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Distribution of PON1 (Q192R) and ENPP1 (K173Q) gene polymorphisms in patients with sickle cell disease, sickle cell trait and healthy individuals

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Abstract. Hemoglobinopathies, such as sickle cell disease (SCD) cause major morbidity; however, non-globin gene variants remain largely underexplored. The present study analyzed 23 patients with SCD, 57 patients with sickle cell heterozygotes, and 66 healthy subjects as the controls. Hemoglobin variants were identified by high performance liquid chromatography. Genotyping for paraoxonase 1 (PON1; Q192R) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1; K173Q) polymorphisms was performed by conventional allele-specific PCR. Genetic association analyses were restricted to comparisons between patients with SCD and the healthy controls only. Allele-based analysis revealed that the R allele of PON1 was significantly less frequent in patients with SCD compared with the controls [odds ratio (OR), 0.36; 95% confidence interval (CI), 0.17=0.73; P<0.001]. For ENPP1, the Q allele was enriched in patients with SCD compared with the controls, corresponding to a 2.89-fold higher odds of disease in Q-allele carriers relative to K-allele carriers (OR, 2.89; 95% CI, 1.41-5.93; P=0.008). Linear regression analysis under an additive model revealed no significant association between the PON1 Q192R or ENPP1 K173Q genotypes and fetal hemoglobin levels. These findings demonstrate population-level differences in the distribution of PON1 and ENPP1 polymorphisms and underscore the need for larger studies to clarify their potential relevance in SCD.

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Introduction

Hemoglobinopathies, mainly β-thalassemia and sickle cell disease (SCD), are among the most prevalent inherited blood disorders and represent a major public health challenge in India (1-4). Although these conditions are caused by mutations in the globin genes, the clinical presentation is highly variable, suggesting that additional genetic modifiers may influence disease heterogeneity (5-7). The burden is particularly high in Western India, including Gujarat, where social practices, such as consanguineous marriage, limited diagnostic access, and the lack of awareness contribute to increased frequency and underdiagnosis (8-11). Despite the high prevalence, the role of genetic modifiers beyond the globin genes remains insufficiently characterized in this population.

Polymorphisms in the paraoxonase 1 *(PON1)* gene have been implicated in modulating oxidative stress, lipid metabolism and inflammatory pathways, which are relevant to the pathophysiology of hemoglobinopathies (12). PON1 is a 354-amino acid enzyme with a molecular mass of 43 kDa, exclusively bound to high-density lipoprotein. Numerous SNPs have been identified within the PON1 gene, with eight in the promoter region and 176 within the genomic sequence (13). Of note, two of the most studied PON1 coding sequence mutations are the leucine/methionine polymorphism at position 55 of the amino acid sequence K173Q or rs854560 and the glutamine/arginine polymorphism at position 192 (Q192R) (14). While the L55 variant has been associated with changes in PON1 serum concentrations, the Q192R polymorphism affects PON1 activity (12,15). These variants have been linked to dyslipidemia, hemolysis and inflammation, and a reduced activity of PON1 has further been associated with complications, such as stroke and splenectomy in patients with SCD (12). Studies have also demonstrated that individuals with the QQ genotype are better protected against low-density lipoprotein oxidation, and are thereby at a lower risk if developing coronary artery disease, atherosclerosis and stroke, whereas individuals with the RR phenotype are more prone to developing atherosclerosis and other related diseases (15,16). This is particularly

relevant in hemoglobinopathies, where chronic hemolysis and oxidative stress already predispose patients to endothelial dysfunction and vascular damage. Additionally, the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) K173Q polymorphism (frequently reported as K121Q due to an incorrect assignment at the start codon in early studies on *ENPP1*) has been demonstrated to play a role in coronary heart disease and diabetes by contributing to vascular and metabolic dysfunction (17,18). This raises the possibility that ENPP1 variants may be relevant to vascular biology in hemoglobinopathies; however, data from Indian populations remain limited.

Fetal hemoglobin (HbF) is a well-established modulator of disease severity in SCD and is influenced by key genetic loci, such as *BCL11A, HBS1L-MYB* and *Xmn1-HBG2*, which regulate erythropoiesis and hematological parameters (19). However, the role of non-globin genetic modifiers, particularly those involved in oxidative stress and vascular dysfunction, in influencing HbF levels remains largely unexplored. Given that oxidative stress is a key driver of stress erythropoiesis, which has been associated with an increased production of HbF, genetic variants influencing oxidative and vascular pathways may indirectly modulate HbF levels. Therefore, polymorphisms in PON1 and ENPP1 were hypothesized to influence HbF expression through their role in regulating oxidative stress and endothelial function.

