Down-regulation of Protein Tyrosine Phosphatase Receptor Gamma (PTPRG) in Chronic Myeloid Leukemia Patients in The State of Qatar is Due to Aberrant DNA Methylation Mechanism

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Research

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Abstract

Background: Several studies showed that the aberrant DNA methylations are involved in leukemia and cancer pathogenesis. PTPRG is a natural inhibitory mechanism that is found to be down-regulated in Chronic Myeloid Leukemia (CML) disease. The mechanism behind its down-regulation has not been fully elucidated.

Aim: The study investigates the role of methylation as a possible mechanism of PTPRG under-expression in CML patients.

Method: Peripheral blood samples from CML patients treated with tyrosine kinase inhibitors (TKIs) and healthy controls were collected. DNA was extracted and treated with bisulfite treatment, followed by PCR and sequencing of 25 of CpG in Promoter and 26 of CpG in the Intronic regions of PTPRG. Bisulfite-sequencing technique as a high-resolution method was employed.

Results : CML groups (New Diagnosed and Failed treatment) have significantly higher methylation levels in the Promoter and Intron regions compared to the healthy group. There were also significant differences in methylation levels of CpG sites in the Promoter and Intronic regions amongst the groups.

Conclusion: Aberrant methylation of PTPRG is documented and potentially is one of the possible mechanisms of PTPRG down-regulation in CML.

Background

Chronic Myeloid Leukemia (CML) is a clonal myeloid stem cell disorder associated with abnormal exponential proliferation of granulocytes and their precursors [1] CML incidence rates are estimated to be 0.7-1.0/100,000[2]. Philadelphia (Ph^+) chromosome, resulting from BCR-ABL fusion oncogene due to t (9; 22) (q34; q11), is the hallmark of CML disease. The translocation juxtaposes the c-abl (ABL1) gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22. The BCR-ABL oncoprotein plays a key role of the constitutive activation of the tyrosine kinase domain [3, 4], therefore it becomes an important target for therapeutic interventions with small molecule tyrosine kinase inhibitors (TKIs) which have the ability to compete with the ATP binding site of the catalytic domain of ABL tyrosine kinase [5]. In the past decade, the introduction of these TKIs has resulted in significant improvement in management and treating Ph-positive (Ph+) CML patients. At present five TKIs namely: Imatinib Mesylate (IM), Nilotinib, Dasatinib, Ponatinib and Bosutinib have been approved by the United States Food and Drug Administration (FDA) for the treatment of patients with CML [6–9]. Despite the treatment efficiency by TKIs achieving major to complete response at the molecular, cytogenetic and hematological levels [6, 10, 11], a significant proportion of patients (between 20–25%) developed resistance to treatments [11–13]. TKIs resistance is either BCR-ABL1-dependent where mechanisms such as point mutations or cellular/biological processes that interfere with TKI bioavailability disrupt the effectiveness of BCR-ABL1 kinase inhibition. On the other hand, BCR-ABL1-independent resistance is attributed to alternative survival pathways operating in the situation of effective TKI inhibition of BCR-ABL1 [14–16]. One of the potential independent inhibitory mechanisms is the epigenetic regulation of protein tyrosine phosphatases. Epigenetic silencing is a phenomenon whereby gene transcription may be suppressed through DNA methylation (a process that may regulate gene function) resulting in decreased protein expression. Several studies have suggested that hypermethylation might play a role in disease progression in CML. Hypermethylation of several genes was associated with the progression of CML, its pathogenesis and the response to therapeutics [17–22].

Protein tyrosine phosphatase (PTP) superfamily of enzymes is a natural regulatory mechanism of the tyrosine kinase family. They have the ability to remove phosphate groups from phosphorylated tyrosine residues leading to equilibrium status in normal populations. Based on their cellular localization, PTPs are classified as receptor-like and non-receptor. Even though receptor-type protein tyrosine phosphatases (PTPRs) share similar basic structure, distinct PTPRs have specific targets and may thus play altered roles in cell regulation [23–25]. Of these, protein tyrosine phosphatase receptor gamma (PTPRG) that was described as a tumor suppressor in several tumors and its expression level was found to be significantly down- regulated in CML patients and cell lines [26]. Moreover, PTPRG expression was restored in patients who responded optimally to the treatment with the tyrosine kinase inhibitors (TKIs), and its expression remained low in patients who failed to respond to the treatment with the TKIs [27]. More recently, we identified a single Nucleotide Polymorphism (SNPs) (rs62620047) in PTPRG (Y92H) in patients who failed Imatinib Mesylate (IM) treatment [28]. While the molecular genetics and flow cytometry characteristics of PTPRG were studied [27], the contributing epigenetic mechanisms influencing disability of PTPRG mechanism in CML patients remains unclear and warrants further investigation. The aim of this study is to investigate the methylation patterns of PTPRG gene in a cohort of CML patients in Qatar where resistance to IM treatment has been reported to be significantly higher than other parts of the world [29].

