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Research paper

Description of PTPRG genetic variants identified in a cohort of Chronic Myeloid Leukemia patients and their ability to influence response to Tyrosine kinase Inhibitors

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ABSTRACT

Tyrosine kinase inhibitors (TKIs) have remarkably transformed Ph+ chronic myeloid leukemia (CML) management; however, TKI resistance remains a major clinical challenge. Mutations in *BCR-ABL1* are well studied but fail to explain 20–40% of resistant cases, suggesting the activation of alternative, *BCR-ABL1*-independent pathways. Protein Tyrosine Phosphatase Receptor Gamma (*PTPRG*), a tumor suppressor, was found to be well expressed in CML patients responsive to TKIs and remained at low level in resistant patients. In this study, we aimed to identify genetic variants in *PTPRG* that could potentially modulate TKIs response in CML patients. DNA was extracted from peripheral blood samples collected from two CML cohorts (Qatar and Italy) and targeted exome sequencing was performed. Among 31 CML patients, six were TKI-responders and 25 were TKI-non-responsive. Sequencing identified ten variants, seven were annotated and three were novel SNPs (c.1602_1603insC, c.85+14412delC, and c.2289-129delA). Among them, five variants were identified in 15 resistant cases. Of these, one novel exon variant (c.1602_1603insC), c.841-29C>T (rs199917960) and c.1378-224A>G (rs2063204) were found to be significantly different between the resistant cases compared to responders. Our findings suggest that *PTPRG* variants may act as an indirect resistance mechanism of *BCR-ABL1* to affect TKI treatment.

Abbreviations: CML, Chronic Myeloid Leukemia; TKIs, Tyrosine Kinase Inhibitors; *BCR-ABL1*, Breakpoint cluster region- ABL Proto-Oncogene 1; *PTPRG*, Protein Tyrosine Phosphatase Receptor Gamma; DNA, Deoxyribonucleic Acid; SNP, Single Nucleotide Polymorphisms; Ph, Philadelphia Chromosome; *CDK1*, cyclin-Dependent Kinase 1; *CDC2*, Cell Division Cycle protein 2 homolog; ATP, Adenosine Triphosphate; IM, Imatinib Mesylate; KD, Kinase Domain; ELN, European LeukemiaNet; PTP, Protein Tyrosine Phosphatase; EDTA, Ethylenediamine Tetraacetic Acid; PGM, Personal Genome Machine; OR, Odds Ratio; CI, Confidence Interval; QGP, Qatar Genome Program.

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1. Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder of primitive hematopoietic progenitor cells caused by the fusion protein kinase derived from a reciprocal translocation between the *ABL1* tyrosine kinase gene at chromosomes 9 and the *BCR* gene at chromosome 22. This translocation results in the creation of a *BCR-ABL1* hybrid oncogene on the derivative Philadelphia (Ph) chromosome and the formation of a constitutively active BCR-ABL protein with enhanced tyrosine kinase activity in CML (Jabbour and Kantarjian, 2020). The *BCR-ABL1* gene is ubiquitously expressed and is regulated by cyclin-dependent kinase 1 (*CDK1*) or cell division cycle protein 2 homolog (*CDC2*)-mediated phosphorylation. Thus, mutations in *CDKI/CDC2* can cause alterations in several cell signaling pathways, leading to loss of regulation of DNA damage response and apoptosis of cells, contributing to the cancerous condition in CML patients (Willis et al., 2005; Vaidya et al., 2015).

Tyrosine kinase inhibitors (TKIs) have remarkably changed CML management by targeting the ATP binding site of the Abl protein kinase leading to stabilization of the inactive form of *BCR-ABL1* (Asnafi et al., 2019). Currently, there are four TKIs approved for CML treatment: the first generation (Imatinib Mesylate) (IM), the second generation (Nilotinib and Dasatinib), and the third generation TKI (Ponatinib) (Gover-Proaktor et al., 2019). In CML patients, mutations in the kinase domain (KD) of *BCR-ABL1* gene result in TKIs resistance (Willis et al., 2005; Milojkovic and Apperley, 2009). Globally, over 25% of CML patients present with various degree of resistance to therapies. Interestingly, a high percentage (54%) was reported in the State of Qatar (Apperley, 2007; Apperley, 2007; Branford et al., 2019; Chandrasekhar et al., 2019; Al-Dewik et al., 2014). Resistance to treatments is defined as failure of CML patients to achieve hematological or cytogenetic or molecular response with European LeukemiaNet (ELN) frame timeline (Baccarani et al., 2019; Hochhaus, 2006). This may lead to an increased risk of disease progression/death. Resistance to TKIs can be classified in *BCR-ABL1* dependent and independent mechanisms. *BCR-ABL* KD mutations represent the most frequent mechanism of acquired resistance through impairment of TKI activity mediated by occupation of a specific drug binding site into the protein. Point mutations account for the most common resistance mechanisms associated with KD mutations (Meenakshi Sundaram et al., 2019). Notably, mutation of *BCR-ABL* KD were shown to present with different sensitivity levels, not only to the first generation of TKIs, but also to the second and the third generation TKIs (Redaelli et al., 2012). Although mutations in exons have been widely investigated, less information is available for mutations in introns (Anna and Monika, 2018; Vaz-Drago et al., 2017).

