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Research Article

Clinical value of the expression levels of protein tyrosine phosphatase non-receptor type 22.6 mRNA in peripheral blood mononuclear cells in Crohn's disease

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Abstract

Objective: To explore the relationship between the expression levels of protein tyrosine phosphatase non-receptor type (PTPN) 22.6 mRNA in peripheral blood mononuclear cells (PBMCs) and the disease activity as well as clinical characteristics in Crohn's disease (CD) patients.

Methods: A total of 480 subjects were enrolled. Data were collected including baseline information, expression levels of PTPN22.6 mRNA in PBMCs for all subjects, C-reactive protein (CRP) levels in serum, clinical characteristics, and disease activity for all patients. Expression levels of PTPN22.6 mRNA in PBMCs, CRP levels in serum, clinical characteristics according to Montreal Classification [8], and Crohn's disease activity index (CDAI) were the primary observation outcomes.

Results: The expression levels of PTPN22.6 mRNA ($P = 0.032$) in PBMCs and serum CRP levels ($P < 0.001$) were significantly higher in active CD patients than in inactive CD patients ($P = 0.032$). Correlation analysis showed that there was a positive correlation between expression levels of PTPN22.6 mRNA and CDAI value ($r = 0.512$, $P = 0.003$), as well as expression levels of PTPN22.6 mRNA and CRP levels in the CD group ($r = 0.456$, $P = 0.006$). There were significantly higher expression levels of PTPN22.6 mRNA in PBMCs in patients with structuring behavior than that in patients with non-stricturing and non-penetrating (NSNP) behaviors ($P = 0.018$) and penetrating behaviors ($P = 0.024$).

Conclusions: The expression levels of PTPN22.6 mRNA can be used as an indicator to help predict CD diagnosis, disease activity, serum CRP level, and behavior type of CD disease.

Keywords: Crohn's disease, protein tyrosine phosphatase non-receptor type 22, peripheral blood mononuclear cells, C-reactive protein

Introduction

Crohn's disease (CD) is a recurrent chronic intestinal inflammatory syndrome that could involve the entire digestive tract and is often accompanied by extraintestinal manifestations, such as oral ulcers, joint pain, erythema nodosum, and so on [1, 2]. Current studies indicate that CD results from intestinal immune imbalances to microbial antigens in hosts with genetic predisposition, in which T cell-mediated immunity plays a critical role in the pathogenesis of CD [1, 2].

Protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene codes a lymphoid-specific tyrosine phosphatase (LYP), which could reduce the activation T cell immunity by dephosphorylation of a few pivotal mediators in T cell receptor (TCR) signaling pathways, such as proto-oncogene tyrosine-protein kinase (Fyn) and lymphocyte-specific protein tyrosine kinase (Lck), as well as TCR zeta and zeta associated protein (ZAP)-70 [3, 4]. Recent studies have discovered another human PTPN22 splice form, namely, PTPN22.6, which lacks almost total phosphatase domain and could function as a dominant-negative isoform of the full-length PTPN22 [5, 6]. PTPN22.6 antagonizes the influence of PTPN22-Csk on T-cell signal transduction as well as weakens the inhibitory effects of PTPN22 on T-cells, resulting in excessive T cell activation, which acts a critical role in the CD pathogenesis [1, 2, 5, 6]. Chang found that the high levels of PTPN22.6 mRNA expression in peripheral blood mononuclear cells (PBMCs) were associated with disease activity in patients with rheumatoid arthritis, suggesting that PTPN22.6 might be a new serum marker in patients with rheumatoid arthritis [6]. While the clearer relationship between abnormal expression of PTPN22.6 mRNA and CD patients in clinical remains to be investigated.

Herein, our study aimed to explore the relationship between the expressions of PTPN22.6 mRNA in PBMCs and the disease activity as well as clinical characteristics in CD patients, further providing a clinical research basis for the diagnosis and treatment of CD disease.