Materials And Methods

Patient recruitment and characteristics

Informed consent was obtained from all participants. The study was approved by both supreme council of health (SCH) and institutional review board of Hamad Medical Corporation (Project No. SCH-HMC-020-2015). This study adhered to the World Medical Association's Declaration of Helsinki (1964–2008) and its amendments for Ethical Human Research including confidentiality, privacy and data management. A total of 26 adult CML patients and 6 matched healthy controls that were confirmed to have normal Complete blood count (CBC) and negative for *BCR-ABL1* translocation were included in this study.

The peripheral blood samples were collected in EDTA tubes for new diagnosis patients. The samples were collected at time of diagnosis before starting TKIs. On the other hand, the peripheral blood samples were collected in EDTA tubes for failed/relapsed patients at time of failure according European Leukemia Net (ELN) guidelines. The CML patients' response to TKIs treatment was assessed based on the haematologic, cytogenetic, and molecular response results according to (ELN- 2013) [30, 31]. Resistant to treatment was defined as showing lack of one of the following; hematological response and/or Ph+ >95% by 3 months, BCR-ABL1 >10% and/or Ph+ >35% by 6 months, BCR-ABL1 >1% and/or Ph+ >0 response by 12 months after the start of the treatment. Thirteen out of the 26 participants were Newly Diagnosed (ND) with CML and 13 were classified as "Failed treatment (F)" group.

Samples collection, DNA isolation and bisulfite conversion

Total DNA was isolated with Maxwell® 16 DNA Purification Kits as per manufacture guidelines [32]. Purity of extracted DNA was assessed by a Nano Drop spectrophotometer 2000 (Thermofisher Scientific), a ratio of 1.8-2.4 was acceptable and for optimal results, an absolute quantity of 200 - 500 ng of DNA was used.

The DNA samples were then treated with sodium bisulfite according to the manufacturer's instructions (EpiTect Bisulfite Kit, QIAGEN, Hilden, Germany). The bisulfite treatment catalyzes the deamination of all the unmethylated cytosine (uC), nucleotides to uracil (U) or thymidine (T) nucleotides and leaves the methylated cytosine (mC) unchanged. For optimal results, the amount of starting DNA in the bisulfite modification process was from 200 to 500 ng.

Primers design, bisulfite sequencing PCR (BSP) and Gradient Polymerase Chain reaction

The University of California, Santa Cruz (UCSC) Genome Browser [33] was utilized to identify the possible "CpG sites" flanking the CpG region followed by "Bio Edit Sequence Aliqment Editor" tool to identify the forward and reverse primers. Finally, BiSearch is a primer-design and search tool utilized to ensure amplification of specific PCR products (Supplement table 1) [34]. Gradient PCR was performed to find the specific annealing temperature for the selected gene. Specific products 321bp and 218bp are detected at 60^0C for Promoter and Intron regions of PTPRG respectively. Bisulfite treatment was performed followed by Sanger sequencing (Supplement table 2).

Bisulfite Sequencing

The PCR products were sequenced with an ABI PRISM BigDye terminator sequencing kit v1.1 (Life Technologies) and directly analyzed by an automated ABI 3130 Genetic Analyzer (Life Technologies).

Methylation Analysis

The analysis of the methylation status of CpG sites in the region amplified by PCR was performed using the ESME (Epigenetic Sequencing Methylation) Analysis Software [35]. The ratio of C to T at CG sites was determined after correction for incomplete conversion to determine methylation. Further, the percentage of methylation was calculated as the peak height of C versus the peak height of C plus the peak height of T for each CpG site as shown in the computer-generated sequencing chromatogram extracted from the Chromas program (Version 2.32, Technelysium). A single C at the corresponding CpG site was considered as 100% methylation, a single T as no methylation and overlapping C and T as partial methylation (0–100%) [36].