Despite great improvements in CML treatment, some patients fail to achieve optimal molecular response or require prolonged duration of treatment time [i.e., >12 months as per the (ELN) guidelines] (Soverini et al., 2019). Others fail treatment due to mechanisms that are independent from the acquisition of resistance-associated mutations in *BCR-ABL1* (Apperley, 2007). It has been estimated that approximately 20–40% of CML cases of clinical TKI failure are due to alternative mechanisms that are independent of *BCR-ABL1* (Wagle et al., 2016).

There has been a growing body of evidence suggesting that Protein Tyrosine Phosphatase Family (PTP) could play a role as potential therapeutic targets (He et al., 2014), as well as independent prognostic factors in solid cancer (Zhangyuan et al., 2018). Furthermore, several studies highlighted the role of PTP variants in various human cancers and drug efficacy (Hendriks and Pulido, 2013; Zhao et al., 2015). Among PTPs, the gene encoding for protein tyrosine phosphatase receptor gamma (*PTPRG*), is currently recognized as a tumor suppressor gene in CML and other types of cancers (Hendriks et al., 2013). In contrast to protein kinases, *PTPRG* has the ability to remove a phosphate group from the phosphorylated amino acid tyrosine present on its substrate protein (Hendriks et al., 2013; Tonks, 2006). *PTPRG* expression was reported to be down-regulated in CML patients at diagnosis (Della Peruta et al., 2010). In addition, the protein level of *PTPRG* determined by flow cytometry on neutrophils was found to be significantly higher in CML patients who responded to treatment with TKIs compared to non-responders (Ismail et al., 2021). Moreover, aberrant DNA methylation of *PTPRG* was reported as one possible mechanism of under-expression (Ismail et al., 2020; Ismail et al., 2018). Furthermore our preliminary data reported four mutations associated to CML patients who failed Imatinib treatment (Al-Dewik et al., 2016).

While the epigenetic mechanism and expression level of *PTPRG* are well studied, an in-depth study of other mechanisms that might affect *PTPRG* activity in CML patients is needed and warrantsfurther investigation. Hence, in this study, we aimed to investigate and identify all possible genetic *PTPRG* variants in two different CML cohorts (treatment-responders vs. failure cohorts) and correlate these variants with the clinical outcome as an indirect prognostic tool for CML patients.

2. Materials and methods

2.1. Patients and sample collection

The World Medical Association's Declaration of Helsinki (1964–2008) for Ethical Human Research, was followed in this research. This study included a total of 44 samples obtained from 31 adult CML patients.

The samples were collected from patients at different intervals during the treatment plan, at day one of diagnosis, and at the time of achieving response or failed/relapsed treatments. The CML patients' response to treatment was evaluated according to the cytogenetic, hematologic, and molecular responses. The responder cohort was defined as the ability to achieve hematological, molecular, and cytogenetic responses, regardless of the time frame. Patients with non-responsive to treatment (failure cohort) were defined as showing a lack of any response following a maximum of 36 months follow-up.

2.2. DNA isolation, NanoDrop, Qubit

gDNA was isolated from patients' whole blood was isolated using the Maxwell® 16 DNA Purification Kits according to the manufacturer's instructions (Khokhar et al., 2011). The purity and the concentration were measured both on Nanodrop 2000c spectrophotometer (Thermo-Fisher Scientific, USA) and on Qubit Fluorimeter using DNA BR assay kit (Invitrogen, UK). DNA Samples that passed the QC with concentrations above 50 ng/μL and purity of a ratio of 1.8–2.0 were selected for *PTPRG* targeted sequencing on Ion Torrent Personal Genome Machine (PGM™) sequencer.

2.3. Ampliseq primer design

For Ion Torrent Sequencing, primers for the full Exonic regions of *PTPRG* gene with 5 bp padding was designed using Ion Ampliseq Designer v4.0 tool. (ampliseq.com), Thermo Fisher, Scientific, USA). The designed primers (supplementary file) covered 94% of all the targeted exonic regions of the *PTPRG* gene with 5 bp Intron-Exon Padding to detect splice mutations. The total bases of *PTPRG* were 4,638, while designed primers covered 4362 bases. Finally, a total of 33 Primer Pairs were received in 2 pools (Design ID: IAD 69908) for Ampliseq Library preparation (Table S1).

2.4. Library preparation and sample pooling

A ten ng each of the sample DNA was amplifed with 2 pools of designed ampliseq primers in each tube using the Ion Library kit v2.0 (Thermo Fisher Scientific as per the manufacturer guidelines). Post amplification follows partial digestion of the primers and phosphorylate the amplicons for adapter ligation with FuPa reagent from the Ion Library Kit. Using the Ion barcode set, each sample was combined with common Ion P1 adaptor and unique barcode X adaptors for sample

identification. The barcoded library was cleaned up with AmpureXp beads (Beckman-coulter). Amplify the barcoded libraries for 6 cycles using the Library amplification mix from the Ion Library Kit as per the maufacturer protocol guide. Again purify the amplified Library using AmpureXp beads (Beckman-coulter) as per the protocol. The final libraries were quantified on Qubit fluorometer using Qubit dsDNA HS Assay Kit and Agilent Bioanalyzer, as per the Library kit user guide and the concentration was adjusted to the final library of 100 pM. The libraries were diluted to ~100 Pm, equal volumes of the barcoded libraries were combined in sets of 16 barcodes in a total volume of 100 μL for the next step of Library Template to Ion Sphere Particles (ISP).

