Original Article

Single nucleotide polymorphism and promoter methylation analysis of protein tyrosine phosphatase 1B in patients with myeloproliferative neoplasms

Jie Zhou[#], Hao Wu[#], Bing Li[#], Lili Zhou, Wenjun Zhang, Yi Ding, Xinyu Zhu, Huina Lu, Bing Xiu, Aibin Liang, Jianfei Fu

Department of Hematology, Tongji Hospital of Tongji University, Tongji University School of Medicine, Shanghai, China

Contributions: (I) Conception and design: J Fu; (II) Administrative support: A Liang; (III) Provision of study materials or patients: J Zhou, L Zhou, W Zhang, Y Ding, X Zhu, H Lu, B Xiu; (IV) Collection and assembly of data: J Zhou, H Wu; (V) Data analysis and interpretation: J Zhou, B Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Jianfei Fu, MD; Aibin Liang, MD. Department of Hematology, Tongji Hospital of Tongji University, Tongji University School of Medicine, No. 389 Xincun Road, Putuo District, Shanghai 200065, China. Email: fjf2017@tongji.edu.cn; lab7182@mail.tongji.edu.cn.

Background: The recurrent somatic mutations in genes such as Janus kinase 2 (JAK2) lead to cytokine-independent activation of the JAK-signal transducer and activator of transcription (STAT) pathway, a crucial factor in the development of classic myeloproliferative neoplasms (cMPNs). Protein tyrosine phosphatase 1B (PTP1B) is a significant regulator in this pathway, while the single nucleotide polymorphism (SNP) and promoter methylation profiles of the *PTP1B* gene in cMPN patients have largely remained unexplored. Therefore, to further explore the SNP and promoter methylation profiles of the *PTP1B* gene in cMPNs, we conducted a comprehensive SNP analysis of the *PTP1B* gene as well as the methylation status detection of the *PTP1B* promoter between cMPN patients and healthy controls.

Methods: Bone marrow (BM) biopsies were collected from a cohort comprising 96 cMPN patients and 50 healthy controls. SNP-specific extension primers were utilized to facilitate single base extension at the SNP site. A MALDI-TOF mass spectrometer and MassARRAY Typer software were used to detected the SNP. The incidence of SNPs within *PTP1B* were calculated in cMPN patients and healthy controls. The promoter region of the *PTP1B* gene were amplified and methylation Bisulfite amplicon sequencing (BSAS) analysis were performed, MethylKIT software was utilized to analyzed the methylation levels at each CpG site of *PTP1B*. Visualization of data was facilitated using the Methylation Plotter software. Statistical analysis of methylation was performed using the Kruskal-Wallis test. Differences of methylation at *PTP1B* gene sites were analyzed by Kruskal-Wallis test. P values <0.05 were considered to be statistically significant.

Results: Our findings revealed seven coding-region SNPs, including a novel variant (g.50579818T>A). Additionally, we identified aberrant hypermethylation and hypomethylation of several CpG islands within the *PTP1B* gene. Notably, the incidence of SNPs was significantly different between cMPN patients and healthy controls, and the methylation level of the *PTP1B* promoter was markedly elevated in cMPN samples compared to healthy controls.

Conclusions: In this study, we identified a novel SNP and observed differences in the frequency of seven SNPs and hypermethylation of *PTP1B* promoters between cMPN patients and normal controls. These results suggest that the *PTP1B* gene might play a critical role in the pathogenesis of cMPNs. Further research exploring more mechanism and larger sample is warranted to fully elucidate the specific role of PTP1B in cMPNs.

Keywords: Protein tyrosine phosphatase 1B (*PTP1B*); single nucleotide polymorphism (SNP); methylation; classic myeloproliferative neoplasms (cMPNs)

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Introduction

Background

Philadelphia chromosome-negative classic myeloproliferative neoplasms (cMPNs), which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are clonal disorders characterized by the hyperplasia of one or more myeloid cell lineages (1). A significant proportion of cMPN patients exhibited cytokine-independent activation of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway due to recurrent somatic mutations in genes such as *JAK2*, calreticulin (*CALR*) and thrombopoietin receptor (*MPL*) (2). Additionally, other somatic mutations implicated in deoxyribonucleic acid (DNA) methylation, histone modification, messenger ribonucleic acid (mRNA) splicing, transcription, and signal transduction have been identified (3-5). Although the pathogenesis of cMPNs is multifactorial, the activation of the JAK/STAT pathway is of critical importance. This pathway is regulated by various inhibitors, including cytokine signaling (SOCS) (6), protein tyrosine phosphatase (PTP) (7) such as PTP1B, Src homology region 2 domain-containing phosphatase (SHP)-1, SHP-2, and cluster of differentiation 45 (CD45), and protein inhibitors of activated STAT (PIAS) (8). However, the specific roles of these negative feedback regulators in the pathogenesis of cMPNs remain poorly understood.

The tyrosine-protein phosphatase non-receptor type 1 gene (*PTP1B*, also known as *PTPN1*) functions as a negative feedback regulator within the PTP family. It encodes the PTPN1 protein (PTP1B), which is widely expressed across various tissues (9-11). The *PTP1B* gene has been implicated in both oncogenic and tumor suppressor activities (12). Huang *et al.* found that PTP1B inhibited cancer stemness and chemoresistance of triple negative breast cancer (13). Liu *et al.* found recurrent somatic mutations and splice variants of *PTP1B* in human B-cell and Hodgkin's lymphoma (14). The *PTP1B* gene can suppress tumorigenesis through direct interactions between its encoded protein PTP1B, and oncoproteins, or by modulating downstream pathways (15).