CpG sites of Promoter and Intronic regions of PTPRG were plotted using Methylation plotter[37]. The methylation levels (0–100%) were converted (0-1) for plotting purposes [38].

BCR-ABL1 and PTPRG Quantification by RQ-PCR

The *BCR-ABL1* and *PTPRG* quantification were carried out using RQ-PCR as previously described [26, 27, 29, 39-42] (Table 1).

Statistical Analysis

Descriptive statistics in the forms of median range and frequency, and percentages were calculated. For continuous outcomes and categorical independent variables, T-test for independent samples was used to test the mean differences for two groups and one-way ANOVA with Bonferroni post hoc analysis were employed to test the mean differences for three groups using SPSS 24. All *P* values presented will be two-tailed, and P values <0.05 will be considered as statistically significant.

Results

Participants' characteristics

Out of the 26 CML patients, 13 were newly diagnosed (ND) with CML and 13 had a failed treatment (F) (Table 1). Patients' age ranged from 25-60 years (mean 37.48 and SD: 9.82) with male to female ratio of 18 (69.2%) males to 8 (30.8%) females. In addition, there were 6 healthy participants who have never had cancer in their lives (H) (Age range: 23-46 years mean: 37.17; SD: 9.58; Gender: 5 (83.3%) male and 1(16.7%) female). Out of the 26 patients, 24patients were in chronic phase (CP) (92.3%), one patient was in accelerated phase (AP) (3.85%) and one patient was in Blast crisis (BC) phase (3.85%).

Hypermethylation of the promoter region of *PTPRG* in patients diagnosed with CML

We performed T-test analysis to study the methylation pattern of Promotor of PTPRG in both cases and controls. The results revealed that there was a significant difference in promoter methylation levels between CML (newly diagnosed and failed treatment groups) and the healthy group ($t(30) = 5.7$, $P < 0.001$) (CML: Mean=6.77, SD: 2.87; Healthy: Mean=0.00, SD: 0.00).

In addition, we tested the differences between the three groups (ND, F and H). One-way ANOVA and Bonferroni post-hoc test results indicated that the ND and F groups had significantly higher methylation compared with the H group ($P<0.001$). There was no significant difference between ND and F groups (Table 2).

Results of the methylation in the 25 CpG sites of Promoter region of *PTPRG*

One-way ANOVA was conducted to compare methylation status in each CpG site in the promoter region between the three groups (ND, F, and H). There were significant differences in the 2 out of 25 CpG sites (13 and 143) among the groups ($F(2, 29) = 7.0$; $P = 0.003$ and $F(2, 29) = 4.35$; $P = 0.022$ respectively). Bonferroni post-hoc test results indicated that methylation in CpG 13 for the ND and the F groups was significantly higher compared to the H group ($P = 0.002$ and $P = 0.035$ respectively). In addition, methylation in CpG 143 for the F group was significantly higher compared to the H group ($P = 0.045$). (Figure 1). No significant differences were found in the rest of the CpG sites.

Figure 1

Hypermethylation at Intronic region of *PTPRG* in CML patients

We performed T-test analysis to study the methylation pattern of Intron of PTPRG in both cases and controls. The results revealed that there was a significant difference in intron methylation levels between CML (newly diagnosed and failed treatment groups) and the healthy group ($t(30) = 10.38$, $P < 0.001$) (CML: Mean=14.62, SD: 3.41; Healthy: Mean=0.00, SD: 0.00).

One-way ANOVA showed significant differences in methylation between the three groups (ND, F and H) for intron region [$F(2, 29) = 53.590$, $P =0.001$]. Bonferroni post-hoc test results indicated that the methylation status for the ND and the F groups was significantly higher than the H group ($P <0.001$). There was no significant difference between the ND and the F groups (Table 2).