2.5. Library template preparation and enrichment

The barcoded libraries was templated with ISP (Ion Sphere Particles) using emulsion PCR and enriched for positive templated libraries for Ion Torrent Sequencing. For the template preparation, 2uL of 100 pM pooled library was combined with 23 μL of nuclease-free water and used as a template for emulsion PCR using the Ion PGM™ Template OT2 200 Kit on the Ion One Touch2 system as per the Manufacturers user guide. The diluted library was combined with Ion OT2 amplification solution and reaction oil. Then loaded into the Ion One Touch 2 system to generate the templated ISP, followed by the enrichment for positive templated ISPs using the Ion OT enrichment system and its kits, as per the manufacturer's protocol. The quality of the final positive templated ISP's was assessed on Qubit Fluorometer 2.0 using Ion Sphere™ Quality Control assay, and the ratio of templated to non-templated ISPs was calculated.

2.6. Ion Torrent personal genome machine (PGM) sequencing of PTPRG gene

Prior to run, the Ion PGM machine was initialized to adjust the pH of the wash 2 buffer to 7.2–7.3 as per the manufacture protocol. After successful pH, individual four nucleotides were added into respective tubes to complete the system initialization. Once the system has passed the initialization, it is ready to run the Ion chip. A five μL of Control Ion Sphere™ Particles was added directly to the entire volume of enriched, template-positive ISPs, centrifuged and supernatant removed, leaving 15 μL volume in the tube. To this Ion sequencing primer was added and incubated at 95 °C for 2 min and then 37 °C for 2 min on the PCR. Ion Chip check was performed on the PGM machine to qualify a new Ion the chip for a run. Once the Chip check was successful, the chip was ready for sample loading.

After primer annealing, the tubes were removed from the thermal cycler, and 3 μL of Ion PGM™ Sequencing 200 v2 Polymerase was added to the ISPs and incubated at room temperature for 5 min. The sample was loaded into the chip programmed for the Ion PGM sequencing run, as per the kit protocol.

2.7. BCR-ABL1 and PTPRG quantification and PTPRG quantitation by flow cytometry application

The *BCR-ABL1* and *PTPRG* quantification were carried out via RT-PCR. PTPRG expression was also examined using the flow cytometry technique as previously described (Al-Dewik et al., 2014; Della Peruta et al., 2010; Ismail et al., 2021; Vezzalini et al., 2017).

2.8. Data analysis and statistical analysis

Variant calling was performed using the Torrent Suite Software v.4.0.2 (Life Technologies) with the variantCaller plugin optimized with the parameters for the *PTPRG* panel. Additional variant analysis was also performed using the GeneGrid (Genomatix, GmbH) cloud variant analysis software to list the variants classifying for dbSNP, allele frequency. Positions of variants and variant effect predictor data were verified by data mining using the Ensembl project utilizing GRCh37 reference sequence. Genotype and allele frequencies for annotated SNPs in the *PTPRG* in CML patients were compared to 1000 Genomes Project (Auton et al., 2015) as well as the Qatar genome program (QGP) as reference (Al Thani et al., 2019).

For comparison of the allele and genotype frequencies of SNPs between CML patients and reference (Control), Odds ratios (ORs) and 95% Confidence Interval (CI) were calculated. The statistical significance was set at $P \leq 0.05$. Logistic regression models (codominant, dominant, and recessive) were conducted. SPSS 25.0 software was employed for data analysis (SPSS Inc., Chicago, IL, USA).

3. Results

Patient demographic and disease characteristics along with quantification of both *PTPRG* and *BCR-ABL* are presented in (Table 1). In total, 31 CML patients (24 males and 7 females) were included in the study; 17 from Qatar and 14 from Italy. The mean age was 44.4 years. Regarding the clinical phase at diagnosis, 29 (93.5%) patients were in the chronic phase (CP) and 2 (6.5%) patients were in the accelerated phase (AP) (CML cases 20 and 23). Out of 29 patients in CP, 28 were treated with imatinib 400 mg/day and the remaining patients were on nilotinib 300 mg/day (CML case 30). Patients in AP were treated with imatinib 600 mg/day (CML cases 20 and 23). The patients' median follow-up was 24.5 months (range: 6 to 36 months). Out of the 31 CML patients treated with TKIs, six patients achieved response (*BCR-ABL* < 1 %) (19%), and 25 patients failed treatment (81%). None of the patients had additional chromosomal anomalies (ACA) during the study (Table 1).

3.1. Molecular genetic findings

Forty-four samples were sequenced with overall good quality metrics, a mean depth of 2,000x. We detected two missense, one synonymous and one frameshift substitution in the PTPRG exons, and six variants in the intronic region.

PTPRG sequencing identified five variants that were distinctive for resistant CML cases only at the time of diagnosis and during follow up. Of the five homozygous variants, c.841-29C>T was identified in three patients, and c.1720G>A; p.Gly574Ser was identified in two patients (Table 2). The variant c.274 T>C; p.Tyr92His was found in homozygous and heterozygous forms in six resistant cases (one homozygous and five heterozygous from the Italian and Qatari cohorts, respectively), while, c.1034-46C>T, was identified as homozygous and heterozygous forms in 2 resistant cases from Qatar cohort. In addition to the above, one novel heterozygous variant was identified in the TKI-resistant CML patient group (c.1602-1603insC; Thr536HisfsX15), which was identified in two CML patients from the Italian cohort (Table 2).

