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Original article

Impact of the rs1050757 C > T variant in the 3'UTR of the *G6PD* gene on mRNA structure and miRNA binding in G6PD deficiency: a nanopore MinION sequencing study

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Abstract

Background: Glucose 6-phosphate dehydrogenase (G6PD) deficiency is a genetic disorder caused by impaired enzyme function or instability due to mutations in the *G6PD* gene, resulting in reduced enzyme activity. This study aimed to investigate mutations within the regulatory regions of the *G6PD* gene using Nanopore MinION sequencing to investigate the potential effects of noncoding variants on G6PD activity.
Methods: Blood samples from 19 males (13 adults and 6 neonates) with G6PD deficiency or intermediate enzyme activity but with unidentified coding sequence mutations were analyzed. Genomic DNA was amplified using degenerate oligonucleotide-primed PCR and sequenced on the Oxford Nanopore MinION platform. Bioinformatic analyses were performed to evaluate the effects of single-nucleotide polymorphisms on G6PD mRNA structure and miRNA binding potential.
Results: The rs1050757 C > T variant in the 3' untranslated region (3' UTR) of the *G6PD* gene was detected in 12 individuals, including 9 individuals with intermediate G6PD activity (7 adults and 2 neonates) and 3 neonates with G6PD deficiency. Among adults, G6PD activity was comparable between carriers of the T (11.5 ± 1.3 U/g Hb, n = 7) and C (11.8 ± 0.8 U/g Hb, n = 6) alleles. However, G6PD activity was more variable in neonates, with mean values of 1.8 ± 4.0 U/g Hb for the T allele (n = 5) and 6.0 U/g Hb for the C allele (n = 1). No cases of hemolytic anemia were observed in individuals carrying the rs1050757 variant, suggesting a limited direct effect on enzyme function. Computational analyses revealed that the variant induced minor alterations in the secondary structure of the G6PD mRNA, resulting in a shift in the Gibbs free energy (ΔG) from −425.0 to −412.1 kcal/mol and the minimum free energy from −441.1 to −440.4 kcal/mol. These changes may subtly influence mRNA loop formation and miRNA binding at the hsa-miR-92b-3p site.
Conclusion: The rs1050757 variant appears to exert a limited effect on G6PD activity and clinical phenotype. Nevertheless, its potential role in modulating gene regulation through alterations in mRNA structure or miRNA interaction warrants further investigation. Improved understanding of noncoding region mutations may improve diagnostic accuracy and facilitate genotype–phenotype correlations, particularly in malaria-endemic regions where G6PD deficiency screening is crucial.

Keywords: 3' UTR, G6PD deficiency, glucose 6-phosphate dehydrogenase, miRNA binding, mRNA secondary structure, nanopore MinION sequencing, noncoding mutations, regulatory regions, rs1050757.

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Glucose 6-phosphate dehydrogenase (G6PD) deficiency is among the most prevalent enzymatic disorders, affecting approximately 400 million individuals worldwide.[1] G6PD plays a crucial role in the pentose phosphate pathway, maintaining the cellular redox balance through the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH).[1] NADPH supports the regeneration of glutathione (GSH), which is a key antioxidant that protects red blood cells (RBCs) from oxidative stress.[1] Mutations in the X-linked *G6PD* gene that impair enzyme activity or stability render RBCs vulnerable to oxidative stress, making individuals with G6PD deficiency particularly susceptible to hemolytic anemia upon exposure to certain drugs, infections, or environmental triggers.[1]

In Southeast Asia, G6PD deficiency is highly prevalent because of its protective effect against malaria;[2] therefore, understanding the genetic mechanisms underlying this disorder is crucial. Although most previous studies have focused on mutations within the coding region that directly affect enzymatic function, an increasing number of cases have reported G6PD deficiency without identifiable mutations in these regions.[3, 4] This suggests that noncoding regions, such as the 5' and 3' untranslated regions (UTRs) and promoters, are potentially involved in gene expression regulation and mRNA stability.[1] Despite their importance, noncoding regions remain understudied in both diagnostic and research settings, as current approaches predominantly target coding region mutations. Observations from several populations, including Saudi Arabia, Thailand, and Malaysia, highlight the potential contributions of regulatory regions to G6PD phenotypic variability and the need for expanded genetic testing.[3-10]

Advances in sequencing technologies, such as the Oxford Nanopore MinION platform, enable comprehensive analysis of coding and noncoding regions. This study is among the first to employ Oxford Nanopore MinION sequencing to investigate the effect of the rs1050757 C > T variant in the 3' UTR of the *G6PD* gene on mRNA structure, miRNA binding, and enzymatic activity in a Thai cohort. By focusing on individuals with G6PD deficiency but no coding region mutations, this study aimed to evaluate the role of regulatory regions in *G6PD* expression. However, unlike previous studies that primarily focused on mutations within the coding region, our study highlights the importance of post-transcriptional regulation in explaining G6PD enzymatic variability. These findings enhance the understanding of noncoding mutations and their implications for diagnostics and treatment strategies, particularly in malaria-endemic regions.