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Methylation in 26 CpG sites of Intronic region of *PTPRG* in CML patients

One-way ANOVA was also conducted to compare methylation levels in each CpG sites in the Intron region between the three groups ND, F, and H. The results indicated that there were significant differences in the 23 out of 26 CpG sites (Figure 2 and Table 3). Bonferroni post-hoc test revealed that the methylation levels were significantly higher amongst the ND and the F groups compared to the H group in most of the Intron CpG sites except for CpG 70, CpG 94, CpG 155 and CpG 161 (in the ND group) and CpG 173 (in the F group). In addition, the F group had significantly higher methylation levels in the CpG sites 94 ($P= 0.003$) and 155 ($P=0.01$) compared to the ND group.

Discussion

This is the first prospective study to evaluate epigenetic mechanisms of PTPRG regulation in CML patients in Qatar where the rate of resistances to IM is higher than international data [16, 29]; and addresses the importance role of PTPRG as a regulatory factor in BCR-ABL-mediated oncogenesis. Our study provides an evidence of the involvement of the epigenetic modification of PTPRG in the pathogenesis of CML. PTPRG was found to be significantly hyper-methylated compared to the control (Figs. 1 and 2).

PTPRG is known to induce a reduction of protein BCR-ABL-specific tyrosine phosphorylation of its direct downstream targets/substrates such as CRKL and of STAT5 [26]. In this study, we expanded the methylation coverage of PTPRG via studying two regions of its Promoter 321 bp and Intron 218 bp and using

advanced molecular technique such as Sanger sequencing. In the same context, Peruta et al 2011 documented earlier that up-regulated PTPRG expression is associated with reduction in methylation levels in 166 bp of PTPRG using Methylation-specific PCR technique [26].Our findings revealed that the methylation occurs frequently higher in the Intronic region compared to Promoter region of CML patients and showing a significant increase of the methylated percentage at the CpG sites in both Promoter and Intronic regions compared to healthy individuals (Table 3). Interestingly, our findings showed and confirmed that the hyper-methylated pattern of PTPRG gene in CML patients acts as an early promoter for CML formation and to be dependent of BCR-ABL1 titers. It may play as a mechanism beyond the BCR-ABL1 function i.e., a BCR-ABL independent resistance molecular event.

We analyzed 51 CpG sites in PTPRG in CML and healthy control groups for methylation. Overall, the frequency of methylated CpG sites was significantly higher in CML cases compared to healthy controls, suggesting the potential involvement of CpG methylation sites in CML (Figs. 1 and 2). Interestingly, two CpG sites in the Intronic region were found to be significantly methylated amongst failed groups compared newly diagnosed. In the newly diagnosed group, the frequency of CpG site methylation was significantly different from the healthy group, suggesting that CpG site methylation have a central role in CML. These findings support the assumption that the CML disease is not mainly driven by the BCR-ABL1 translocation. In addition, we also observed a significantly higher methylated CpG sites in the failed group compared to the healthy group, indicating that CpG site methylation may be important for disease progression (Table 3).

Several studies documented about the effect of DNA methylation pattern of regulatory genes on various cellular activities such as cell proliferation and survival, as well as cell-signaling molecules in CML [43]. Jelinek et al 2011 studied the Methylation levels of 10 genes in CML patients and found that the frequency of methylated genes ranged from 11%- 86% as follows: ABL1 (86%), CDH13 (79%), NPM2 (74%), PGRA (66%), TFAP2E (63%), DPYS (54%), PGRB (52%),OSCP1 (30%), PDLIM4 (21%) and CDKN2B (11%), This indicates that there is aberrant methylation of DNA associated with the progression of CML [44]. Another study using a whole methylome approach in 36 CML patients, found that 31 genes were uniquely hyper methylated in CML and 42 genes that became hyper methylated with the progression of CML. Interestingly, the same group showed that utilizing DNA methylation inhibitor such as azacytidine in blastic crisis CML patients resistant to Imatinib Mesylate (IM) could reverse the aberrant hypermethylation associated with progression of CML to blast crisis and supports the use of this drug as an epigenetic therapy [45]. In another study with CML cell line K562 and its IM resistant variant (K562-R) the methylation level was found to be significantly higher and the gene expression level significantly lower for MLH1, RPRM, FEM1B, and THAP2 in K562-R cells when compared to parental K562 cells. Moreover, treatment of the K562-R cells with methylation inhibitors, such as 5-azacytidine (AzaC) and trichostatin A (TSA), reduced resistance to Imatinib [46]. In another study, SOX30 methylation has been correlated with disease progression in patients with chronic myeloid leukemia [47].