Apart from above variants, two homozygous variants (c.2448C>T and c.86–13T>C) were identified in thirty one and eight CML cases, respectively. Furthermore, five heterozygous variants (c.1378-224A>G, c.86–13 T>C, c.84129C>T, c.1720G>A, c.85+14412delC, and c.2289-129delA) were identified in CML patients numbers, thirty one, seventeen, ten, twelve, twenty five and twenty one, respectively (Table 2). These variants were identified at the time of diagnosis and during follow-up.

3.2. Association of PTPRG variants with CML disease

Genotyping and allele analysis showed that two out of seven *PTPRG* variants were significantly associated with CML disease (Table 3). The rs199917960 variant had three genotypes as C/C, C/T and T/T with frequencies 58%, 32.3% and 9.7%, respectively among the CML patients' group, whereas in the 1000 Genomes Project control the frequencies were C/C: 99.96%, C/T: 0.04% and T/T: 0%, respectively as well as QGP CC: 100%, C/T and T/T: 0%. On the other hand, the three genotypes of rs2063204 were A/A, A/G and G/G with frequencies of

Table 1
CML patient's characterization according to clinical phase, TKIs and overall response timeline.

Patients	BCR-ABL1(IS)	*PTPRG(%)*	*PTPRG* ExpressionNeutrophil (MFI)	Response	follow up Timeline(Months)	Remarks
CML case 01.	100	0.01	0.90	Failed	12	
CML case 02.	42.20	1.20	0.80	Failed	36	4 subsequent samples
CML case 03.	100	0.01	1.10	Failed	15	
CML case 04.	63.40	0.01	1.20	Failed	36	2 subsequent samples
CML case 05.	8.20	0.04	1.20	Failed	36	2 subsequent samples
CML case 06.	100	N/A	1.10	Failed	36	
CML case 07.	100	0.01	0.90	Failed	36	
CML case 08.	100	0.10	1.20	Failed	24	
CML case 09.	87.10	0.01	1.10	Failed	18	
CML case 10.	100	0.10	0.90	Failed	20	
CML case 11.	100	0.01	1.1	Failed	36	
CML case 12.	100	0.01	1.40	Failed	24	2 subsequent samples
CML case 13.	100	0.02	1.50	Failed	12	
CML case 14.	73.40	0.01	1.10	Failed	36	
CML case 15.	60	0.01	0.90	Failed	36	
CML case 16	100	0.01	0.90	Failed	–	Single time point
CML case 17*.	100	0.37	1.00	Failed	36	
CML case 18*.	86	1.78	1.10	Failed	36	
CML case 19*.	100	0.37	1.20	Failed	36	
CML case 20*.	91	N/A	1.30	Failed	12	
CML case 21*.	101	0.04	1.20	Failed	36	
CML case 22*.	113	1.52	0.90	Failed	12	
CML case 23*.	19	0.15	1.10	Failed	36	
CML case 24*.	100	0.05	1.30	Failed	36	1 subsequent sample
CML case 25*.	84	0.03	0.80	Failed	24	
CML case 26*.	100	0.13	1.40	Responder	24	1 subsequent sample
CML case 27*.	100	4.15	1.32	Responder	24	
CML case 28*.	34	0.46	1.40	Responder	15	
CML case 29*.	62	0.001	1.20	Responder	36	1 subsequent sample
CML case 30*.	65	0.01	1.10	Responder	6	
CML case 31.	89	0.01	1.51	Responder	12	

* Indicates CML patients from the Italian cohort. CML case 31- achieved molecular response at 1st follow up (3 months), while *PTPRG* protein expression restored its expression on 2nd follow up (6 months).

Table 2
PTPRG Genetic variants identified in CML patients.

RefSeq no.	Location	cDNA	Amino Acid Change	Zygosity	No patients
rs199917960*	Intron 7	c.841-29C>T	NA	Homo	3*
				Hetro	10
rs62620047*	Exon 3	c.274T>C	p.Tyr92His	Homo	1*
				Hetro	5*
rs2292245*	Exon 12	c.1720G>A	p. Gly574Ser	Homo*	2*
				Hetro	12
rs57829866*	Intron11	c.1034-46C>T	NA	Homo	1*
				Hetro	1*
Novel 1*	Exon 12	c.1602-1603insC	p.Thr536HisfsX15	Hetro	2*
rs1352882	Exon15	c.2448C>T	p.Ile816Ile	Homo	31
rs2063204*	Intron 11	c.1378-224A>G	NA	Hetro	29
				Homo	2
rs3821880	Intron 1	c.86–13T>C	NA	Hetro	17
				Homo	8
Novel 2	Intron 1	c.85+14412delC	NA	Hetro	25
Novel 3	Intron 13	c.2289-129delA	NA	Hetro	21

* Denotes variants associated with TKI-resistant cases.

0%, 93.4 % and 6.5%, respectively, among CML patients, and frequencies of 0.08%, 2.8% and 97.12%, respectively in the 1000 Genomes Project control as well as QGP A/A: 0.03% A/G: 0.9% and G/G 99.07%.