The *PTP1B* gene is involved in hematopoiesis and plays a crucial role in regulating erythropoiesis. It downregulates the erythropoietin (EPO)-mediated JAK/STAT pathway (16), thereby influencing the production of red blood cells. Additionally, the *PTP1B* gene promotes the progression of monocyte-phagocytic cells into fully differentiated macrophages (17) by downregulating the colony-stimulating factor-1 receptor (CSF1R)-mediated signaling pathway. This gene is also associated with various myeloid neoplasms. Up-regulation of *PTP1B* gene expression prevents the transformation of Rat-1 cells induced by breakpoint cluster region (BCR)-breakpoints in the Abelson (ABL) and promotes the differentiation of BCR-ABL-expressing cells (18). Furthermore, the deficiency of *PTP1B* gene specifically in myeloid cells is sufficient to promote the development of acute myeloid leukemia (19).

Rationale and knowledge gap

Studies have shown that increased methylation of the *PTP1B* gene promoter is a risk factor for cancer. Hypermethylation of normally unmethylated CpG islands of tumor suppressor

Highlight box

Key findings

- In this study, we identified a novel single nucleotide polymorphism (SNP) and observed differences in the frequency of seven SNPs and hypermethylation of protein tyrosine phosphatase 1B (*PTP1B*) promoters between classic myeloproliferative neoplasms (cMPNs) patients and normal controls.

What is known and what is new?

- PTP1B downregulates the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway; however, the specific roles of these negative feedback regulators in the pathogenesis of cMPNs remain poorly understood.
- We identified a novel SNP and observed differences in the frequency of seven SNPs between cMPN patients and normal controls. Additionally, we noted hypermethylation of *PTP1B* promoters compared to normal controls. These findings suggest that the *PTP1B* gene is disrupted in cMPNs and may contribute to the pathogenesis of these conditions.

What is the implication, and what should change now?

- PTP1B expression is disrupted in cMPNs and may contribute to the pathogenesis of these conditions. Therefore, PTP1B could be a new potential therapeutic target for cMPN patients.

genes is associated with transcriptional silencing and plays a critical role in cancer development and progression (20). Mutations in the *PTP1B* gene result in a loss of phosphatase activity and increase in the phosphorylation of JAK and STAT family members. This leads to heightened cytokine sensitivity, elevated JAK-STAT signaling, and alterations in gene expression (21).

The *PTP1B* gene, located on human chromosome 20 within the region q13.1–q13.2, frequently undergoes deletion [del(20q)] in Philadelphia chromosome-negative cMPNs. This deletion affects the *PTP1B* gene locus, leading to loss of its expression and thereby disrupting its negative feedback regulation of the JAK/STAT signaling pathway (22). Despite its significance, research on the role of the *PTP1B* gene in cMPNs remains limited.

Objective

Building on this background, we propose that abnormalities in the *PTP1B* gene contribute to cMPNs by impairing its negative feedback regulation of the JAK/STAT pathway. This dysregulation may lead to increased activation of the JAK/STAT pathway, similar to the effects observed with the *JAK2*V617F mutation. To investigate this hypothesis, we conducted a study analyzing single nucleotide polymorphisms (SNPs) and methylation levels of *PTP1B* gene in cMPN patients, utilizing DNA sequencing and Methylation MassArray analysis to confirm the role of the *PTP1B* gene in the pathogenesis of cMPNs.

Methods

Patients and samples

Bone marrow (BM) biopsies were collected from a cohort comprising 96 patients diagnosed with chronic myeloproliferative neoplasms (cMPNs) and 50 healthy controls. Samples were obtained from the hematology departments of hospitals in Shanghai, including Tongji Hospital of Tongji University and Ruijin Hospital. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by both ethics committees of Tongji Hospital of Tongji University (No. 2021-KYSB-177) and Ruijin Hospital (No. SHDC2020CR5002-2021-105). Informed consent was obtained from all individual participants.

Diagnosis of cMPNs was based on the 2016 World Health Organization (WHO) diagnostic criterial (23). Mononuclear cells were isolated from BM samples, and genomic DNA was extracted using the Axygen Scientific DNA Extraction Kit. Subsequent analyses, including SNP genotyping and methylation Bisulfite amplicon sequencing (BSAS) analysis, were performed on the extracted DNA using the EZ DNA Methylation-Gold Kit (Zymo Research Corp., California, United States) and VAHTS Turbo DNA Library Prep Kit for Illumina® (ND102-0102) (Vazyme, Nanjing, China), following the manufacturers' protocol.

Identification the PTP1B gene SNPs

The *PTP1B* gene sequence was retrieved from GenBank and primers were designed using Primer Premier 5.0 software. The selected primers were subsequently compared on the National Center for Biotechnology Information (NCBI) website for sequence alignment using the Basic Local Alignment Search Tool (BLAST) tool. Primer specificity was confirmed through sequence alignment with the NCBI BLAST tool, ensuring that homology with non-target sequences was less than 70%. The synthesis of primers was carried out by Shenzhen Huada Gene Company, and their sequences are provided in *Table 1*. Polymerase chain reaction (PCR) amplification was conducted, and the resulting products were analyzed using 2% agarose gel electrophoresis. The purification of the PCR products involved treatment with shrimp alkaline phosphatase and exonuclease. SNP-specific extension primers were utilized to facilitate single base extension at the SNP site.