PTPRG expression has been shown to be down-regulated by RAS activation, while its up-regulation has been observed in hypo-methylation condition in in childhood acute lymphoblastic leukemia (ALL) [48]. Finally, PTPRG methylation has also been reported in solid cancer [49, 50]. Eddy et al., suggested that PTPRG inton1 methylation could be a biomarker for early detection of colorectal cancer [51].

Conclusion

Hypermethylation acts as a molecular mechanism independent of BCR-ABL1 function in CML patients. Our data contributes to the crucial role of aberrant DNA methylation in CML disease initiation and progression. However, further studies are needed on the validation of specific aberrant methylation of PTPRG and its prognostic and predictive values for the response to therapy in the CML patients.

Abbreviations

ABL1
Abelson Murine Leukemia; ALL:Acute Lymphoblastic Leukemia; AP:Accelerated Phase; ANOVA:Analysis Of Variance; ATP:Adenosine Triphosphate; AzaC:5-azacytidine ; BC:Blast Crisis Phase; BCR:Breakpoint Cluster Region; CBC; Complete Blood Count; CDH13:Cadherin 13; CDKN2B:Cyclin Dependent Kinase Inhibitor 2B; CML:Chronic Myeloid Leukemia; CP:Chronic Phase; CpG:Cytosine Phosphate Guanine; CRKL:Crk-like protein; DNA:Deoxyribonucleic acid; DPYS:Dihydropyrimidinase; EDTA:Ethylenediaminetetraacetic acid; ELN:European LeukemiaNet; ESME:Epigenetic Sequencing Methylation; F:Failed Treatment; FDA:Food and Drug Administration; FEM1B:Fem-1 Homolog; H:Healthy; HMC; Hamad Medical Corporation; IM:Imatinib Mesylate; mC:Methylated Cytosine; MLH1:MutL homolog 1; ND:New Diagnosis; NPM2:Nucleoplasmin 2; OSCP1:Organic Solute Carrier Partner 1; PCR:Polymerase Chain Reaction; PDLIM4:PDZ And LIM Domain 4; Ph+:Philadelphia chromosome; PGR-A:Progesterone Receptor-A; PGR-B:Progesterone Receptor-B; PTP:Protein tyrosine phosphatase; PTPRG:Protein Tyrosine Phosphatase Receptor Gamma; PTPRs:Protein Tyrosine Phosphatases Receptor-Type; RPRM:Reprimo, TP53 Dependent G2 Arrest Mediator Homolog; RT-PCR:Reverse Transcription- Polymerase Chain Reaction; SD:Standard deviation; SCH; Supreme Council of Health; SOX30; Sry-related high-motility group box 30; SPSS:Statistical Package for the Social Sciences; STAT5:Signal Transducer and Activator of Transcription 5; T:Thymidine; TFAP2E:Transcription Factor AP-2 Epsilon; THAP2:THAP domain containing 2; TKIs:Tyrosine Kinase Inhibitors; TSA:Trichostatin A; U:Uracil; Uc:Unmethylated Cytosine; UCSC:The University of California, Santa Cruz.

Declarations

ETHICAL APPORVAL AND CONSENT APPROVAL

Informed consent was obtained from all participants. The study was approved by both supreme council of health (SCH) and institutional review board of Hamad Medical Corporation (Project No. SCH-HMC-020-2015). This study adhered to the World Medical Association's Declaration of Helsinki (1964–2008) for Ethical Human Research including confidentiality, privacy and data management.

CONSENT FOR PUBLICATION

Consent for publication was obtained through ethics approval and consent to participate.

AVAILABILITY OF DATA AND MATERIALS

This is a research article and all data generated or analyzed during this study are included in this publication.

COMPETING / CONFLICTS OF INTEREST

The authors declare that there are no conflicts of / or competing interests.

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AUTHORS' CONTRIBUTIONS

MI was responsible for performing the experiments; MI, MS and ND were responsible for analyzing the data MI and ND designed the experiment MI, ND, MS performed statistical analysis. MI, MY and ND involved in patient's recruitment. MI, ND, AS, MS, MV, MM, MS, HM, WQ, HZ, RC, CS, HM and ND conceptual work, framework, draft write-up and editing. All authors read and approved the final manuscript.