The frequency of the major (C) allele in rs199917960 variant was 74.2% and 99.9% for the CML and control group respectively, while the minor (T) allele frequency was 25.8% and 0.01% for the CML patients and control group respectively. Notably the distribution of allele frequency was significant between the indicated groups (Table 3). Further, the frequencies of the major (A) and the minor (G) alleles in rs2063204 variant were 46.8% and 53.2% vs. 1.5% and 98.5% among the CML patients' and control groups, respectively.

Similar findings of genotyping and allele analysis for rs199917960 and rs2063204 were also obtained when compared to QGP (Table 3). In addition; rs62620047 and rs57829866 were significant when compared to QGP only. Rs62620047 variant showed three genotypes of CML patients as T/T, T/C and C/C with frequencies 80.6%, 16.2% and 3.2%. While the genotypes frequencies of QGP controls were 58.2%, 34.9% and 6.9% respectively. Then again, three genotypes of rs57829866 variant of CML patients as C/C, C/T and T/T with frequencies 93.6%, 3.2 % and 3.2 % which corresponding to 96.8%, 3.0% and 0.2% in QGP controls. The frequency of the major (T) allele in rs62620047 variant was 88.7% and 75.7% for the CML patients and control group

Table 3
Genotype and allele frequencies of variants of *PTPRG* in CML patients in comparison to 1000 Genomes Project and QGP.

Genetic variants	Genotype	CML patients N = 31	1000 Genomes Project Reference N = 2504	*P* value	QGP Reference N = 14669	*P* value	Allele	CML	Reference	*P* value	QGP Reference	*P* value
rs199917960	C/C	18 (58%)	2503 (99.96%)	**<0.0001**	14,669 (100%)	**0.0001**	C	46 (74.2%)	5007 (99.9%)	**<0.0001**	29,338 (100%)	**0.0001**
	C/T	10 (32.3%)	1 (0.04%)		0 (0%)		T	16 (25.8%)	1 (0.01%)		0 (0%)	
	T/T	3 (9.7%)	0 (0%)		0 (0%)							
rs62620047	T/T	25 (80.6%)	2092 (83.54%)	0.5400	8538 (58.2%)	**0.0400**	T	55 (88.7%)	4568 (91.2%)	0.4949	22,196 (75.7%)	**0.0168**
	T/C	5 (16.2%)	384 (15.34%)		5120 (34.9%)		C	7 (11.3%)	440 (8.8%)		7142 (24.3%)	
	C/C	1 (3.2%)	28 (1.12%)		1011 (6.9%)							
rs2292245	G/G	17 (54.8%)	1565 (62.5%)	0.6699	7616 (51.9%)	0.8840	G	46 (74.2%)	3919 (78.3%)	0.4399	20,995 (71.6%)	0.7781
	G/A	12 (38.7%)	789 (31.5%)		5763 (39.3%)		A	16 (25.8%)	1089 (21.7%)		8343 (28.4%)	
	A/A	2 (6.5%)	150 (6.0%)		1290 (8.8%)							
rs57829866	C/C	29 (93.6)	2245 (89.7%)	0.2654	14,206 (96.8%)	**<0.0001**	C	59 (95.2%)	4723 (94.3%)	>0.9999	28,858 (98.4%)	0.0820
	C/T	1 (3.2%)	233 (9.3%)		446 (3.0%)		T	3 (4.8%)	285 (5.7%)		480 (1.6%)	
	T/T	1 (3.2%)	26 (1.0%)		17 (0.2%)							
rs1352882*	C/C	0	9 (0.3%)	0.5214	4 (0.03%)	0.8264	C	0	101 (2.2%)	0.6457	182 (0.6%)	>0.9999
	C/T	0	92 (3.7%)		174 (1.19%)		T	62 (100%)	4898 (97.8%)		29,116 (99.4%)	
	T/T	31 (100%)	2403 (96%)		14,471 (98.78%)							
rs2063204	A/A	0	2 (0.08%)	**<0.0001**	3 (0.03%)	**<0.0001**	A	29 (46.8%)	73 (1.5%)	**<0.0001**	139 (0.5%)	**<0.0001**
	A/G	29 (93.4%)	69 (2.8%)		133 (0.9%)		G	33 (53.2%)	4935 (98.5%)		29,199 (99.5%)	
	G/G	2 (6.5%)	2433 (97.12%)		14,533 (99.07%)							
rs3821880	T/T	6 (19.4%)	415 (16.6%)	0.5144	2757 (18.8%)	0.500	T	29 (47.8%)	2023 (40.4%)	0.3622	12,214 (41.6%)	0.4403
	T/C	17 (54.8%)	1193 (47.6%)		6700 (45.7%)		C	33 (53.2%)	2985 (59.6%)		17,124 (58.4%)	
	C/C	8 (25.8%)	896 (35.8%)		5212 (35.5%)							

* Total number for rs1352882 was 14,649 at QGP. **Bold** value denotes statistically significant differences between CML group and reference group (1000 Genomes Project).

respectively. While the minor (C) allele frequency was 11.3% and 24.3% as well for CML patients and control group respectively. Notably the distribution of allele frequency was significant between mentioned groups (Table 3). The variant rs57829866 major (C) allele frequency was 95.2% (CML patients) vs. 98.4% (Control group), and the minor (T) allele frequency was 4.8% (CML patients) vs. 1.6% (Control group). However, there was no significant differences in the allele frequency amongst groups (Table 3).