Sample preparation involved co-crystallization with chip matrices, followed by placing the crystal in the vacuum tube of the mass spectrometer. An intense nanosecond (10^{-9} s) laser was used to excite the nucleic acid molecules, desorbing and transforming them into singly charged ions. Detection and analysis were then conducted using a MALDI-TOF mass spectrometer and MassARRAY Typer software.

Methylation BSAS analysis of PTP1B gene

Sequencing procedures targeting the promoter region of the *PTP1B* gene were detailed in *Figure 1A,1B*. The target fragment comprises two base sequences, denoted as pair 1 and pair 2, with primer sequences highlighted in yellow and green, respectively. The amplified fragment includes a CG site marked in red font. Relative positions of the CG site with respect to chromosome positions are summarized in *Table 2*.

Methylation detection protocols employing BSAS are outlined in *Figure 1C*, with subsequent result descriptions referencing relative position annotations. The MethylKIT

Table 1 Primer sequences for identification of the *PTP1B* gene SNPs

Primer	Sequence
PTP1B-1-F	GGTTGACATCAAGAACCAGC
PTP1B-1-R	CATCGAATCCTCAAGCAGTA
PTP1B-2-F	ACCTCTGAATTATCACCTTGC
PTP1B-2-R	CGTCATAAACCTCTGCTACATT
PTP1B-3-F	ATCTCAACTAAAACAGGGCTTC
PTP1B-3-R	GCTGAAATCCTGACCTTCTAA
PTP1B-4-F	AGAAAATGGAGCTGCAGTTA
PTP1B-4-R	GGACGAAAATGGTAACTATATG
PTP1B-5-F	GAGTTATCATGAAGCTTGTGG
PTP1B-5-R	TGGTAGGTACACAAGTAAGCTC
PTP1B-6-F	TATTTGTTGACTGGGTGTGTG
PTP1B-6-R	ACGCAAAAACAGACTAACACA
PTP1B-7-F	TTAACCAGCTCTCTTGTGAAT
PTP1B-7-R	TCGTCTTCCTATCAATGCTCT
PTP1B-8-F	AGAGCATTGATAGGAAGACGA
PTP1B-8-R	TTTTCAGTACCAGCGTGTGTT
PTP1B-9-F	TCATCCAACTCTGTCTACACC
PTP1B-9-R	GCACCACAGAACTGAATCCTA
PTP1B-10-F	GCTCATCTGAACTGTTTGGT
PTP1B-10-R	GGGAAGATGGGTTTTAGTGC

PTP1B, protein tyrosine phosphatase 1B; SNP, single nucleotide polymorphism; F, forward primer; R, reverse primer.

software (http://www.bioconductor.org/packages/release/bioc/html/methylKit.html) was utilized to perform methylation calling on the processed data, enabling the acquisition of site-specific methylation information. Methylation levels at each CpG site were calculated as the ratio of methylated reads to total reads (methylated plus unmethylated), yielding a value between 0 and 1. Additionally, the average methylation value for all sites in each sample was calculated. Visualization of data was facilitated using the Methylation Plotter software.

Statistical analysis

Statistical analysis of methylation at *PTP1B* gene sites was performed using the Kruskal-Wallis test due to non-normal distribution of the samples. Differences of methylation at *PTP1B* gene sites were compared and tested for statistical significance with the Kruskal-Wallis test. P values <0.05 were considered to be statistically significant.

Results

Characteristics of cMPN patients

Specimens were obtained from 96 patients diagnosed with cMPNs, comprising 50 male and 46 female patients, with a mean age of 47 years (range: 13 to 85 years). Diagnosis was established according to the WHO diagnostic criteria, with 40 cases of PV, 52 cases of ET, and 4 cases of PMF. The control group included 50 healthy volunteers. The average peripheral blood platelet count was 460×10^9/L and the average white blood cell count was 16.7×10^9/L.

SNPs of PTP1B gene detected in cMPN patients

Details of the identified SNPs within the *PTP1B* gene among cMPN patients and healthy controls are presented in *Table 3* and *Figure 2*. A total of seven coding-region SNPs were identified in our study. Among these, one SNP (g.50579818 T>A) was novel, with a frequency of 1% in cMPN patients and was absent in healthy controls. The remaining six SNPs (rs75493894, rs1885177, rs2082849587, rs1364576352, rs2282147, rs2230604) had been previously reported. Their frequencies in cMPN patients were 1%, 31%, 3%, 5%, 15%, and 12%, respectively, compared to frequencies of 8%, 60%, 0%, 0%, 44%, and 36%, respectively, in healthy controls. Notably, some SNPs (rs2082849587, rs1364576352, and g.50579818 T>A) showed higher frequencies in cMPN patients than in healthy controls, whereas others (rs75493894, rs1885177, rs2282147, rs2230604) exhibited lower frequencies. Importantly, all identified SNPs were synonymous.