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AUTHORS' INFORMATION

Not applicable.

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Tables

ıts	Gender Male (M), Female (F)	Age (years)	Clinical phase	BCR-ABL1(IS)	PTPRG/ABL*100	Treatment	Response
ıe 01.	M	45	CP	100%	0.02%	No treatment.	Newly diagnosed
ıe 02.	M	23	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 03.	M	28	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 04.	M	38	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 05.	M	43	AP	100%	0.01%	No treatment.	Newly diagnosed
ıe 06.	F	45	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 07.	M	46	CP	100%	0.02%	No treatment.	Newly diagnosed
ıe 08.	F	28	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 09.	M	40	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 10.	M	34	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 11.	M	58	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 12.	F	43	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 13.	M	32	CP	100%	0.01%	No treatment.	Newly diagnosed
ıe 14.	F	49	CP	37%	0.2%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 15.	F	35	CP	86%	0.01%	Imatinib(400mg), then shift to Dasatinib (50 mg)	Failed treatment
ıe 16.	M	23	CP	35%	0.3%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 17.	M	25	CP	12%	0.3%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 18.	F	34	CP	45%	0.2%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 19.	M	31	CP	33%	0.2%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 20.	F	29	CP	25%	0.3%	Imatinib (400mg) No changes in treatment	Failed treatment

ıe 21.	F	35	CP	68%	0.1%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 22.	M	38	CP	80%	0.02%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 23.	M	38	CP	60%	0.1%	Imatinib (400mg) No changes in treatment	Failed treatment
ıe 24.	M	34	CP	55%	0.1%	Imatinib (400mg)	Failed treatment
ıe 25.	M	61	CP	11%	0.4%	Nilotinib (300mg) No changes in treatment	Failed treatment
ıe 26.	M	40	BC	15%	0.3%	Dasatinib (70 mg) No changes in treatment	Failed treatment

Table 1 CML patients' characteristics according to gender, age, clinical phase, the type and total dose of TKIs received and response to treatments. CP; Chronic phase. AP; Accelerated phase. BC; Blast crisis phase.

Region	Groups	N	Mean ± SD	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Promotor	Newly Diagnosed (ND)	13	7.3 ± 3.0	5.5	9.2
	Failed (F)	13	6.1 ± 2.6	4.5	7.7
	Healthy (H)	6	0.00	.00	0.00
Intron	Newly Diagnosed (ND)	13	14.13± 3.6	11.95	16.312
	Failed (F)	13	15.11± 3.3	13.147	17.08
	Healthy (H)	6	.00	0.00	0.00

Table 2. Descriptive analysis for methylation levels of the whole promoter region and whole intron region.

