lower than that in nonresponse patients ($P = .002$), baseline JKAP expression also exhibited a good predictive value for clinical response to IFX in CD patients with an AUC of 0.699 (95% CI: 0.590–0.808). The sensitivity and specificity were 67.6% and 71.4% at best cut-off point, respectively (Fig. 6B).
3.8. Logistic regression model analysis of factors affecting IFX treatment response

Univariate logistic regression was applied for analyzing factors affecting IFX treatment response in CD patients, which revealed that serum JKAP expression ($P = .003$) was correlated with worse IFX treatment response, while CRP ($P = .006$) and IL-17 levels ($P = .019$) were correlated with better IFX treatment response (Table 2). All factors were further analyzed via multivariate logistic regression, and the results elucidated that JKAP expression ($P = .008$) was an independent factor for predicting worse IFX treatment response, while CRP level ($P = .016$) was an independent factor for predicting better IFX treatment response.

4. Discussion

In the present study, we discovered that: serum JKAP level disclosed a great predictive value for decreased CD risk, and it was negatively associated with disease activity and inflammatory cytokines levels. All CD patients exhibited increased JKAP level after IFX treatment, and the decreased baseline JKAP level was an independent factor for predicting better IFX treatment response. JKAP belongs to the low molecular weight atypical DUSPs family, which is involved in a variety of biological and pathological processes.\cite{15-19} Recently, several studies have reported the predictive value of JKAP for disease risk and its negative correlation with disease activity and inflammation level in several immune diseases.\cite{11-13} It is reported that JKAP-
knockout mice are more susceptible to experimental autoimmune encephalomyelitis (EAE) compared to wild-type mice. What is more, JKAP-knockout EAE mice exhibit increased serum interferon (IFN)-γ and IL-17 levels, they also present with greater numbers of infiltrating CD4+ T cells, IFN-γ+ Th1 cells, and IL-17+ Th17 cells in their brain tissues compared to JKAP-knockout healthy mice or wild-type EAE mice, implying that the decreased JKAP level correlates with higher risk of immune disease and elevated inflammation level. Another study discovers that JKAP expression in T cells could be served as a diagnostic biomarker for SLE complicated with active lupus discases that JKAP expression in T cells could be served as a diagnostic biomarker for SLE complicated with active lupus.

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**Table 2**

Factors affecting infliximab treatment response by logistic regression model analysis.

| Parameters                  | Univariate logistic regression | | Multivariate logistic regression |
|-----------------------------|-------------------------------|------------------|-------------------------------|
|                             | P     | OR Lower | 95% CI Lower | OR Higher | 95% CI Higher |
| JKAP expression (M0)        | .003  | 0.971    | 0.953 - 0.990 | .008      | 0.963 - 0.991 |
| Age                         | .405  | 0.982    | 0.940 - 1.025 | .569      | 0.981 - 1.020 |
| Gender (female)             | .715  | 0.844    | 0.339 - 2.102 | .787      | 0.841 - 2.240 |
| Disease duration            | .258  | 0.950    | 0.869 - 1.038 | .151      | 0.906 - 1.792 |
| CRP                         | .006  | 1.044    | 1.013 - 1.077 | .016      | 1.054 - 1.010 |
| ESR                         | .315  | 1.015    | 0.986 - 1.044 | .429      | 1.018 - 0.974 |
| CDAI                        | .924  | 1.000    | 0.990 - 1.010 | .798      | 1.184 - 0.324 |
| TNF-α                       | .838  | 1.001    | 0.995 - 1.006 | .154      | 0.994 - 1.002 |
| IL-1β                       | .398  | 1.021    | 0.973 - 1.071 | .204      | 0.933 - 0.839 |
| IL-6                        | .690  | 1.000    | 1.000 - 1.000 | .749      | 1.000 - 1.000 |
| IL-10                       | .833  | 0.986    | 0.984 - 1.015 | .690      | 0.995 - 0.972 |
| IL-17                       | .019  | 1.007    | 1.001 - 1.014 | .286      | 1.004 - 0.996 |
| IL-23                       | .545  | 1.002    | 0.996 - 1.007 | .550      | 0.998 - 0.990 |

Data were presented as P value, OR, and 95% CI. Factors affecting IFX treatment response were determined by logistic regression model analysis. P < .05 was considered significant.

CDAI = Crohn disease activity index, CI = confidence interval, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, IFX = infliximab, IL-1β = interleukin-1β, IL-10 = interleukin-10, IL-17 = interleukin-17, IL-23 = interleukin-23, JKAP = dual specificity protein phosphatase 22, OR = odds ratio, TNF = tumor necrosis factor.

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As previously described, a few studies that focus on the role of JKAP in immune disease diagnosis and monitoring have been reported, whereas only 1 study investigates the predictive value of JKAP level for treatment response to IFX in CD patients. In that previous study, both mRNA and protein levels of JKAP in intestinal mucosa are upregulated after IFX treatment compared to that before IFX treatment in response patients. Meanwhile, mRNA expression of JKAP is lower in patients who achieve response than that in patients who fail to achieve response.[11,12,19] However, in that study, JKAP expression is assessed in intestinal mucosa where JKAP level is less feasible to be determined; besides, the sample size is too small, with only 25 patients included. Therefore, whether serum JKAP expression is capable of predicting treatment response to TNF-α in CD patients remains unknown. In the present study, we discovered that all CD patients exhibited increased JKAP level after IFX treatment, and baseline JKAP level was an independent factor for predicting lower possibility of IFX treatment response. The result might be due to that: as a TNF-α inhibitor, IFX was able to inhibit TNF-α-mediated inflammatory activity via repressing TNF-α level, and then reduced disease activity and inflammation level of CD; thus, JKAP expression was elevated after IFX treatment. Response patients presented with greater reduction of inflammation level after treatment compared to nonresponse patients, so the response patients exhibited higher elevation of JKAP level after IFX treatment compared to nonresponse patients. Firstly, treatment response to IFX was defined as a decrease of CDAI score equal or above 70 after IFX treatment, and patients with higher baseline JKAP level tended to present with decreased CDAI score and corresponding lower gap for CDAI decrement. Secondly, patients with higher JKAP level exhibited less disease activity and inflammation level, which might reduce the efficacy of anti-inflammation treatment (including IFX). Hence, patients with increased JKAP expression
at baseline were less likely to obtain clinical response to IFX treatment.

There were some limitations in the present study: the observation period of treatment response was only 12 weeks, thus the predictive value of serum JKAP expression for a long-term IFX treatment response in CD patients needed additional investigation in the future studies. We only investigated the value of JKAP in predicting response to IFX, while its predictive value for response to other TNF inhibitors (such as adalimumab and golimumab) was not explored. Considering that there is no relevant study investigating the value of JKAP for predicting response to other TNF inhibitors either, additional studies are required in the future. Most of the patients in the present study were from Middle China, which might lead to selection bias. The sample size in this study might not be sufficient enough, which might be due to the relatively low incidence of CD and the extremely high cost of IFX in China. Besides, a proportion of eligible CD patients were excluded from the study because they declined to sign the informed consents or other personal reasons. The association of serum JKAP level with fecal calprotectin level in CD patients also needed additional investigations.

In conclusion, circulating JKAP expression correlates with decreased disease risk, activity, and inflammation level, and it could be served as a novel biomarker for reduced clinical response to TNF-α inhibitor in CD patients.

**Author contributions**

Conceptualization: Xue Shi.

Formal analysis: Wei Yang.

Investigation: Xue Shi.

Methodology: Nian Wang.

Writing – original draft: Xue Shi.

Writing – review & editing: Junyi Zhu.

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