Methylation level of each CpG site in the promoter region of PTP1B gene

To assess *PTP1B* promoter methylation, 33 samples (including 25 samples from healthy controls and 8 from cMPN patients) were successfully amplified, and BSAS methylation analysis was conducted. For *PTP1B* pair 1, average methylation levels of target fragments in each sample are depicted in *Figure 3A*, with blue representing normal controls and purple representing cMPN patient samples. Methylation profiles of all sites within pair 1 for normal

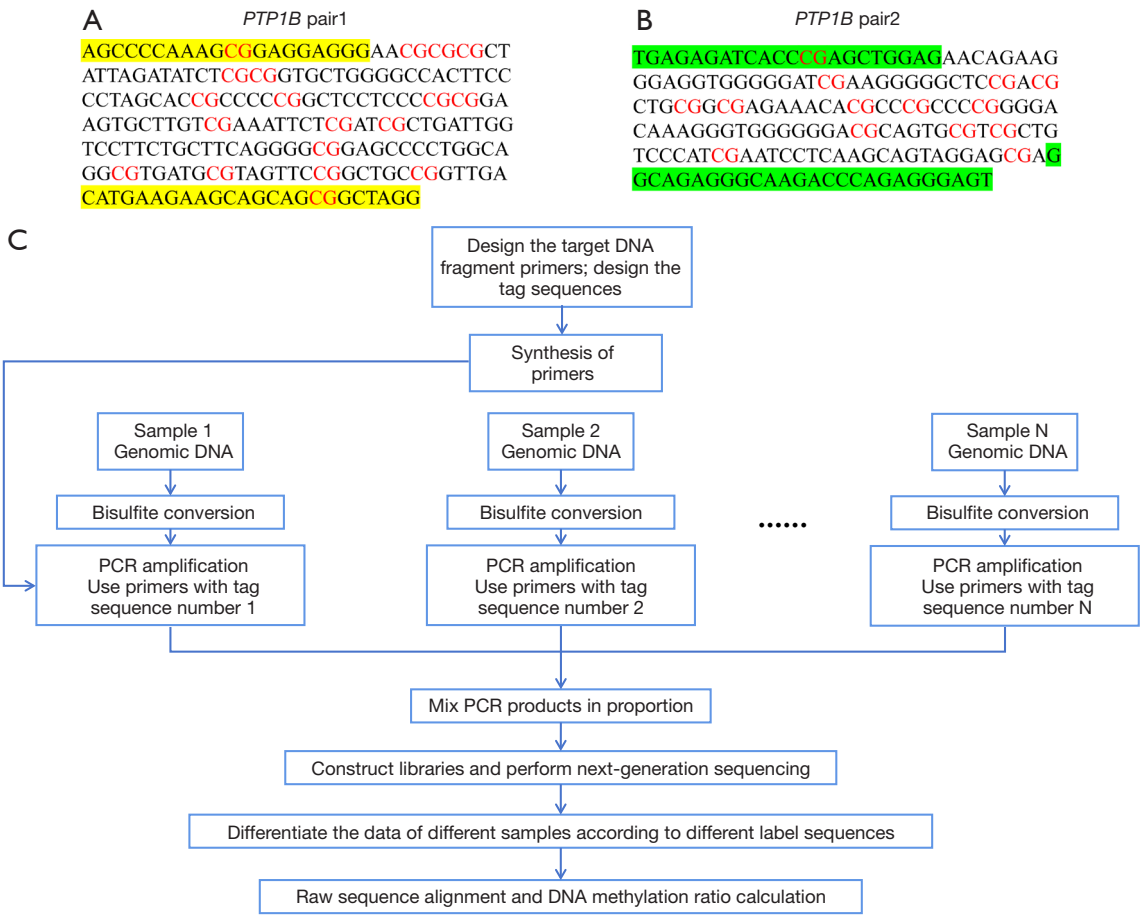

Figure 1 Sequence characteristics, primer design and procedure of methylation detection by MassArray. (A,B) The two promoter region of *PTP1B* gene (the primers of target sequences were marked in yellow and green, respectively). And the CG marked in red were the detection sites. (C) Protocols of methylation detection by MassArray. *PTP1B*, protein tyrosine phosphatase 1B; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; CG, cytosine-guanine.

controls and cMPN patients are illustrated in *Figure 3B*. A dendrogram displaying methylation levels across all sites of pair1 for normal control and cMPN samples is presented in *Figure 3C*. Box plots detailing methylation levels at all sites of pair1 for both groups are shown in *Figure 3D*. Schematic representation of methylation information across all sites of pair 1 is provided in *Figure 3E* and summarized in *Table 4*. Our analysis revealed statistically significant differences in methylation levels across multiple sites in *PTP1B* pair1 between normal control subjects and cMPN patients. *Figure 3E* and *Table 4* provide a schematic representation and detailed data of methylation levels for all sites in pair1 across all samples. Specifically, cMPN patients exhibited significantly higher methylation levels in several sites of *PTP1B* pair 1 compared to normal controls.

For *PTP1B* pair2, average methylation levels of target fragments are shown in *Figure 4A*, distinguishing between normal controls (blue) and cMPN patients (purple). Methylation profiles across all sites of pair2 for both groups are shown in *Figure 4B*. A dendrogram illustrating methylation levels at all sites of pair2 is depicted in *Figure 4C*, followed by box plots in *Figure 4D*. Methylation information for all sites of pair2 in normal controls and cMPN samples is presented schematically in *Figure 4E* and detailed in *Table 5*. Our analysis revealed statistically significant differences in methylation levels at multiple sites in *PTP1B* pair2 between normal controls and cMPNs patients. Specifically, cMPN patients exhibited significantly higher methylation levels in several sites of *PTP1B* pair2 compared to normal controls.

Table 2 The relative position of the CG site of the amplified fragment and the position of the chromosome (the following are marked with relative positions)

Relative position	Chromosome 20 location (hg19)
PTP1B pair1	
11	50515235
23	50515247
42	50515266
71	50515295
77	50515301
88	50515312
103	50515327
112	50515336
116	50515340
143	50515367
159	50515383
166	50515390
174	50515398
181	50515405
204	50515428
PTP1B pair2	
13	50515860
44	50515829
56	50515817
59	50515814
64	50515809
67	50515806
76	50515797
80	50515793
85	50515788
106	50515767
113	50515760
116	50515757
127	50515746
148	50515725

PTP1B, protein tyrosine phosphatase 1B; CG, cytosine-guanine.