In light of this, the present hospital-based case-control study was designed to assess the distribution of PON1 Q192R and ENPP1 K173Q polymorphisms among patients with SCD and healthy controls, and to explore their association with HbF levels as a preliminary hematological parameter.

Patients and methods

Study setting. The present study was conducted at Parul Sevashram Hospital (PSH) that is a tertiary care hospital affiliated with Parul University, in Vadodara, Gujarat, India. This was a hospital-based case-control study, and all eligible patients with SCD attending the center during the study period of 1 year, who fulfilled the inclusion criteria were recruited (n=80), along with 66 unrelated healthy controls.

Study design and reporting compliance. The diagnosis of hemoglobinopathy was performed using high-performance liquid chromatography (HPLC) to measure the HbF, HgA2, HgA0 and HbS levels. Polymorphisms in PON1 and ENPP1 were detected using conventional allele-specific PCR and allele frequencies were calculated. The research adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational research.

Ethical considerations. The present study was approved by the Institutional Review Board of Parul Sevashram Hospital (Approval no. PUIECHR/PIMSR/00/081734/8703). All procedures followed the ethical principles of the 1964 Declaration of Helsinki and its later amendments, and written informed consent was obtained from all participants prior to inclusion.

Categorization of patients. The classification of hemoglobinopathies was based on standard HPLC diagnostic criteria described in the literature (20-22). In brief, sickle cell disorders were defined on the basis of relative proportions of HbS, HbA and HbF (20). Using these criteria, patients were classified as having SCD (n=23) or sickle cell trait (SCT; n=57). Out of the 23 patients with SCD, genotyping was successfully performed in 21 samples due to inadequate DNA quality. This was likely attributable to minor pre-analytical variations, including short-term storage at 4˚C and variable processing times, which may have affected DNA integrity and resulted in occasional amplification failure in allele-specific PCR. All controls were recruited from the same geographical region (Vadodara, Gujarat) and belonged to the same ethnic background as the patients, in order to minimize the effect of population stratification on allele frequency comparison. All control individuals exhibited normal HPLC profiles, and this matching was intended to minimize the potential effects of population stratification on allele frequency comparisons.

Sample collection and DNA extraction. Trained personnel collected peripheral venous blood under sterile conditions using EDTA-coated vacutainer tubes. A portion of the blood sample was analyzed for hemoglobin fractionation via HPLC, while the remaining sample was used for genomic DNA extraction. DNA was isolated using a QIAGEN DNA isolation kit according to the manufacturer's protocol (cat. no. 51104; Qiagen, Inc.). Allele-specific polymerase chain reaction (PCR) was used to genotype PON1 Q192R and ENPP1 K173Q polymorphisms.

Conventional allele-specific PCR. PCR was performed in a 10-μl reaction mixture containing genomic DNA and primers using GoTaq master mixes (Promega Corporation) according to the manufacturer instructions. Allele-specific primers were used to detect the PON1 Q192R polymorphism. Due to primer competition, allele-specific amplifications were performed in separate PCR reactions for each allele. The primer sequences for the R allele were as follows: Forward, 5'-TGTTCCATTATAGCTAGCACGA-3' and reverse, -5'-TTTCTTGACCCCTACTTCCA-3'. For amplification of the Q allele, the primers used were the following: Forward, 5'-TTTCACCCCCTGAAAAATTA-3' and reverse, 5'-CAA ATACATCTCCCAGGCTC-3'. The PCR cycling conditions were as follows: 5 min at 95˚C; 30 cycles of 30 sec at 95˚C, 30 sec at 55˚C and 40 sec at 72˚C; 10 min at 72˚C Each reaction was verified on a 3% agarose gel. The expected PCR product sizes were 302 bp for the QQ (AA) genotype, 236 bp for the RR genotype, and both bands for the heterozygous QR genotype.