In comparison to the 1000 Genomes Project, rs199917960 was significantly associated with CML disease in all models: co-dominant (C/T vs C/C: OR = 1390.5556, 95% CI: 169.0438–11438.7216), $P < 0.0001$; T/T vs. C/C: OR = 947.2703, 95% CI: 47.2406–18994.7026), $P < 0.0001$), dominant (OR = 1807.7, 95% CI: 224.4522–14559.2698, $P < 0.0001$); recessive (OR = 615.1404, 95% CI: 31.0530–12185.5527), $P < 0.0001$), and over–dominant (OR = 1191.9, 95% CI: 145.9485–9733.8255), $P < 0.0001$) (Table 4). On the other hand, rs2063204 was significantly associated with CML disease in the recessive and over-dominant models only (G/G vs. A/A-A/G: OR = 0.002, 95% CI: 0.0005–0.0086, $P < 0.0001$; A/G vs. A/A–G/G: OR = 511.7, 95% CI: 119.7–2187.5, $P < 0.0001$) (Table 4).

In comparison to QGP, rs199917960 was significantly associated with CML diesase in all models: co-dominant (C/T vs C/C: OR = 16651.8649, 95% CI: 940.8474–294717.9492, $P < 0.0001$; T/T vs. C/C: OR = 5550.6216, 95% CI: 276.8696–111277.6754, $P < 0.0001$), dominant (OR = 21409.5405, 95% CI: 1226.9387 to 373587.0534, $P < 0.0001$); recessive (OR = 3603.0351, 95% CI: 181.9240–71358.7125, $P < 0.0001$), and over–dominant (OR = 14328.3488, 95% CI: 813.6619–252318.0504, $P < 0.0001$) (Table 4). Again, rs2063204 was significant in the co-dominant, recessive, and over-dominant models only (G/G vs. A/A: OR = 0.0012, 95% CI: 0.0000–0.0299, $P < 0.0001$, A/A-A/G vs. G/G: OR = 0.0006, 95% CI: 0.0002–0.0027, $P < 0.0001$; A/A–G/G vs. A/G: OR = 1584.7519, 95% CI: 374.3251–6709.2436, $P < 0.0001$) (Table 4).

Table 4
Logistic regression analysis of the association between *PTPRG* variants (rs199917960 and rs2063204) and TKI response in CML patients.

SNP	Model	Genotype	*1000 Genomes Project Reference N = 2504	**QGP Reference N = 14669	CML patients N = 31	*OR (95% CI)	P value	**OR (95% CI)	P value
rs199917960	Co-dominant	C/C	2503 (99.96%)	14,669 (100%)	18 (58.06%)	Reference		Reference	
		C/T	1 (0.04%)	0 (0%)	10 (32.26%)	1390.5556 (169.0438–11438.7216)	**<0.0001**	16651.8649 (940.8474–294717.9492)	**<0.0001**
		T/T	0 (0%)	0 (0%)	3 (9.68%)	947.2703 (47.2406–18994.7026)	**<0.0001**	5550.6216 (276.8696–111277.6754)	**<0.0001**
	Dominant	C/C	2503 (99.96%)	14,669 (100%)	18 (58.06%)	Reference		Reference	
		C/T-T/T	1 (0.04%)	0 (0%)	13 (41.94%)	1807.7 (224.4522–14559.2698)	**<0.0001**	21409.5405 (1226.9387 to 373587.0534)	**<0.0001**
	Recessive	C/C- C/T	2504 (100%)	14,669 (100%)	28 (90.32%)	Reference		Reference	
		T/T	0 (0%)	0 (0%)	3 (9.68%)	615.1404 (31.0530–12185.5527)	**<0.0001**	3603.0351 (181.9240–71358.7125)	**<0.0001**
	Over dominant	C/C-T/T	2503 (99.96%)	14,669 (100%)	21 (67.74%)	Reference		Reference	
		C/T	1 (0.04%)	0 (0%)	10 (32.26%)	1191.9 (145.9485–9733.8255)	**<0.0001**	14328.3488 (813.6619–252318.0504)	**<0.0001**
rs2063204	Co-dominant	A/A	2 (0.08%)	3 (0.02%)	0 (0%)	Reference		Reference	
		A/G	69 (2.76%)	133 (0.91%)	29 (93.55%)	2.1223 (0.0988–45.5729)	0.4810	1.5468 (0.0778–30.7580)	0.7749
		G/G	2433 (97.12%)	14,533 (99.07%)	2 (6.45%)	0.0051 (0.0002–0.1365)	0.0051	0.0012 (0.0000–0.0299)	**<0.0001**
	Dominant	A/A	2 (0.08%)	3 (0.02%)	0 (0%)	Reference		Reference	
		A/G-G/G	2502 (99.92%)	14,666 (99.98%)	31 (100%)	0.0629 (0.0030–1.3379)	0.0762	0.0150 (0.0008 to 0.2971)	0.0058
	Recessive	A/A–A/G	71 (2.84%)	136 (0.93%)	29 (93.55%)	Reference		Reference	
		G/G	2433 (97.16%)	14,533 (99.07%)	2 (6.45%)	0.002 (0.0005–0.0086)	**<0.0001**	0.0006 (0.0002–0.0027)	**<0.0001**
	Over-dominant	A/A-G/G	2435 (97.24%)	14,536 (99.09%)	2 (6.45%)	Reference		Reference	
		A/G	69 (2.76%)	133 (0.91%)	29 (93.55%)	511.7 (119.7–2187.5)	**<0.0001**	1584.7519 (374.3251–6709.2436)	**<0.0001**