Materials and methods

Sample collection

A total of 19 leftover blood samples collected as part of two earlier studies were used. These originated from 13 adult males (average age: 40 years) and 6 male neonates (gestation ≥ 37 weeks; birth weight ≥ 2,000 g) at King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand, between February and April 2021.[3, 11] All samples were collected in EDTA-coated tubes, a standard anticoagulant for enzymatic and genetic analyses. These samples were previously identified as G6PD-deficient or intermediate by enzymatic testing, without mutations in the coding regions of the *G6PD* gene, thus providing a suitable cohort for investigating potential noncoding region variants (**Figure 1**). To ensure clear genotype–phenotype correlations, only male participants were included. Because *G6PD* is an X-linked gene, hemizygous males exhibit a uniform G6PD activity phenotype (deficient or normal), whereas heterozygous females can exhibit variable enzyme activity because of random X-chromosome inactivation, potentially confounding analyses in this exploratory study. This was a retrospective analysis of previously collected samples; thus, no prospective sample size calculation was performed. The study was designed as a preliminary investigation of noncoding variants associated with altered G6PD activity. Ethical approval for this study was obtained from the Institutional Review Board on Human Research, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA no.: 0265/2024; IRB no.: 0056/67).

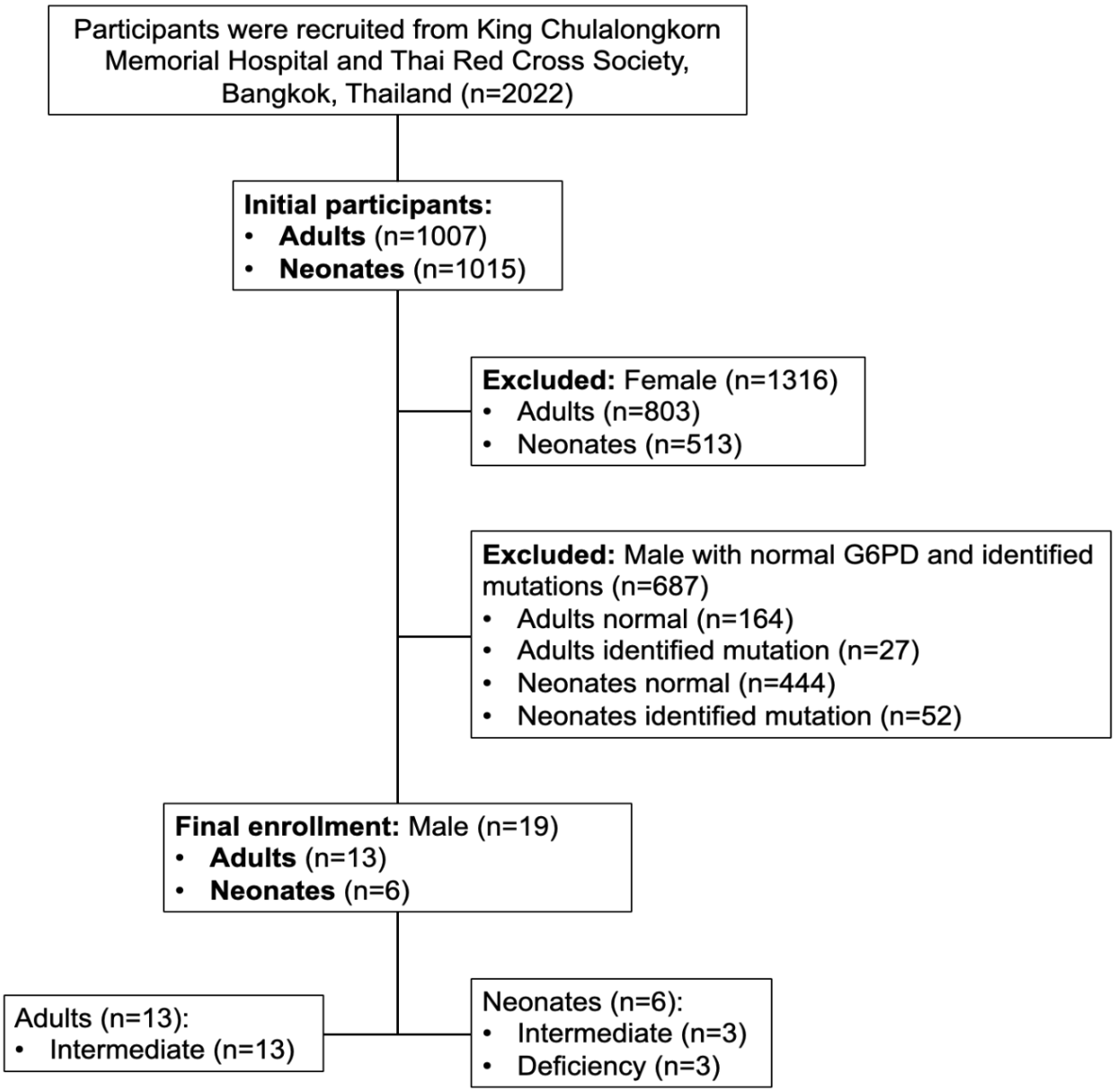


Figure 1. Flow diagram for patient enrolment. G6PD, glucose 6-phosphate dehydrogenase.

G6PD activity and hematological data