Similarly, genotyping for the ENPP1 K173Q polymorphism was conducted using a conventional allele-specific PCR in separate PCR reactions for each allele. The reaction mixture contained genomic DNA, a common forward primer (5'-GGACTTGCAACAAATTCAGGTGTGGT-3'), and one of the two reverse primers specific to either the K allele (5'-AGTTGCTGCAGCAGTCGCGCTT-3') or the Q allele (5'-AGAACTGTTAATGATGCAGCAGTC GACCTG-3'), along with standard PCR reagents. PCR

Table I. Distribution of demographic and clinical characteristics in the cases and controls.

Variables	Patients with SCD (n=23)	Individuals with SCT (n=57)	Controls (n=66)	P-value (patients with SCD vs. controls)	P-value (patients with SCD vs. those with SCT)
Age	23.41±2.76	24.76±1.26	20.75±0.69	>0.05	>0.05
HbF	13.7±1.2	1.07±0.14	1.66±0.54	<0.0001	<0.0001
VOC					
Yes	6 (26.08%)	1 (1.75%)	0	0.0012	0.0004 (SCD vs. SCT)
No	17 (73.92%)	56 (98.25%)	66 (100%)		
Sex					
Female	15 (65.21%)	49 (85.96%)	38 (57.58%)	0.47	0.035
Male	8 (34.78%)	8 (14.03%)	28 (42.42%)		

SCD, sickle cell disease; SCT, sickle cell trait; HbF, fetal hemoglobin; VOC, Vaso-occlusive crises.

amplification was carried out with an initial denaturation step at 95˚C for 10 min, followed by 37 cycles of denaturation at 95˚C for 1 min, primer annealing at 54˚C for 2 min, and extension at 72˚C for 1 min. This was followed by a final extension at 72˚C for 7 min. The resulting amplicons were separated on a 3% agarose gel stained with ethidium bromide [Sisco Research Laboratories Pvt Ltd (SRL)] and visualized under UV light using a transilluminator (iBright, Thermo Fisher Scientific, Inc.). The expected PCR product sizes were 99 bp for the KK genotype, 107 bp for the QQ genotype, and both bands for the heterozygous KQ genotype. This method enabled clear distinction between the K and Q alleles based on the presence or absence of allele-specific bands. To validate the allele-specific PCR results, Sanger sequencing was performed for a representative sample for the PON1 Q192R polymorphism, and concordance between the two methods was observed. Briefly, PCR-amplified products were purified and subjected to bidirectional sequencing based on the chain termination method. Sequencing was carried out using an automated capillary electrophoresis system (Applied Biosystems Genetic Analyzer, Thermo Fisher Scientific, Inc.) at a commercial facility (Barcode Biosciences, Bangalore, India). However, sequencing validation was not performed for ENPP1 due to resource limitations.

Statistical analysis. OriginPro (version 2019b) was used to perform statistical analysis. Continuous variables such as age and HbF levels are represented as the mean and standard error of mean (SEM), while categorical variables such as sex are represented as percentages and proportions. Comparisons of continuous variables among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. Categorical variables were analyzed using Fisher's exact test. The association between HbF levels and genotypes of PON1 and ENPP1 was assessed using linear regression under an additive genetic model. The association between age and the presence of PON1 (QR/RR) and ENPP1 polymorphism (KQ/QQ genotypes) was performed using binary logistic regression analysis. The calculation of the genotypic and allele frequencies was carried out by the gene-counting method. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of study participants. The demographic and clinical profiles of the study participants demonstrated several notable differences across groups. Although the mean age was comparable between patients with SCD and individuals with SCT, as well as between patients with SCD and the controls (P>0.05), one-way ANOVA revealed an overall significant difference among the groups, with post hoc analysis indicating a significant difference between individuals with SCT and the controls (P<0.05) (Table I). The sex distribution also differed, with more females in the SCT group than patients with SCD (85.96 vs. 65.21%; P=0.035). Female representation was comparable, yet not statistically significant between patients with SCD and the controls (65.21 vs. 57.58%; P=0.47). A significant difference was observed in the level of HbF in patients with SCD compared to those with SCT (13.7±1.2 vs. 1.07±0.14%; P<0.0001). Compared to controls, the patients with SCD also had higher concentrations of HbF (13.7±1.2 vs. 1.66±0.54; P<0.0001). No significant difference was observed between the SCT and control groups (P>0.05). Vaso-occlusive crises (VOC) were significantly more frequent in patients with SCD than in those with SCT (26.08 vs. 1.75%; P=0.0004, Fisher's exact test). Collectively, these findings highlight clear clinical and hematological differences between individuals with SCD and those with SCT (Table I).