Discussion

Key findings

Numerous studies have established the pivotal role of JAK-STAT pathway activation in the pathogenesis of cMPNs (24-26). While much attention has been directed towards positive regulators, the significance of negative feedback regulators within this pathway has been underexplored. A variety of negative feedback regulators are essential for maintaining balance in this regulatory network. In the present study, we conducted SNP and methylation analyses of the *PTP1B* gene in cMPN patients. We identified seven coding-region SNPs and aberrant hypermethylation of the *PTP1B* gene in cMPN patients for the first time.

Strengths and limitations

The discovery of a new SNP, differences in the frequency of seven SNPs between cMPN patients and normal controls and hypermethylation of *PTP1B* promoters collectively suggest that the *PTP1B* gene is disrupted in cMPNs and may contribute to the pathogenesis of these conditions. However, there are limitations in this study, including a limited sample size and lack of *in vitro* validation. Further research is warranted to fully elucidate these findings and their implications.

Comparison with similar researches and explanations of findings

The *PTP1B* gene, located on human chromosome 20q13.1-q13.2, is a critical negative feedback regulator of the JAK-STAT pathway. Chromosomal abnormalities, including deletions affecting chromosome 20q, are frequently associated with cMPNs. Abnormalities in the *PTP1B* gene have been implicated in various hematopoietic malignancies (20,27). Building upon this knowledge, we propose a novel hypothesis: abnormalities in the the *PTP1B* gene contribute to dysregulated negative feedback, thereby disrupting the balance and leading to enhanced JAK-STAT pathway activation in cMPNs. To test our hypothesis, we conducted SNP and methylation analyses of the *PTP1B* gene in cMPN patients. Our findings aim to elucidate the role of *PTP1B* gene alterations in the pathophysiology

Table 3 Different SNPs detected in cMPN patients and healthy controls

No.	SNP	Description	Exon	Number of patients	Incidence of patients	Number of controls	Incidence of controls
1	rs75493894	g.50568291 C>T	Exon 4	1	1%	2	8%
2	rs1885177	g.50574691 A>C	Exon 5	30	31%	15	60%
3	rs2082849587	g.50578698 T>C	Exon 7	3	3%	0	0%
4	rs1364576352	g.50578957 G>T	Exon 7	5	5%	0	0%
5	rs2282147	g.50579630 T>C	Exon 8	14	15%	11	44%
6	rs2230604	g.50579747 C>T	Exon 8	12	12%	9	36%
7	g.50579818 T>A	g.50579818 T>A	Exon 8	1	0.01	0	0

SNP, single nucleotide polymorphism; cMPN, classic myeloproliferative neoplasm.