Data on G6PD activity and hematological parameters, including hemoglobin (Hb), RBC count, hematocrit (HCT), reticulocyte count (RET), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration, and bilirubin levels [total (TB), direct (DB), and indirect (IB)], were obtained from previous studies[3, 11] G6PD activity in neonates was measured using temperature-controlled spectrophotometric assays at 37 °C over 5 min (cat no. G7583; Pointe Scientific Inc., Canton, Michigan, USA) with standardized controls (G6888, G5888; Trinity Biotech, Bray, Co. Wicklow, Ireland).[3] In adults, G6PD activity was assessed using a clinical chemistry analyzer (BS-360E, Mindray Medical International, China) with manufacturer-provided quality controls for normal and deficient levels.[11] Both methods were performed under rigorous quality control measures. G6PD activity was categorized based on established thresholds: < 30.0% of the normal median was defined as deficient, 30.0%–80.0% as intermediate, and > 80.0% as normal. In neonates, G6PD activity < 7.8 U/g Hb was considered deficient, and 7.8–20.8 U/g Hb was considered intermediate. In adults, the intermediate range was defined as 4.6–15.3 U/g Hb. [3, 11]

Table 1. G6PD primer sequence

Site	Primer name	Primer sequence (5'-3')	PCR product size (bp)
5'UTR	5UF	5'-TTT CTG TTG GTG CTG ATA TTG CTG TCA GCA GAG TCC GTC AG-3'	1016
	5UR	5'-ACT TGC CTG TCG CTC TAT CTT GAC GTG CGG GGT ATA AAG GG-3'	
3'UTR	3UF	5'-TTT CTG TTG GTG CTG ATA TTG TGG AGC TAC CTC ATG CCT CT-3'	870
	3UR	5'-ACT TGC CTG TCG CTC TAT CTT GGA AGT CAT CTT GGG TGG GG-3'	

*Underline: Anchor barcode for nanopore. UTR, untranslated region.

Coding region mutation screening

Data on the coding region mutations in the sample were obtained from previous studies.[3, 11] The G6PD Mahidol^{G497A} and G6PD Viangchan^{G871A} variations were screened using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA), while other coding region mutations from exons 3–12 were identified via Sanger sequencing, comparing the results with the *G6PD* reference sequence (NC 000023.11). Genomic DNA was extracte from the blood samples of individuals without identified coding region mutations using the Nucleospin Blood kit (MACHEREY–NAGEL GmbH & Co. KG, Düren, Germany).