Allele specific-PCR for PON1 Q192R polymorphism in hemoglobinopathies groups. The distribution of PON1 Q192R genotypes (QQ, QR and RR) varied across the hemoglobinopathy subgroups and controls. Representative agarose gel

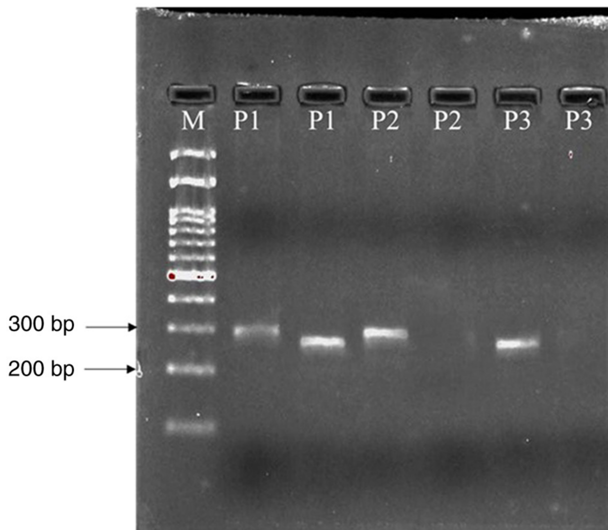

Figure 1. Representative agarose gel electrophoresis image illustrating allele-specific PCR amplification for the PON1 Q192R polymorphism. Lane M, 100 bp DNA ladder; lanes P1, heterozygous AG genotype (bands at ~236 bp and ~302 bp); lanes P2, homozygous AA genotype (single band at ~302 bp); lanes P3, homozygous GG genotype (single band at ~236 bp). PCR products were resolved on a 3% agarose gel and visualized under UV illumination. The expected band sizes correspond to the presence of Q (A allele, 302 bp) and R (G allele, 236 bp) variants.

electrophoresis images illustrating the genotyping patterns of the PON1 Q192R polymorphism are presented in Fig. 1. There was a significant disparity in the distribution of PON1 Q192R genotypes between SCD, SCT and the control groups. The QR genotype was the most common in patients with SCD (71.42%), and the proportion of QQ and RR genotypes was equal (14.28% each). However, among the individuals with SCT, the QR genotype was most frequent (49.12%), followed by a higher frequency of the RR genotype (29.82%) and a lower proportion of the QQ genotype (21.05%). By contrast, the control subjects exhibited a distinct distribution, with the RR and QR genotypes being most frequent (48.48%), while the QQ genotype was rare (1.52%) (Table II).

The genotype distribution and allele frequencies of the PON1 A>G Q192R polymorphism are summarized in Table III. Allele-based analysis revealed that the R allele was significantly less frequent in patients with SCD compared with the controls [odds ratio (OR), 0.36; 95% confidence interval (CI), 0.17-0.73; P<0.001]. Conversely, the Q allele exhibited a higher frequency in the patients with SCD compared with the controls. The genotype distribution of the PON1 Q192R polymorphism in the control group (n=66) deviated from the Hardy-Weinberg equilibrium (χ^2=4.91, P=0.027) (Table III). To validate the allele-specific PCR results, Sanger sequencing was performed for a representative sample for the PON1 Q192R polymorphism, and concordance between the two methods was observed (Fig. 2).

Regression analysis for PON1 Q192R. Linear regression analysis using an additive genetic model revealed a non-significant trend toward lower HbF levels with increasing R-allele dosage (β: -1.43, SE: 0.73, P=0.054) (Table IV). However, the observed borderline P-value (P=0.054) suggests a potential trend toward association, which may not have reached statistical significance due to the limited sample size. Larger studies with increased statistical power are warranted to further investigate this association. Overall, this analysis indicates that PON1 Q192R variation is not significantly associated with the HbF concentration. In addition, logistic regression demonstrated no association of either age or sex with the PON1 192RR genotype (age-coefficient, -0.0209; OR, 0.98; 95% CI, 0.94-1.02; P=0.311; sex-coefficient, 0.157; OR, 1.17; 95% CI, 0.57-2.40; P=0.65) (Table IV).