Site	Groups	Mean ± SD	95% Confidence Interval Range	P value
CpG 59	F	17.00 ± 10.47	10.67 ± 23.32	.013
	ND	16.07 ±13.66	7.82±24.33	.019
CpG 70	F	21.38±24.21	6.75±36.02	.034
	ND	15.00±5.89	11.44±18.56	.204
CpG 77	F	8.30±5.089	5.23±11.38	.001
	ND	8.30±3.79	6.01±10.60	.001
CpG 86	F	5.46±3.69	3.23±7.69	.001
	ND	4.62±1.61	3.64±5.59	.003
CpG 88	F	2.69±2.39	1.25±4.14	.027
	ND	2.85±1.86	1.72±3.97	.018
CpG 91	F	1.92±3.59	-.25±4.09	.352
	ND	1.00±1.08	.34±1.65	1.000
CpG 94	F	11.38±6.70	7.33±15.43	.000
	ND	4.92±2.22	3.58±6.26	.109
CpG 111	F	2.08±6.64	-1.94±6.09	1.000
	ND	.15±.55	-.18±.49	1.000
CpG 117	F	1.84±5.18	-1.29±4.98	.817
	ND	.23±.44	-.03±.50	1.000
CpG 155	F	11.30±11.78	4.19±18.43	.016
	ND	1.77±1.48	0.87±2.66	1.000
CpG 161	F	9.46±11.23	2.68±16.25	.041
	ND	2.69±1.75	1.63±3.75	1.000
CpG 173	F	4.00±2.97	2.204±5.80	.169
	ND	5.85±5.59	2.46±9.22	.021
CpG 189	F	12.38±6.51	8.45±16.32	.042
	ND	15.07±13.44	6.95±23.20	.011
CpG 191	F	13.85±10.97	7.22±20..47	.002
	ND	10.92±3.33	8.91±12.93	.016
CpG 193	F	11.38±4.37	8.74±14.02	.000
	ND	12.70±4.73	9.83±15.55	.000
CpG 199	F	8.00±4.14	5.50±10.50	.000
	ND	7.85±4.02	5.42±10.27	.001
CpG 226	F	14.62±7.10	10.32±18.90	.000
	ND	13.62±4.23	11.06±16.17	.000
CpG 228	F	23.62±9.82	17.68±29.55	.000
	ND	24.46±6.92	20.28±28.65	.000
CpG 236	F	17.62±9.22	12.05±23.18	.000
	ND	17.62±5.58	14.25±20.98	.000
CpG 238	F	17.54±9.47	11.81±23.26	.000
	ND	17.62±5.55	14.26±20.97	.000
CpG 243	F	36.23±13.23	28.24±44.23	.000
	ND	43.23±14.76	34.21±52.15	.000
CpG 246	F	28.54±10.71	22.07±35.01	.000
	ND	28.54±7.48	24.02±33.06	.000
CpG 252	F	19.77±8.05	14.90±24.64	.000
	ND	20.23±4.21	174.70±22.77	.000
CpG 260	F	28.00±11.23	21.21±34.79	.000
	ND	29.69±7.03	25.45±33.94	.000
CpG 281	F	37.23±15.54	27.84±46.62	.000
	ND	39.69±14.37	31.01±48.38	.000
CpG 288	F	27.31±12.60	19.70±34.92	.000
	ND	22.77±12.50	15.21±30.33	.001

Table 3 Methylation levels of the 23 CpG sites in the Intron region amongst F and ND groups compared to H group.