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Materials and methods

Subjects

From 1 January 2012 to 30 December 2018, a total of 480 subjects were enrolled in this retrospective study. The CD group included 180 patients with CD disease in the Central Hospital of Wuhan. The control group included 300 healthy volunteers recruited from The Central Hospital of Wuhan. This study protocol was formulated in accordance with the requirements of the Declaration of Helsinki of the World Medical Association. It was approved by the Ethics Committee of the Central Hospital of Wuhan (NO.2014IECS002).

Inclusion and exclusion criteria

Inclusion criteria: CD patients diagnosed in accordance with the criteria of Lennard-Jones [7].;

Exclusion criteria: 1. Control group with a family history of CD; 2. Control group with the symptoms of chronic bowel disorders.

Data collection

Data were collected including baseline information, expression levels of PTPN22.6 mRNA in PBMCs for all subjects, C-reactive protein (CRP) levels in serum, clinical characteristics, and disease activity for all patients.

Clinical characteristics were described according to the Montreal classification [8], and the main factors included: disease location (L1: terminal ileum, L2: colon, L3: ileocolon, L4: isolated upper gastrointestinal) age at diagnosis (A1: <16, A2: 17–40, A3: >40) and disease behavior (B1: non-stricturing and non-penetrating (NSNP), B2: stricturing, B3: penetrating).

Crohn's disease activity index (CDAI) was used to evaluate the clinical activity of the patients with CD [9]. While a CDAI score ≥150, the CD patients were in an active phase.

Expression levels of PTPN22.6 mRNA in PBMCs, CRP levels in serum, clinical characteristics according to Montreal Classification [8], CDAI was the primary observation outcomes.

Preparation of PBMCs

PBMCs were separated from heparinized venous blood of controls and the patients with CD via Ficoll-Hypaque density gradient centrifugation (AXIS-SHIELD, Oslo, Norway), and then the concentration of cell was adjusted to 2×10^6 ml.

RNA isolation and qRT-PCR of PTPN 22.6 mRNA

The procedure for RNA isolation of the PBMCs and qRT-PCR (qReal-time PCR) of PTPN22.6 mRNA using SYBR Green PCR Kit was carried out according to our previous study [10]. The primer sequences were as follows: PTPN22.6—forward 5′-TTT GCC CTA TGA TTA TAG CCG-3′, and reverse 5′-GTT CTC AGG AAT TAT AAG GAC ACT-3′ [6]. The β-actin was used as an internal control gene. The primer sequences of β-actin and PTPN22 were in accordance with our previous study [10]. The relative changes in expression of PTPN22.6 mRNA levels in the PBMCs were calculated according to comparative CT-value method [11, 12].

Measurement of CRP levels in serum

A fasting venous blood sample was collected from the healthy controls and CD patients, and then centrifuged (L2-6K desktop low-speed centrifuge, Hunan Kecheng Instrument Equipment Co., LTD.) at room temperature at 1000*g* for 15 min. The serum was collected and stored in a 1.5 ml sterile EP tube at −80°C prior to analysis.

The serum CRP levels (mg/L) were measured according to the immunonephelometry method based on the manufacturer's guidelines [13]. Each sample was measured in triplicate.

Statistical analysis

All the data collected in this study were analyzed using SPSS 20.0 software. The normality of continuous variables was tested by the Shapiro–Wilk test as well as the graphical illustration of histograms and Q–Q plots. Normally distributed measurement data were expressed as mean ± SD, while non-normally distributed measurement data were expressed as median (interquartile range), and the comparisons were examined by Student's *t*-test and Mann–Whitney test (non-parametric distribution). The categorical data were expressed as *n* (%), and the differences between the two groups were examined by Chi-square analysis or Fisher's Exact Test. Spearman's correlation test was performed for the correlation analysis. The statistical significance level was set at 0.05 for a two-sided test.