Allele specific-PCR for K173Q ENPP1 polymorphism in hemoglobinopathies groups. For the *ENPP1* gene, all three possible genotypes KK (wild-type), KQ (heterozygous) and QQ (mutant) were identified in the patient group (n=23), whereas only KK (wild-type) and KQ (heterozygous) genotypes were observed in the control group (n=66). Representative agarose gel electrophoresis images illustrating the genotyping patterns of the ENPP1 K173Q polymorphism are presented in Fig. 3. The distribution of ENPP1 K173Q genotypes exhibited marked differences across the SCD, SCT and control groups. In both the SCD and SCT groups, the heterozygous KQ genotype was overwhelmingly predominant, observed in 90.47% of patients with SCD and 89.47% of the individuals with SCT. The wild-type KK genotype was rare among the patients with SCD (4.76%) and SCT (10.52%), but was the most frequent genotype in the controls (48.48%). The mutant QQ genotype was detected in only 1 patient with SCD (4.76%) and was absent in both the patients with SCT and the controls (Table V).

The distribution of ENPP1 (K173Q; rs1044498) genotypes and allele frequencies among patients with SCD and the controls is presented in Table VI. Allele-based association analysis demonstrated that the Q allele of ENPP1 was significantly more frequent in patients with SCD compared with the controls (OR, 2.89; 95% CI, 1.41-5.93; P=0.008). This indicates that individuals carrying the Q allele have a 2.89-fold higher odds of disease compared to those carrying the K allele, suggesting that the Q allele may function as a potential risk allele in the studied population. The genotype distribution of the ENPP1 K173Q polymorphism in the control group (n=66) also exhibited deviation from the Hardy-Weinberg equilibrium (χ^2=7.84, P=0.005) (Table VI). Sequencing validation was not performed for ENPP1 due to resource limitations.

Regression analysis for ENPP1 K173Q. Linear regression analysis using an additive model for ENPP1 K173Q revealed no significant association with HbF levels (β: 0.14, standard error: 0.21, P=0.52). Overall, this analysis indicates that ENPP1 variation does not influence fetal hemoglobin concentration. In addition, logistic regression demonstrated no association of either age or sex with the ENPP1 QQ genotype (age coefficient, -0.312; P=0.99; sex coefficient, 18.47; P=0.99) (Table VII).

Discussion

The present study examined the distribution of PON1 Q192R and ENPP1 K173Q polymorphisms in patients with hemoglobinopathy from the West Indian Gujarat population and explored their association with HbF levels. Of note, two key findings emerged: The distribution of both PON1 Q192R and

Table II. Distribution of PON1 genotypes in patients with haemoglobinopathies.

Genotype	Patients with SCD[a] (n=21)	SCT (n=57)	Control (n=65)[b]
QQ (wild-type)	3 (14.28%)	12 (21.05%)	01 (1.52%)
QR	15 (71.42%)	28 (49.12%)	32 (48.48%)
RR (mutant)	3 (14.28%)	17 (29.82%)	32 (48.48%)

[a]Genotyping could not be performed in 2 patients with SCD due to inadequate DNA quality. [b]One sample could not be genotyped for PON1 due to assay-specific amplification failure. PON1, paraoxonase 1; SCD, sickle cell disease; SCT, sickle cell trait.

Table III. Allelic frequencies and association analysis of PON1 Q192R polymorphism in patients with SCD and controls.

		Patients with SCD	Controls	OR (95% CI)	P-value
Allele frequency	Q	0.50 (50%)	0.2615 (26.15%)	Reference	
	R	0.50 (50%)	0.7385 (73.85%)	0.36 (0.17-0.73)	<0.001
Hardy-Weinberg equilibrium	χ^2	-	4.91	-	0.027

PON1, paraoxonase 1; SCD, sickle cell disease.