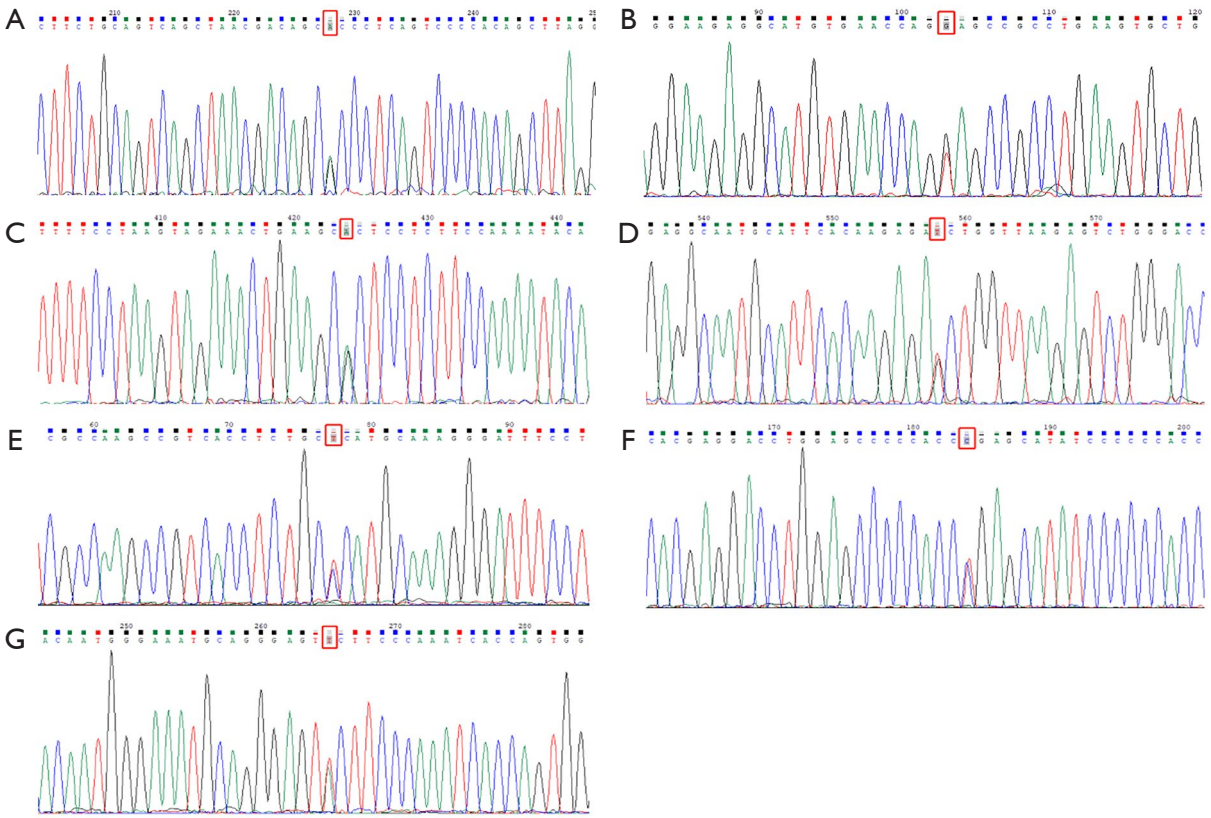

Figure 2 Different SNPs detected in cMPN patients. (A) rs75493894; (B) rs1885177; (C) rs2082849587; (D) rs1364576352; (E) rs2282147; (F) rs2230604; (G) newly discovered SNP. SNP, single nucleotide polymorphism; cMPN, classic myeloproliferative neoplasm.

of cMPNs, potentially highlighting new avenues for therapeutic intervention.

In our study, we identified seven coding-region SNPs in the *PTP1B* gene (*Figure 2*). Among these, rs1885177 (exon 5), rs2282147 (exon 8) and rs2230604 (exon 8) were present in 31%, 15% and 12% of the 96 subjects, respectively. The occurrence of these SNPs in cMPN patients was lower compared to healthy volunteers. Additionally, the SNPs rs75493894 (exon 4) and rs1364576352 (exon 7) were detected in only one and five patients, respectively, with

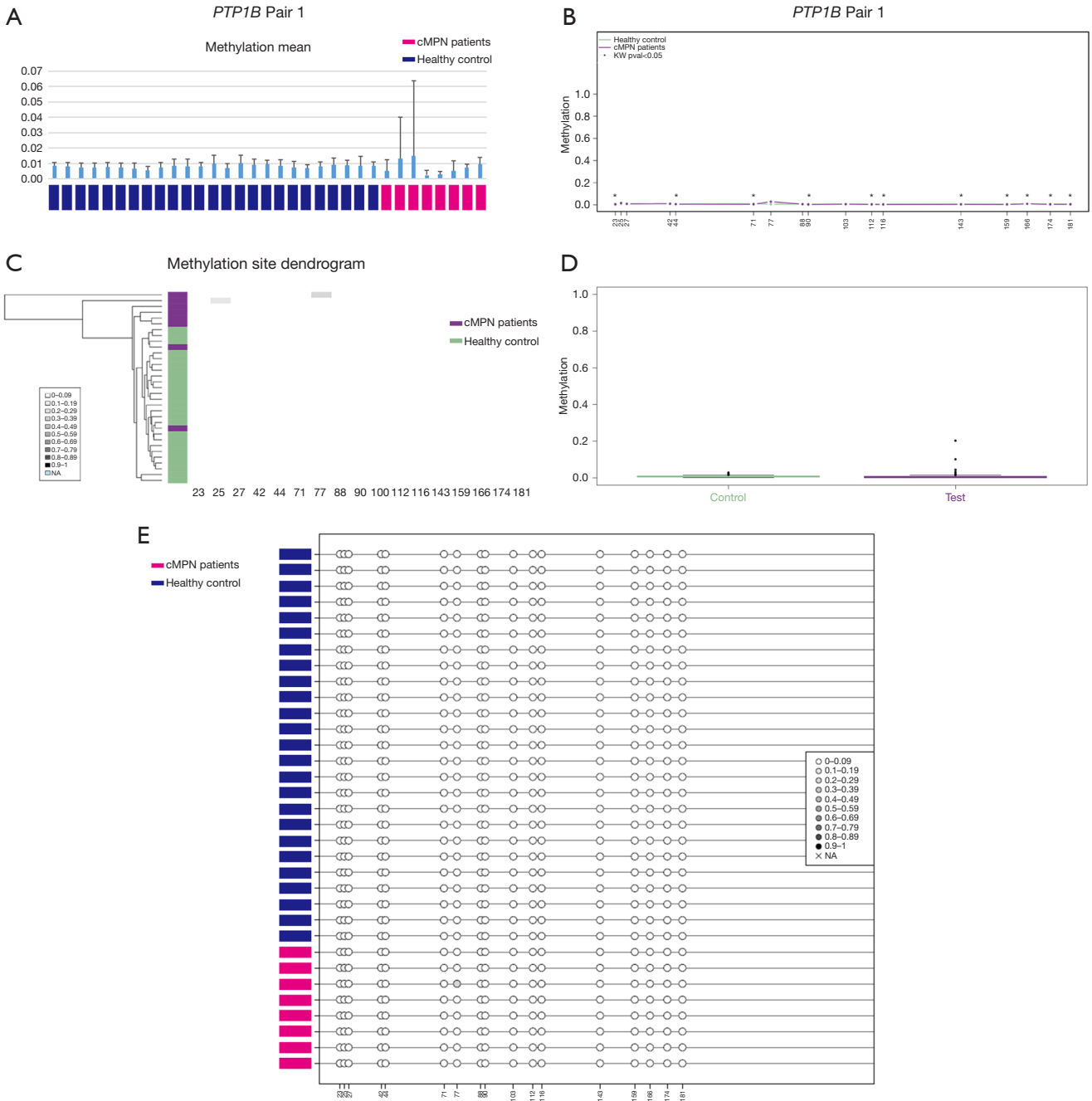

Figure 3 Methylation level of each CpG site in the promoter region of *PTP1B* pair 1. (A) For *PTP1B* pair 1, average methylation levels of target fragments in each sample are depicted, with blue representing normal controls and purple representing cMPN patient samples; (B) methylation profiles of all sites within pair 1 for normal controls and cMPN patients are illustrated; (C) methylation levels across all sites of pair 1 for normal control and cMPN samples is presented in the dendrogram; (D) detailing methylation levels at all sites of pair 1 for both groups are shown in the box plots; (E) schematic representation of methylation information across all sites of pair 1 is provided. *, P<0.05. CpG, cytosine-phosphate-guanine; NA, not applicable; cMPN, classic myeloproliferative neoplasm.