Results

Demographic characteristics of subjects

Baseline data and clinical characteristics of all subjects are presented in Table 1. Of all the 480 subjects included, 289 (60.2%) were males. The CD group included 104 (57.8%) males with an average age of 37.21 ± 14.78 yrs. The control group included 185 (61.7%) males with an average age of 39.09 ± 11.17 yrs. In the CD group, 50 (27.8%) patients had extraintestinal manifestations. Among CD patients, 131 (72.8%) patients received sulfasalazine or 5-aminosalicylates, 83 (46.1%) patients received an addition of steroid for relief of symptoms, 26 (14.4%) received azathiopurine/6-mercaptopurine/methotrexate, 13 (7.2%) received Infliximab and 50 (27.8%) received operation treatment.

Comparison of the expression levels of PTPN22.6 mRNA

The expression levels of PTPN22.6 mRNA in PBMCs were significantly higher in the CD group compared with that in the control group ($P = 0.015$) (Fig. 1), and the expression levels of PTPN22.6 mRNA in PBMCs were significantly higher in active CD patients than that in inactive CD patients ($P = 0.032$) (Fig. 1). Correlation analysis showed that there was a positive correlation between expression levels of PTPN22.6 mRNA and CDAI value in the CD group ($r = 0.512$, $P = 0.003$).

We also detected the PTPN22 mRNA expression levels in PBMCs in CD patients by quantitative PCR. The result showed that PTPN22 mRNA expression levels were not significantly different between active Crohn's disease and controls ($P > 0.05$).

Table 1 : demographic characteristics and clinical characters of subjects

	CD	Healthy controls
	n = 180	n = 300
Male	104 (57.8 %)	185 (61.7 %)
Age (yrs) [mean ± SD]	37.21 ± 14.78	39.09 ± 11.17
Age at diagnosis (yrs) [mean ± SD]	33.45 ± 14.28	
A1:< 16	9 (5.0 %)	
A2:17–40	119 (66.1 %)	
A3:> 40	52 (28.9 %)	
Disease location		
L1: Terminal ileum	52 (28.9 %)	
L2: Colon	38 (21.1 %)	
L3: Ileocolon	79 (43.9 %)	
L4:Isolated upper GI	11 (6.1 %)	
Disease behaviour		
B1: non-stricturing, non-penetrating	86 (47.8 %)	
B2: stricturing	44 (24.4 %)	
B3: penetrating	50 (27.8 %)	
CDAI [mean ± SD]	172.3 ± 84.1	
Active disease	70 (38.9 %)	
Inactive disease	110 (61.1 %)	
Extra-intestinal manifestations	50 (27.8 %)	
Treatment		
5-ASA/ SASP	131 (72.8 %)	
Steroid	83(46.1 %)	
Azathiopurine/6-mercaptopurine/ methotrexate	26 (14.4 %)	
Infliximab	13 (7.2 %)	
Operation	50 (27.8 %)	

CD: Crohn's disease; CDAI: Crohn's disease activity index;SD: standard deviation; 5-ASA: 5-aminosalicylate; SASP: sulfasalazine.

The relationship between expression levels of PTPN22.6 mRNA and CRP levels in the CD patients

Serum CRP levels were significantly increased in active CD patients than that in inactive CD patients ((9.34 ± 2.15) mg/l vs. (4.67 ± 1.34) mg/l, $P < 0.001$). Correlation analysis showed that there was a positive correlation between expression levels of PTPN22.6 mRNA and CRP levels in CD group ($r = 0.456$, $P = 0.006$).

Comparison of expression levels of PTPN22.6 mRNA in CD patients with different clinical characteristics

The behavior of disease, age at diagnosis, and disease location were taken as dependent variables, respectively to compare the expression levels of PTPN22.6 mRNA in CD patients. The results showed that there were no significant differences in expression levels of PTPN22.6 mRNA among patients at different ages of diagnosis and disease locations (both $P > 0.05$). Analysis of different disease behaviors showed significantly higher expression levels of PTPN22.6 mRNA in PBMCs in patients with stricturing behavior than that in patients with NSNP behaviors ($P = 0.018$) and penetrating behaviors ($P = 0.024$) (Fig. 2).