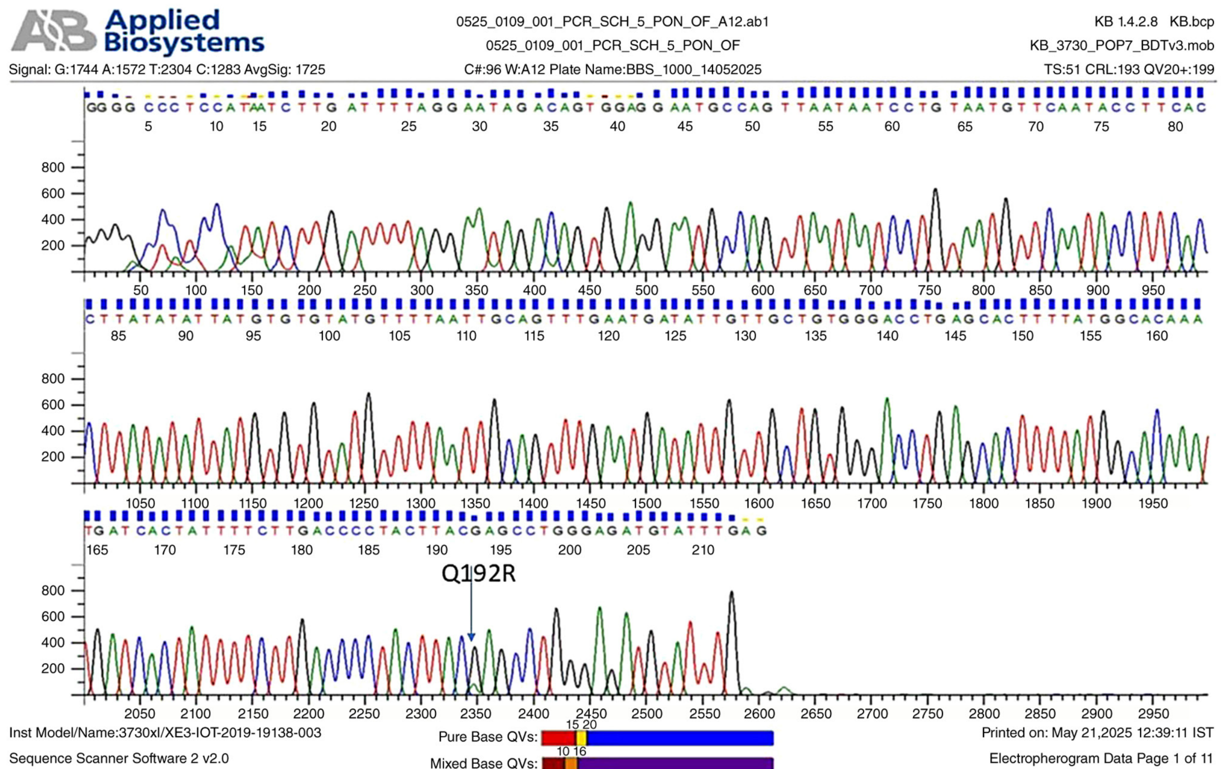

Figure 2. Sanger sequencing chromatogram illustrating the Q192R (rs662) polymorphism in the PON1 gene. The zoomed electropherogram highlights codon 192. The presence of overlapping G and A peaks at the second position (C(G/A)A) indicates a heterozygous genotype (QR), corresponding to both glutamine (CAA) and arginine (CGA) alleles.

ENPP1 K173Q genotypes differed markedly between patients with SCD and the controls, and HbF levels exhibited no significant association with either genetic variant.

Previous studies have demonstrated that variations at the PON1 Q192R locus may influence oxidative stress regulation, lipid metabolism, or inflammatory pathways, which

Table IV. Linear and logistic regression analysis of PON1 (Q192R) polymorphism.

Analysis	Variable	β/coefficient	SE	OR	95% CI	P-value
Linear regression (HbF)	Q allele dosage	-1.43	0.73	-	-	0.054
Logistic regression (QQ genotype)	Age	-0.0209	-	0.98	0.94-1.02	0.311
	Sex	0.157	-	1.17	0.57-2.40	0.65

PON1, paraoxonase 1; HbF, fetal hemoglobin; OR, odds ratio; CI, confidence interval.