Noncoding region analysis

Degenerate oligonucleotide-primed PCR (DOP-PCR)[12]
was used to amplify the 5' UTR and 3' UTR regions of the *G6PD* gene using modified primers (**Table 1**). The PCR mixture (10 µL total) consisted of 5 µL of 2× Ultra HiFidelity Mix, 2 µL of PCR enhancer, 0.25 µL of primers (65 µM), and 50 ng/µL of genomic DNA, with nuclease-free water added to adjust the final volume. The reaction was performed in a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following cycling conditions: initial denaturation at 94 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 65 °C for the 5' UTR or 57 °C for the 3' UTR for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. A second round of PCR was performed using 5 µL of 2× Ultra HiFidelity Mix, 2 µL of PCR enhancer, 0.125 µL of primer barcode, and 1 µL of the first PCR product, with nuclease-free water added to a final volume of 10 µL. The second PCR was performed with an initial denaturation at 94 °C for 2 min, followed by five cycles of denaturation at 98 °C for 10 s, annealing at 60 °C for 30 s, and extension at 68 °C for 45 s, with a final extension at 68 °C for 5 min. The PCR products were analyzed by 1.0% agarose gel electrophoresis, visualized using the Molecular Imager Gel Doc XR+ with Image Lab Software (BioRad), and purified with AMPure XP Beads (Beckman Coulter, Brea, CA, USA).

Nanopore library construction and sequencing

The purified PCR products were quantified using a Qubit dsDNA kit (Thermo Fisher, Waltham, MA, USA) and prepared for sequencing using a PCR Barcoding V14 kit (SQK-LSK114; Oxford Nanopore, Oxford, UK). The libraries were pooled, barcoded, and sequenced on a MinION Mk1C system R10.4.1 (FLO-MIN114) flow cell, with base calling performed using the Guppy software pipeline (MinION v6.1.7; Oxford Nanopore). Adapter sequences were removed using Porechop (v0.2.4; ILRI Research Computing, Nairobi, Kenya), and sequence quality was assessed using Fastp software (v0.23.1).[13] The filtered reads were mapped to the G6PD reference genome (NC_000023.11) using Unipro UGENE v33.0,[14] and alignment was performed using the software MEGA X (MEGA Developer Team, USA).

Bioinformatic analyses of mRNA 2 structure

Changes in the mRNA secondary structure associated with single-nucleotide polymorphisms (SNPs) in the regulatory region were analyzed using the UNAFold[15] and RNAfold web servers.[16] The Gibbs free energy (ΔG) values were computed for each algorithm to compare the stability shifts. The lowest minimum free energy (MFE) structures were further analyzed for altered loop formation and RNA accessibility.

miRNA binding site prediction

Alterations in miRNA binding sites were evaluated using multi-tool comparison across five bioinformatics platforms, including TargetScanHuman[17], miRanda[18], Diana Tools[19], miRTarBase[20], miRWalk[20], and miRbase to identify the potential effects of the SNPs on miRNA interactions.

Table 2. Demographic data of sample enrolled in the study based on G6PD status.

Group	G6PD status	G6PD activity range (U/g Hb)
Adult	Intermediate (n = 13)	5.1–12.2
Newborn	Intermediate (n = 3)	10.4–20.7
	Deficiency (n = 3)	0.3–5.5

G6PD, glucose 6-phosphate dehydrogenase.

Statistical analysis

Statistical analyses were performed using SPSS (v29.0, IBM Corp., Armonk, New York, USA). Data distribution was assessed using the Kolmogorov–Smirnov test or Shapiro–Wilk test. Differences in G6PD activity and the hematological parameters between the rs1050757 alleles (T and C) were analyzed using the Mann–Whitney *U* test and Kruskal–Wallis test. Non-normally distributed data are presented as the median ± interquartile range (IQR). A P-value < 0.05 was considered statistically significant.

Results

Demographic data of the participants

The study included adult and newborn participants categorized based on their G6PD status. Previous studies have documented that normal G6PD activity in the population varies between 20.8 and 64.0 U/g Hb in newborns and between 12.3 and 46.9 U/g Hb in adults. [3, 11] In this study, G6PD activity varied according to G6PD status, with a clear distinction between intermediate and deficient individuals (**Table 2**). The observed enzyme activity levels are consistent with those of previous reports, thus supporting the classification of G6PD deficiency.

SNP identification in G6PD regulatory regions

No genetic alterations were identified in the promoter region or the 5' UTR of the *G6PD* gene. However, an rs1050757 C > T variant was detected in the 3' UTR (position 154531643; dbSNP: rs1050757) (**Supplementary Figure S1**).

Effect of rs1050757 C > T on G6PD activity and hematological parameters

Because the rs1050757 C > T variant was identified, G6PD activity and hematological parameters were assessed in individuals carrying either the T or C alleles (**Table 3**). Although the sample size was limited (allele T, n = 7; allele C, n = 6), a statistically significant difference was observed in the MCV (P = 0.032); however, no significant differences were observed in other parameters. All measured hematological values, including Hb levels, RBC counts, and bilirubin levels, remained within normal ranges, and no evidence of hemolytic anemia was detected in any participant.