Bold value denotes statistically significant results

3.3. Correlation of PTPRG SNPs with overall outcome of CML patients

Afterwards, the rs199917960 and rs2063204 were investigated amongst the responsive and non-responsive groups. Rs199917960 was significantly associated with non-responders in the co-dominant (T/T vs. C/C: OR = 259.0000 (4.3655 to 15366.1413, $P = 0.0076$), dominant (C/T-T/T vs. C/C: OR = 32.0667 (1.5981 to 643.4412, $P = 0.0234$), and recessive (T/T vs. C/C- C/T: OR = 51.0000 (2.1489 to 1210.3845, $P = 0.015$) models when compared to the responders group. However, no significant association was observed in the over-dominant model (Table 5). The variant rs2063204 was significantly associated with non-responsive group in the recessive and over-dominant models (G/G vs. A/A–A/G: OR = 28.3333 (1.1578 to 693.3716, $P = 0.0404$) and (A/G vs. A/A–G/G: OR = 0.0353 (0.0014 to 0.8637$P = 0.0404$) respectively. However, there were no significant differences in the co-dominant and dominant models in comparison to responsive group (Table 5).

Table 5
Logistic regression analysis of the association between *PTPRG* variants (rs199917960 and rs2063204) and overall response.

SNP	Model	Genotype	Non-responders N = 25	Responders N = 6	OR (95% CI)	*P* value
rs199917960	Co-dominant	C/C	18 (72%)	0 (0%)	Reference	
		C/T	7(28%)	3(50%)	17.2667 (0.7917 to 376.5659)	0.0701
		T/T	0 (0%)	3 (50%)	259.0000 (4.3655 to 15366.1413)	**0.0076**
	Dominant	C/C	18 (72%)	0 (0%)	Reference	
		C/T-T/T	7(28%)	6 (100%)	32.0667 (1.5981 to 643.4412)	**0.0234**
	Recessive	C/C- C/T	25 (100%)	3(50%)	Reference	
		T/T	0 (0%)	3(50%)	51.0000 (2.1489 to 1210.3845)	**0.0150**
	Over-dominant	C/C-T/T	18 (72%)	3(50%)	Reference	
		C/T	7(28%)	3(50%)	2.5714 (0.4154 to 15.9180)	0.3099
rs2063204	Co-dominant	A/A	0 (0%)	0 (0%)	Reference	
		A/G	25 (100%)	4 (66.7%)	0.1765 (0.0031 to 10.0893)	0.4008
		G/G	0 (0%)	2 (33.3%)	5.0000 (0.0351 to 711.8657)	0.5247
	Dominant	A/A	0 (0%)	0 (0%)	Reference	
		A/G-G/G	25 (100%)	6 (100%)	0.2549 (0.0046 to 14.1061)	0.5044
	Recessive	A/A-A/G	25 (100%)	4 (66.7%)	Reference	
		G/G	0 (0%)	2 (33.3%)	28.3333 (1.1578 to 693.3716)	**0.0404**
	Over-dominant	A/A-G/G	0 (0%)	2 (33.3%)	Reference	
		A/G	25 (100%)	4 (66.7%)	0.0353 (0.0014 to 0.8637)	**0.0404**

Bold value denotes statistically significant results.

4. Discussion

Despite the great progress that has been made in the treatment of many CML patients with the *BCR-ABL1* TKIs in the past years, drug resistance continues to be a major issue for CML patients, suggesting that the activation of alternative, *BCR-ABL1*-independent survival pathways may be linked to TKI efficacy and disease progression (Harrington et al., 2017; Chaitanya et al., 2017; Gullipalli et al., 2013; Boni and Sorio, 2021). While *BCR-ABL1* mutations have been extensively studied, studies on *BCR-ABL1*-independent mechanisms have not received equivalent attention.

In this study, we aimed to identify possible genetic variants in *PTPRG* that could potentially modulate response to TKIs in CML patients. To our knowledge, the present study is the first to identify homozygous variants in *PTPRG* in association with Imatinib and Nilotinib response among CML patients. Of the 31 CML patients; 6 were TKI-responders; and 25 were TKI-non-responsive. We identified ten variants in *PTPRG*, of which seven were previously annotated and three were novel variants (Table 2). Of the ten identified *PTPRG* variants, five were distinctive for the TKI-non-responsive CML patient's group (Table 2), and six were found to be shared among responders and non-responsive CML patients (Table 2). None of the identified variants were previously reported in ClinVar database (ncbi.nlm.nih.gov/clinvar).

Variants in the intronic region do not encode proteins. However, intronic variants were reported to influence gene and protien expression (Thi Tran et al., 2005; Do et al., 2010; Deng et al., 2017; Verhaegh et al., 2008). In the present study, we were able to identify six intronic variants (Table 2). A significant difference was observed for the intronic variants rs199917960 and rs2063204 between CML patients in comparison to 1000 Genomes Project as well as the Qatar genome program (Control) (Tables 3 and Table 4). These findings were further confirmed when we compared responder and non-responders groups (Table 5).