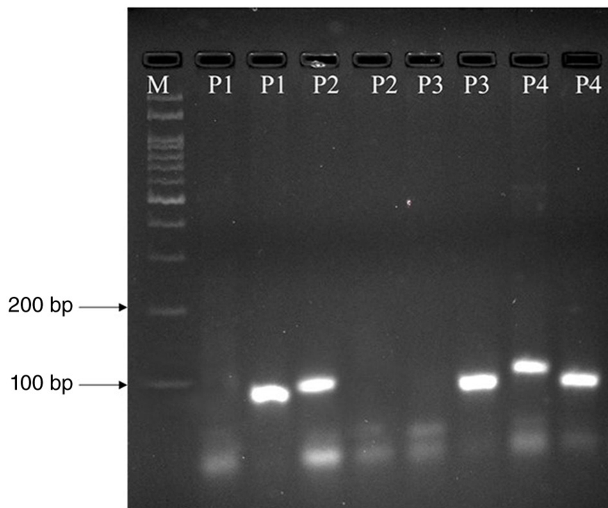

Figure 3. Representative 3% agarose gel electrophoresis image illustrating allele-specific PCR amplification for the ENPP1 K121Q polymorphism. Lane M, 100 bp DNA ladder; lanes P1 and P3, homozygous KK genotype, indicating a single band at 99 bp; lanes P2, homozygous QQ genotype, indicating a single band at 107 bp; lanes P4, heterozygous KQ genotype, with two distinct bands at 99 bp and 107 bp. PCR products were resolved on a 3% agarose gel, stained with ethidium bromide, and visualized under UV light. Band sizes correspond to the presence of allele-specific amplification products for the K and Q variants.

could modulate disease risk. For example, Menezes *et al* (12) reported that PON1c.192Q>R and PON1c.55L>M polymorphisms influence enzyme activity, with a reduced activity of PON1 linked to dyslipidemia, hemolysis and inflammation, and further associated with stroke and splenectomy in patients with SCD. Consistent with these observations, in the present study, the allele-based analysis revealed that the PON1 R allele was less frequent among patients with SCD than the healthy controls. However, despite these differences in allele frequencies, the findings indicated that the PON1 RR genotype did not exhibit a significant association with HbF levels, suggesting that these polymorphisms do not influence fetal hemoglobin expression in this cohort. Post hoc power analysis indicated that the current sample size (n=23) was sufficient to detect large effect sizes (Cohen's f^2=0.35), but underpowered to detect smaller associations. Therefore, the lack of significant association between the studied polymorphisms and HbF levels should be interpreted with caution, as subtle effects may not have been detectable. Although HbF is a well-established modulator of disease severity in SCD, other hematological parameters, such as total hemoglobin, reticulocyte count and markers of hemolysis may provide additional insight into the pathophysiology of the disease. Nonetheless, the key aim of the present study was to examine the association between PON1 and ENPP1 polymorphisms and HbF levels as a key disease modifier. Hence, the current analysis did not cover the other hematological parameters. Future research that considers these parameters is justified to enhance the understanding of their contribution as a potential disease modifier.

For ENPP1, the majority of individuals in the present study carried the heterozygous KQ genotype, while the QQ genotype was absent in the controls, but was detected in 1 patient with SCD. Allele-based analysis further demonstrated that the Q allele was enriched in patients with SCD compared with the controls, corresponding to a 2.89-fold higher odds of disease in Q-allele carriers relative to K-allele carriers. The increased frequency of the Q allele may reflect its potential role as a genetic modifier influencing disease severity or clinical outcomes in SCD, such as VOC or risk of stroke. Previous studies have demonstrated that the ENPP1 QQ genotype was significantly associated with stroke risk and increased cerebral blood flow velocities in children with SCD (23-25). Additionally, ENPP1 variants have also been linked to vascular diabetes complications which may increase vascular risk in SCD through impaired endothelial function (26). The present study also observed a higher frequency of vaso-occlusive crises in SCD patients compared with other groups. This is consistent with the known pathophysiology of SCD, where sickled red blood cells interact abnormally with endothelial cells, leukocytes, platelets, and plasma proteins (27-29). These interactions facilitate inflammation, vascular adhesion and microvascular obstruction, which eventually cause vaso-occlusion. Vaso-occlusion is considered the signature phenomenon in SCD and a major cause of most of its clinical phenotypes, such as pain events, organ injury and elevated morbidity.

To further contextualize the findings, allele frequencies were compared with global populations reported in the 1000 Genomes Project (https://www.internationalgenome.org/). Comparing the results with the global allele frequencies of the 1000 Genomes Project, it was evident that there were inter-population differences in both polymorphisms. For PON1 (Q192R), the R allele exhibited the highest frequency in African (0.66) and East Asian (0.65) populations, followed by Latin Americans (0.45) and South Asians (0.38). In the case of ENPP1 (K173Q; rs1044498), the Q allele had a significant level of diversity amongst populations, 0.75 in Africans, 0.29 in Latin Americans, 0.17 in South Asians, and 0.10 in East Asians. The Q allele of ENPP1 was 0.46 and the R allele of PON1 was 0.50 in the Gujarat population which was higher

Table V. Genotypic distribution of the ENPP1 K173Q polymorphism across the study groups.