Table 4 Results of statistical analysis of methylation at *PTP1B* pair1 test *vs.* control sample sites

Position	Control methylation mean	Test methylation mean	Control methylation standard deviation	Test methylation standard deviation	P value (Kruskal-Wallis)
23	0.008852	0.004513	0.003995	0.004399	0.03*
25	0.010204	0.017275	0.003569	0.034423	0.10
27	0.011492	0.008725	0.005199	0.007671	0.36
42	0.008748	0.010575	0.005069	0.014671	0.58
44	0.007704	0.006588	0.003182	0.01051	0.01*
71	0.010604	0.004875	0.004282	0.007211	0.008*
77	0.006016	0.02885	0.002023	0.07038	0.25
88	0.007416	0.006225	0.002822	0.00695	0.12
90	0.006132	0.003513	0.002857	0.002745	0.02*
103	0.0077	0.006238	0.002479	0.003984	0.15
112	0.007036	0.003713	0.003498	0.002777	0.02*
116	0.007256	0.0032	0.002325	0.002701	0.001*
143	0.007452	0.004275	0.002771	0.002742	0.02*
159	0.006788	0.003363	0.00223	0.002375	0.002*
166	0.008816	0.009738	0.002731	0.013912	0.02*
174	0.007884	0.004513	0.003145	0.00318	0.02*
181	0.008128	0.004713	0.003991	0.004638	0.02*

*, indicates P value <0.05 for Kruskal-Wallis analysis. *PTP1B*, protein tyrosine phosphatase 1B.

even lower occurrence in healthy volunteers. Furthermore, we identified a new SNP, g.50579818 T>A (exon 8), present in one case among cMPN patients, and absent in healthy volunteers. Despite their synonymous nature, these SNPs may still influence gene function (28). For instance, Möhlendick *et al.* demonstrated that the synonymous SNP rs7121 is associated with either tumor progression or prolonged survival in cancer patients (29), while Ovsyannikova *et al.* reported that the synonymous SNP rs2230604 in the *PTP1B* gene correlates with significantly lower T-cell receptor excision circle (TREC) level (30). Tan *et al.* provided an excellent example that a synonymous SNP can alter epidermal growth factor receptor (EGFR) dependency (31). Therefore, while synonymous coding SNPs do not directly alter the amino acid sequence of proteins, they can indirectly impact gene expression, regulation, and protein function through mechanisms including impact on mRNA splicing, alteration of mRNA stability, affect the ribosome's translation speed, and impact microRNA binding.

Moreover, studies have indicated that abnormal *PTP1B* gene expression results in reduced phosphatase activity and increased phosphorylation of proteins in the JAK/STAT pathway (32,33). In Hodgkin's lymphoma cell line KM-H2, silencing of the *PTP1B* gene via RNA interference leads to hyperphosphorylation and overexpression of downstream oncogenes (34). Taken together with our findings on the new SNPs and their frequency difference between cMPN patients and normal controls, our data support the hypothesis that *PTP1B* gene abnormalities in cMPN patients may disrupt negative feedback regulation.

For the methylation MassARRAY analysis, a total of 30 CpG sites were detected in the promoter region of *PTP1B* gene. Significant differences were observed in the methylation levels of several sites in *PTP1B* between the normal control group and the cMPN patient group. Abnormal methylation patterns have been widely associated with cancer development and progression. Our study represents the first report of hypermethylation in the *PTP1B* gene and its potential pathogenic implications in cMPNs. Hypermethylation of the *PTP1B* promoter region