In the present study, the major allele (C) of rs199917960 predominated in the reference group with a frequency of 99.9% in comparison to 74.2% in CML patients' group ($P < 0.0001$), whereas the minor allele (T) frequency was 25.8% in the CML group which was significantly higher compared to the reference group (0.01%) ($P < 0.0005$). The percentage of reference allele (C/C) was higher in reference group (99.96%), while the percentage of heterozygous (C/T) allele and c and homozygous alternate allele (T/T) was significantly higher in CML group (C/C: 32.3% and T/T: 9.7%) in comparison to reference group (C/C: 0.04% and T/T: 0%). The variant was significantly associated with CML disease risk in all models (Table 4). While in the QGP, the major allele (C) was 100% and the minor allele (T) was absent.

The major allele (A) of rs2063204 variant was found to be significantly predominant in the CML patients' group with an allelic frequency of 46.8% compared to 1.5% in the reference group (Table 3). In contrast, the minor allele (G) frequency was significantly higher in the reference group (98.5%) in comparison to the CML group (53.2%) ($P< 0.0001$). The percentage of reference allele (A/A) and heterozygous allele (A/G) was almost same in reference and CML groups, while the percentage of recessive allele (G/G) was higher in reference group 97.2% in comparison to CML group 6.5 % ($P < 0.0005$). The variant was significantly associated with CML disease risk in the recessive and over-dominant models, however, no significant difference was found in the co-dominant and dominant models (Table 4). These findings are in concordance with the database from QGP and may explain the presence of rs2063204 in the responder and non-responder patient groups. Interestingly, the same findings were also obsreved for rs199917960 when we compared the responders to non-responder groups, the variant was significantly associated with CML disease risk in the recessive and over-dominant models only (Table 5).

In the current study, we identified three novel variants, among which one was a frameshift (c.1602-1603insC) that was identified in two patients from the Italian cohort, located in the coding region of *PTPRG* (Table 2). The remaining two variants (c.85+14412delC and c.2289-129delA) are in intronic regions, and both are deletion variants (Table 2). We identified *PTPRG* variants in both cohort groups (Qatar and Italy) that may influence CML responses to TKIs therapy. Of these, we found one homozygous variant (rs1352882; c.2448C>T), in the promoter region of *PTPRG* which was identified in all 31 CML patients. In addition, we identified two missense variants (c.1720G>A and c.274T>C), in the coding region of *PTPRG*. Interestingly, the c.274T>C was identified in homozygous alleles in the Italian group, but present in heterozygosity in Qatari group. However, no significant differences were observed, when compared to the 1000 Genomes Project reference (Control).

A limitation of this study was the small sample size. However, this is one of the few studies on *BCR-ABL1*-independent mechanisms and the first study to identify homozygous mutations in *PTPRG* that are associated with Imatinib and Nilotinib response in patients with CML.

5. Conclusions

CML is one the most common type of adult leukemia in the world. In the present study, we intended to identify genetic variants in the *PTPRG* gene in CML patients from two independent cohorts based on potential related clinical outcomes. Our findings identified variants in *PTPRG* that may act through an indirect resistance mechanism of *BCR-ABL1* to affect TKI response. These findings suggest that *PTPRG* could serve as an independent prognostic tool for CML. The identified variants could influence drug efficacy and disease progression, and thus might help provide a better understanding of mechanisms of resistance.

In-silico investigations on the structural changes in the *PTPRG* protein due to the mutation are warranted and will pave the way for understanding molecular mechanisms involved. In addition, further investigations of genetic variants in *PTPRG* gene in a larger group of CML patients are warranted and could lead to design of better therapeutic modalities such as dose elevation or earlier consideration of second and third generation drugs and these can ultimately help to improve the treatment outcomes and long-term prognosis in CML patients. The homozygous variants could affect the *PTPRG* structure causing protein loss of function. However, the pathogenicity of these variants in the *PTPRG* gene is uncertain and requires detailed characterization for functional consequences in appropriate disease models. These findings, provide further support on the importance of *PTPRG* and its variants for optimizing the therapeutic strategies for CML patients.

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Institutional review board statement

The study was approved by the Ministry of Public Health and the Institutional Review Board (IRB) at Hamad Medical Corporation-Qatar (Protocol No. 11118/11), Kingston University London's Ethical Committee (UK) and Local Ethics Committee of ASL Nuoro-Italy (Permit Number: 1276).

Informed consent statement

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

Data availability statement

This is a research article and all data generated or analyzed during this study are included in this published article [and its supplementary information file].

Credit authorship contribution statement

Mohamed. A. Ismail: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing-original draft, Writing-review &editing, Correspondence. **Gheyath K. Nasrallah:** Writing-review &editing, Resources. **Maria Monne:** Writing-review &editing, Resources. **Ali AlSayab:** Project administration. **Mohamed A. Yassin:** Visualization, Funding acquisition. **Govindarajulu Varadharaj:** Software, Data curation. **Salma Younes:** Resources, Data curation, Writing-review &editing. **Claudio Sorio:** Conceptualization, Writing-review &editing, Funding acquisition. **Richard Cook:** Writing-review &editing. **Helmout Modjtahedi:** Conceptualization, Writing-review &editing. **Nader I. Al-Dewik:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Funding acquisition, Visualization, Supervision, Writing-original draft, Writing-review &editing, Correspondence.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

The following supplementary materials are available online, Table S1: Primer sequences are manufactured precisely for custom with the Ion Ampliseq Kits and enclose proprietary modifications. Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2021.146101.

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