Genotype	Patients with SCD (n=21)[a]	SCT (n=57)	Control (n=66)
KK (wild-type)	1 (4.76%)	6 (10.52%)	32 (48.48%)
KQ	19 (90.47%)	51 (89.47%)	34 (51.52%)
QQ (mutant)	1 (4.76%)	0	0

[a]Genotyping could not be performed in 2 patients with SCD due to inadequate DNA quality. SCD, sickle cell disease; SCT, sickle cell trait; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1.

Table VI. Genotype and allelic frequencies of ENPP1 K173Q for patients with SCD and the controls.

		Patients with SCD	Controls	OR (95% CI)	P-value
Allele frequency	K	0.5385 (53.85%)	0.7424 (74.24%)	Reference	
	Q	0.4615 (46.15%)	0.2576 (25.76%)	2.89 (1.41–5.93)	0.008
Hardy-Weinberg equilibrium	χ^2	-	7.84	-	0.005

SCD, sickle cell disease; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1.

Table VII. Linear and logistic regression analysis of the ENPP1 (K173Q) polymorphism.

Analysis	Variable	β/coefficient	SE	OR	95% CI	P-value
Linear regression (HbF)	Q allele dosage (0,1,2)	0.14	0.21	-	-	0.52
Logistic regression (QQ genotype)	Age	-0.312	-	-	-	0.99
	Sex	18.47	-	-	-	0.99

ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; HbF, fetal hemoglobin; OR, odds ratio; CI, confidence interval.

than the corresponding reference value of the South Asian population, indicating some enrichment or local variation of Western India. These observations emphasize the significance of the genetic diversity of a population in shaping the alleles distribution and support the importance of genetic profiling of a region when interpreting a genetic association study in hemoglobinopathies. Such population-level diversity can be used to offer a bigger perspective in the analysis of inter-individual and inter-population variation in the genetic backgrounds of different ancestral populations.

Deviation from the Hardy-Weinberg equilibrium in control populations has been reported in several genetic studies and may arise due to factors such as population stratification, inbreeding, selection bias, or genotyping variability (30-32). The deviation observed in the control group of the present study for both polymorphisms may be attributed to several factors. These include the relatively small sample size, hospital-based recruitment, and potential population stratification, which are known to influence genotype distribution in genetic association studies. Additionally, the absence of the homozygous variant genotype in ENPP1 may have contributed to the observed deviation.

The present study has several limitations which should be mentioned. PON1 enzyme activity and concentration were not measured, preventing the functional confirmation of the genetic findings. Hospital-based recruitment may have also biased the cohort toward more symptomatic patients. The relatively small sample size limited the statistical power to detect meaningful associations. Future community-based studies with larger sample sizes and functional assays are required to confirm allele frequency patterns and to better clarify whether PON1 or ENPP1 polymorphisms contribute to disease susceptibility or clinical heterogeneity in hemoglobinopathies.

In conclusion, the present study demonstrates differences in the distribution of PON1 Q192R and ENPP1 K173Q polymorphisms between patients with SCD and healthy controls in a Western Indian population. Even though there was a difference in the allele frequencies, neither polymorphism was significantly associated with HbF levels in this cohort. These results demonstrate a population-specific variation in the non-globin genetic polymorphisms, and suggest the necessity to conduct larger, well-powered studies with functional and clinical outcome measures to clarify their possible role in sickle cell disease.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

All authors (CJ, DV, MP, SP, AJ and RG) contributed to the conception and design of the study. DV and CJ were involved in sample collection, material preparation, DNA isolation, PCR and data analysis. AJ was involved in patient diagnosis and hematological assessments. MP and SP were involved in PCR analysis, and arranged the literature. RG prepared the first draft of the manuscript and supervised the study. CJ, DV and RG confirm the authenticity of all the raw data. All authors reviewed and commented on previous versions of the manuscript, and have read and approved the final version.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Parul Sevashram Hospital (Approval no. ECR/702/Inst/GJ/2015/RR-21/8703). All procedures followed the ethical principles of the 1964 Declaration of Helsinki and its later amendments, and written informed consent was obtained from all participants prior to inclusion.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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