Figures

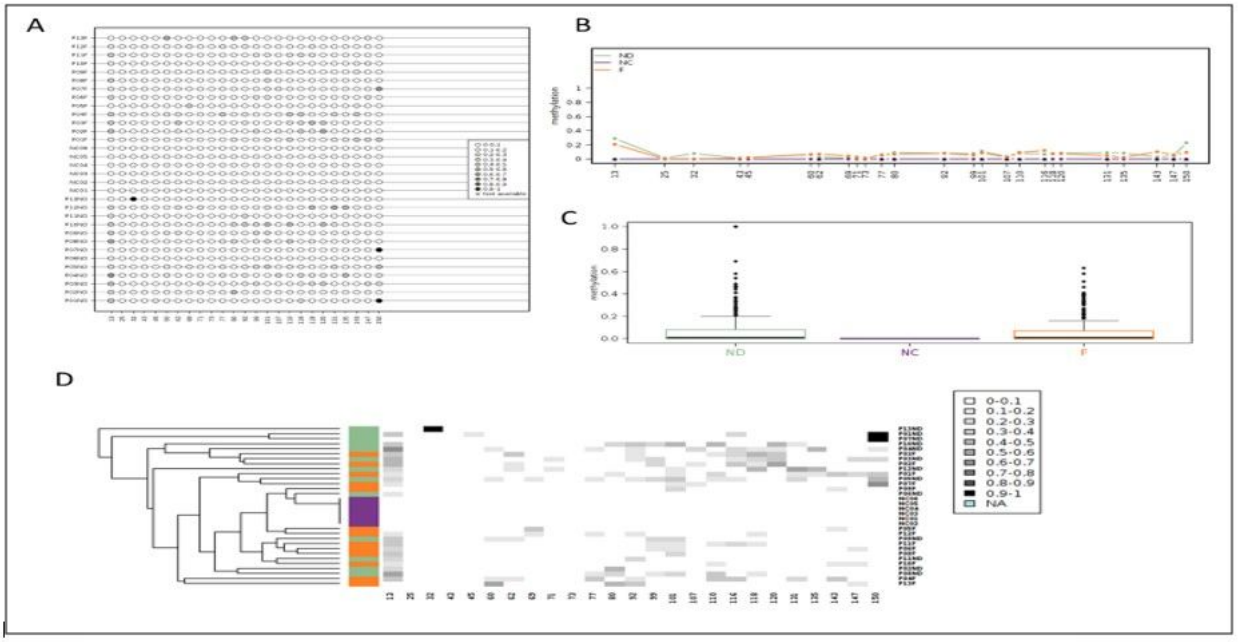

Figure 1

Data visualization with Methylation plotter for 25 sites of Promoter region of PTPRG. A, Lollipop-like visualization of methylation sites B, methylation profiling plot reflecting with asterisks those positions for which significant differences between groups were detected. C, boxplots for each group showing the methylation data distribution. D, unsupervised hierarchical clustering of the data; sample label colors reflect groups classification.

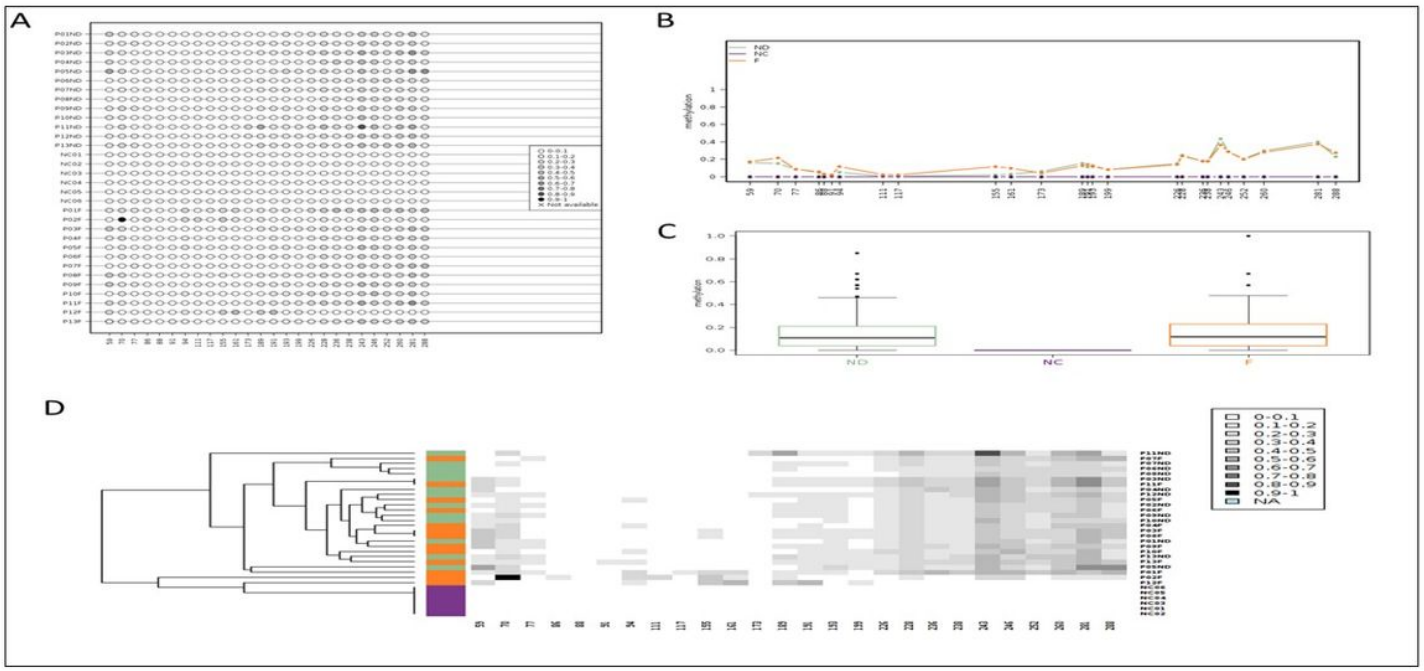

Figure 4

Data visualization with Methylation plotter for 26 sites of Intron region of PTPRG. A, Lollipop-like visualization of methylation sites B, methylation profiling plot reflecting with asterisks those positions for which significant differences between groups were detected. C, boxplots for each group showing the methylation data distribution. D, unsupervised hierarchical clustering of the data; sample label colors reflect the groups classification.

Supplementary Files

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