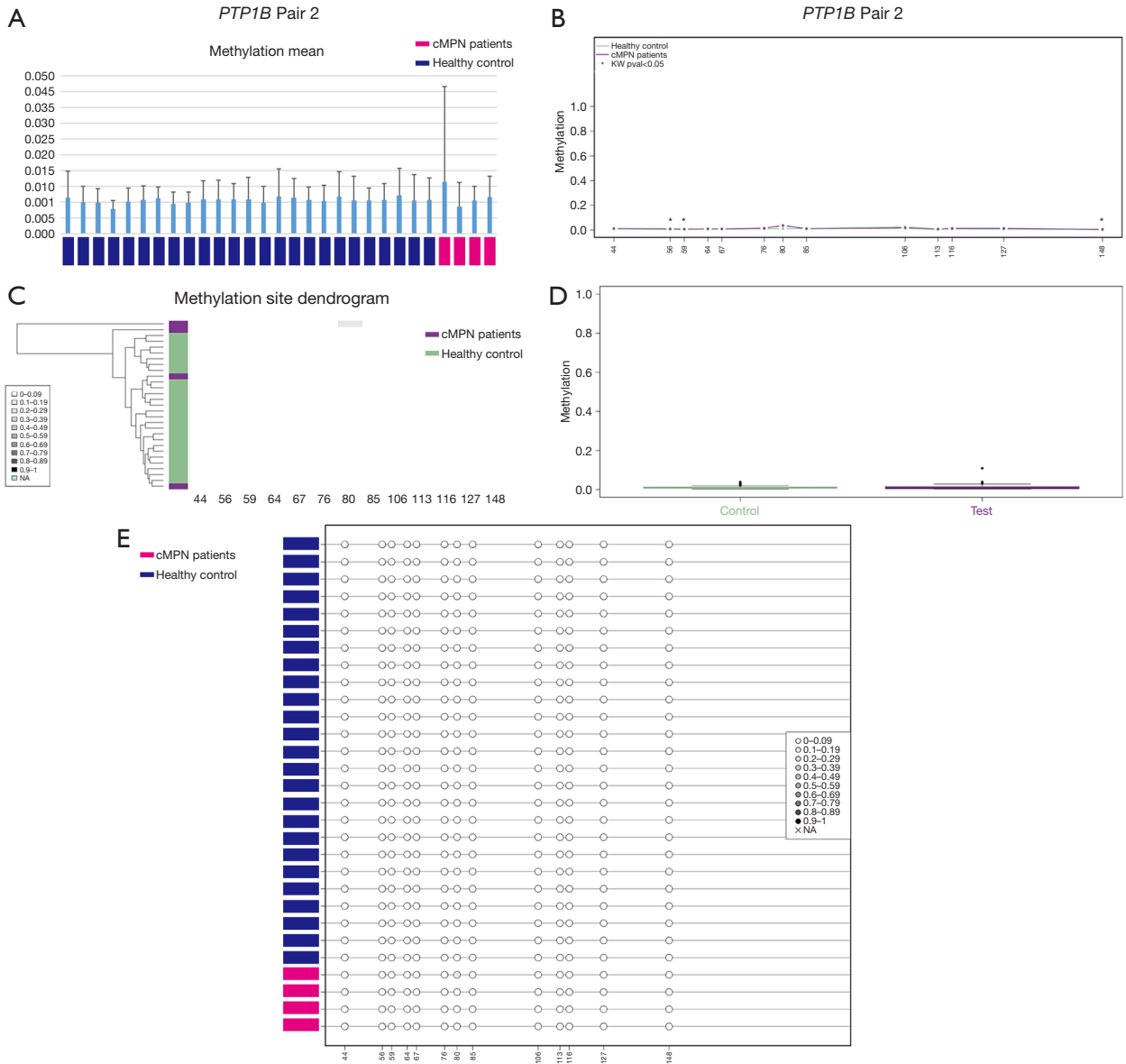

Figure 4 Methylation level of each CpG site in the promoter region of *PTP1B* pair 2. (A) For *PTP1B* pair 2, average methylation levels of target fragments in each sample are depicted, with blue representing normal controls and purple representing cMPN patient samples; (B) methylation profiles of all sites within pair 2 for normal controls and cMPN patients are illustrated; (C) methylation levels across all sites of pair 2 for normal control and cMPN samples is presented in the dendrogram; (D) detailing methylation levels at all sites of pair 2 for both groups are shown in the box plots; (E) schematic representation of methylation information across all sites of pair 2 is provided. *, P<0.05. CpG, cytosine-phosphate-guanine; NA, not applicable; cMPN, classic myeloproliferative neoplasm.

may lead to reduced expression of the PTP1B protein, thereby impairing its ability to dephosphorylate proteins in the JAK/STAT pathway. The reduction of PTP1B expression due to higher methylation of promoter region of *PTP1B* might attenuate the inhibition of the JAK/STAT pathway, thereby promote the cMPNs pathogenesis.

Conclusions

In summary, we have identified seven coding-region SNPs and aberrant hypermethylation of the *PTP1B* gene in cMPN patients for the first time. Notably, we discovered a new SNP and observed differences in the frequency of these seven SNPs

Table 5 Results of statistical analysis of methylation at *PTP1B* pair2 test *vs.* control sample sites

Position	Control methylation mean	Test methylation mean	Control methylation standard deviation	Test methylation standard deviation	P value (Kruskal-Wallis)
44	0.013644	0.01055	0.003525	0.009838	0.53
56	0.011812	0.006875	0.003146	0.003629	0.01*
59	0.00978	0.005725	0.002856	0.001852	0.004*
64	0.007884	0.00905	0.002815	0.005167	0.47
67	0.007496	0.008175	0.002417	0.004387	0.80
76	0.009888	0.014275	0.003026	0.016357	0.28
80	0.010116	0.035975	0.002786	0.048894	0.53
85	0.011968	0.010225	0.003099	0.006944	0.66
106	0.025264	0.0164	0.007389	0.008129	0.06
113	0.008572	0.0059	0.002832	0.002601	0.11
116	0.00864	0.013125	0.002362	0.010434	0.49
127	0.006848	0.01305	0.001929	0.01243	0.28
148	0.00616	0.00395	0.001689	0.001526	0.03*

*, indicates P value <0.05 for Kruskal-Wallis analysis. *PTP1B*, protein tyrosine phosphatase 1B.

between cMPN patients and normal controls. Additionally, we found hypermethylation of *PTP1B* promoters in cMPN patients compared to normal controls. These findings collectively suggest that the *PTP1B* gene is disrupted in cMPNs and may contribute to the pathogenesis of cMPNs. Further research is warranted to fully elucidate these findings.

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Footnote

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1338/dss

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by both ethics committees of Tongji Hospital of Tongji University (No. 2021-KYSB-177) and Ruijin Hospital (No. SHDC2020CR5002-2021-105). Informed consent was obtained from all individual participants.

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