Discussion

The PTPN22 gene is located on human chromosome 1p13 and encodes LYP [3, 4]. Numerous studies have shown that

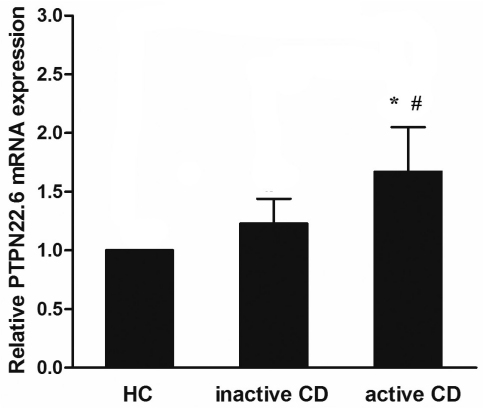

Figure 1: relative expression levels of protein tyrosine phosphatase non-receptor type (PTPN) 22.6 mRNA in peripheral blood mononuclear cells (PBMCs) from inactive Crohn's disease (CD) patients, active CD patients and healthy controls (HC), utilizing arbitrary units. PTPN22.6 mRNA expression levels were normalized to the expression in healthy controls in which PTPN22.6 mRNA expression levels were set arbitrary as 1.0. Data are expressed as means ± SD. c vs. a, $P < 0.05$, c vs. b, $P < 0.05$, b vs. a, P = NS. NS: not significant.

LYP was a negative regulator of T cell signaling pathways [3, 4]. PTPN22 may be involved in the regulation of T-cell differentiation and resistance to the inflammatory response [14, 15]. Currently, many studies found that the PTPN22 gene was associated with human susceptibility to type I diabetes, rheumatoid arthritis, Graves's disease, and inflammatory bowel disease [16–18]. The PTPN22 gene generates two different isoforms: a full-length PTPN22 (namely PTPN22.1) and PTPN22.6 in which the phosphatase domain is lost [19]. Ronninger and Chang found that PTPN22.6 may be an important immunoregulator, which could weaken the inhibitory effects of PTPN22 on T-cells, resulting in excessive T-cell activation [5, 6]. Excessive activation of T cells acts a critical role in CD pathogenesis [19, 20]. We hypothesize that higher expressions of PTPN22.6 could disrupt this potential the balance between PTPN22.1 and PTPN22.6, and result in excessive activation of T-cell immunity, hence bringing about the immune phenotype characteristic of CD.

The current study showed that PTPN22.6 mRNA expression levels in PBMCs were increased in patients with CD, and were positively related to CDAI and the serum levels of CRP. PTPN22.6 mRNA levels were increased in active CD patients than that in non-active CD patients. Several recent reports supported our results showing that PTPN22.6 mRNA expression levels were increased in rheumatoid arthritis PBMCs and that high PTPN22.6 mRNA expression levels were associated with disease activity in rheumatoid arthritis [6]. In addition, the imbalance at the level of the spliced form of PTPN22 was different in patients with rheumatoid arthritis compared with controls [5]. Chang [21] indicated that patients with systemic lupus erythematosus (SLE) had increased expressions of PTPN22 and lower expressions of PTPN22.6 mRNA compared with healthy controls, as well as increased expressions of PTPN22 were negatively related to the Damage Index of SLE. This discrepancy in PTPN22.6 mRNA expression may originate from the different pathogenesis between CD and SLE. PTPN22.6 almost lacks the total phosphatase domain, which is an alternatively spliced form of PTPN22 with different expressions and functions [5, 6]. Spalinger found that

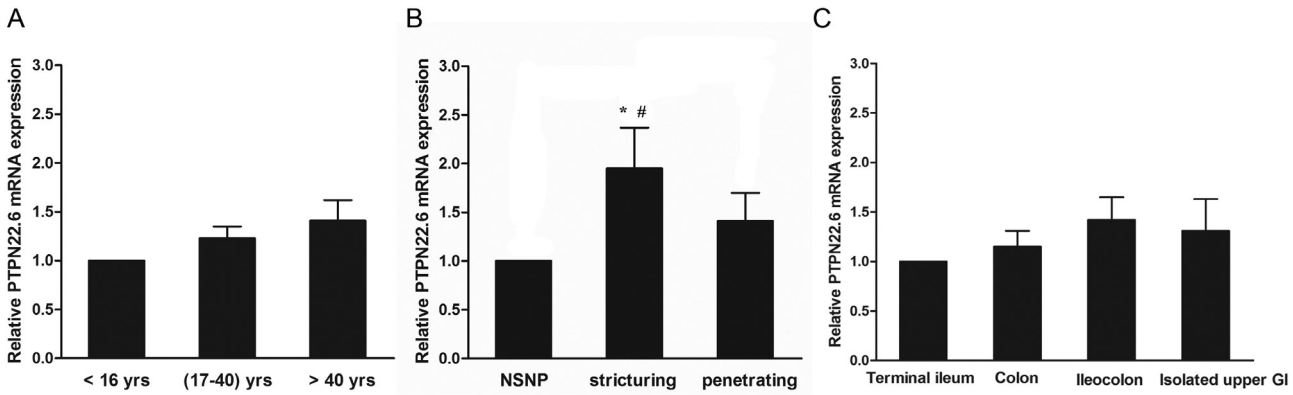

Figure 2 : relative expression levels of protein tyrosine phosphatase non-receptor type (PTPN) 22.6 mRNA in peripheral blood mononuclear cells (PBMCs) in patients with Crohn's disease (CD) after stratification by age at diagnosis (yrs) (A), disease behaviour (B) and disease location (C), utilizing arbitrary units. PTPN22.6 mRNA expression levels were normalized to the expression < 16yrs group, non-stricturing, non-penetrating (NSNP) disease behavior group, and terminal ileum group in which PTPN22 mRNA expression levels were set arbitrary as 1.0, respectively. Data are expressed as means ± SD. e vs. d, $P < 0.05$, e vs. f, $P < 0.05$, f vs. d, P = NS. NS: not significant.

PTPN22 mRNA expression levels were decreased in intestinal tissue samples from patients with CD [22].

Furthermore, we evaluated the possible relationship between PTPN22.6 mRNA expression levels in CD patients and clinical disease characteristics. The result showed that the higher expression levels of PTPN22.6 mRNA in PBMCs were not associated with the factors of the location of disease and age at diagnosis, and were affected by disease behavior in CD patients. The patients with stricturing behavior had higher expression levels of PTPN22.6 mRNA than that in other disease behaviors. Stricturing behavior was considered to be a more severe disease type with higher rates of surgical operation, which provided a new support for abnormally increased expressions of PTPN22.6 mRNA in PBMCs as additional indicators for the need for surgical operation in the patients with CD. Relatively limited information exists on the relationship between clinical disease characteristics of CD and expression levels of PTPN22.6 mRNA to date.

One of the limitations was that the small sample size may weaken the generalizability of the results. Another limitation was that the lack of treatment information limited the exploration of possible influencing factors. In the next study, patients with different expression levels of PTPN22.6 mRNA should be included in a random, blind, and large sample size to explore possible influencing factors and relationships.

Conclusion

In summary, the expression levels of PTPN22.6 mRNA can be used as an indicator to help predict CD diagnosis, disease activity, serum CRP level, and behavior type of CD disease.

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Conflict of interests

The authors declare that they have no competing interests.

Authors contributions

H.M. and Z.H. contributed to the conception and design of the study; C.Z.T., L.Y.S., W.J., and Z.D. performed the experiments, collected and analyzed data; H.M. and Z.H. wrote the manuscript; all authors reviewed and approved the final version of the manuscript.

Ethics approval and consent to participate

This study protocol was formulated in accordance with the requirements of the Declaration of Helsinki of the World Medical Association. It was approved by the Ethics Committee of the Central Hospital of Wuhan (NO.2014IECS002).

Consent for publication: Not applicable.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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