In silico analysis of mRNA secondary structure

Computational modeling revealed that the rs1050757 C > T variant slightly altered the secondary structure of G6PD mRNA. In addition, the ΔG values shifted slightly from -425.0 kcal/mol (wildtype C allele) to -412.1 kcal/mol (variant T allele), whereas the MFE values changed marginally from -441.1 kcal/mol to -440.4 kcal/mol. These small differences suggest that it had a minimal effect on mRNA stability (**Figure 2**). Moreover, structural predictions showed alterations in the number and configuration of hairpin loops and other features of the G6PD 3'UTR (**Figure 3**).

In silico analysis of miRNA binding site

The rs1050757 C > T variant disrupted the seed region of hsa-miR-92b-3p, thereby reducing its binding capacity. Cross-analysis using five computational tools revealed that hsa-miR-92b-3p had three binding sites (positions 198, 488, and 541) in mRNA transcripts from individuals with the C allele but only two binding sites (positions 198 and 541) in those with the T allele (**Figure 4**). These changes suggest potential alterations in the miRNA-mediated regulation of G6PD expression.

Table 3. G6PD activity and hematological profile in adult and newborn patients with rs1050757 allele T and C.

Parameter	Group	rs1050757		*P*-value[a]	Normal range
		Allele T (n = 12)	Allele C (n = 7)		
G6PD activity (U/g Hb)	Adult (n = 13)	11.5 ± 1.3 (n = 7)	11.8 ± 0.8 (n = 6)	0.116	> 20.8
	Newborn (n = 6)	1.8 ± 4.0 (n = 5)	6.0 (n = 1)	NA	> 12.3
RBCs (x10^6/µl)	Adult (n = 13)	4.7 ± 0.8 (n = 7)	4.4 ± 0.3 (n = 6)	0.317	4.6–6.0
	Newborn (n = 6)	5.1 ± 0.9 (n = 5)	4.79 (n = 1)	NA	3.9–5.9
Hb (g/dl)	Adult (n = 13)	12.6 ± 2.0 (n = 7)	13.1 ± 1.4 (n = 6)	0.474	13.0–17.0
	Newborn (n = 6)	19.3 ± 3.3 (n = 5)	15.2 (n = 1)	NA	13.4–19.9
HCT (%)	Adult (n = 13)	37.6 ± 5.9 (n = 7)	39.3 ± 3.1 (n = 6)	0.568	39.0–51.0
	Newborn (n = 6)	55.6 ± 9.9 (n = 5)	44.7 (n = 1)	NA	42.0–65.0
MCV (fl)	Adult (n = 13)	80.3 ± 6.3 (n = 7)	87.8 ± 2.7 (n = 6)	**0.032**	80.0–100.0
	Newborn (n = 6)	105.9 ± 1.6 (n = 5)	93.5 (n = 1)	NA	88.0–123.0
RET (%)	Adult (n = 13)	1.6 ± 0.4 (n = 7)	1.3 ± 0.3 (n = 6)	0.352	1.0–2.0
	Newborn (n = 6)	5.9 ± 1.8 (n = 5)	4.0 (n = 1)	NA	2.5–6.5
MCHC (g/dl)	Adult (n = 13)	32.4 ± 1.0 (n = 7)	33.5 ± 1.1 (n = 6)	0.114	33.0–37.0
	Newborn (n = 6)	34.6 ± 0.4 (n = 5)	34.0 (n = 1)	NA	28.0–36.0
Total Bilirubin (mg/dL)	Newborn (n = 6)	11.5 ± 2.6 (n = 5)	12.3 (n = 1)	NA	1.2–15.0
Direct Bilirubin (mg/dL)	Newborn (n = 6)	0.5 ± 0.1 (n = 5)	0.4 (n = 1)	NA	< 0.5
Indirect Bilirubin (mg/dL)	Newborn (n = 6)	11.0 ± 2.5 (n = 5)	11.9 (n = 1)	NA	0.7–14.5

[a]Samples with Viangchan^{G871A} or Mahidol^{G487A} were enrolled to act as controls for known *G6PD* mutation cases. NA, not applicable. G6PD, glucose 6-phosphate dehydrogenase; Hb, hemoglobin; HCT, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBCs, red blood cells; RET, reticulocyte count.

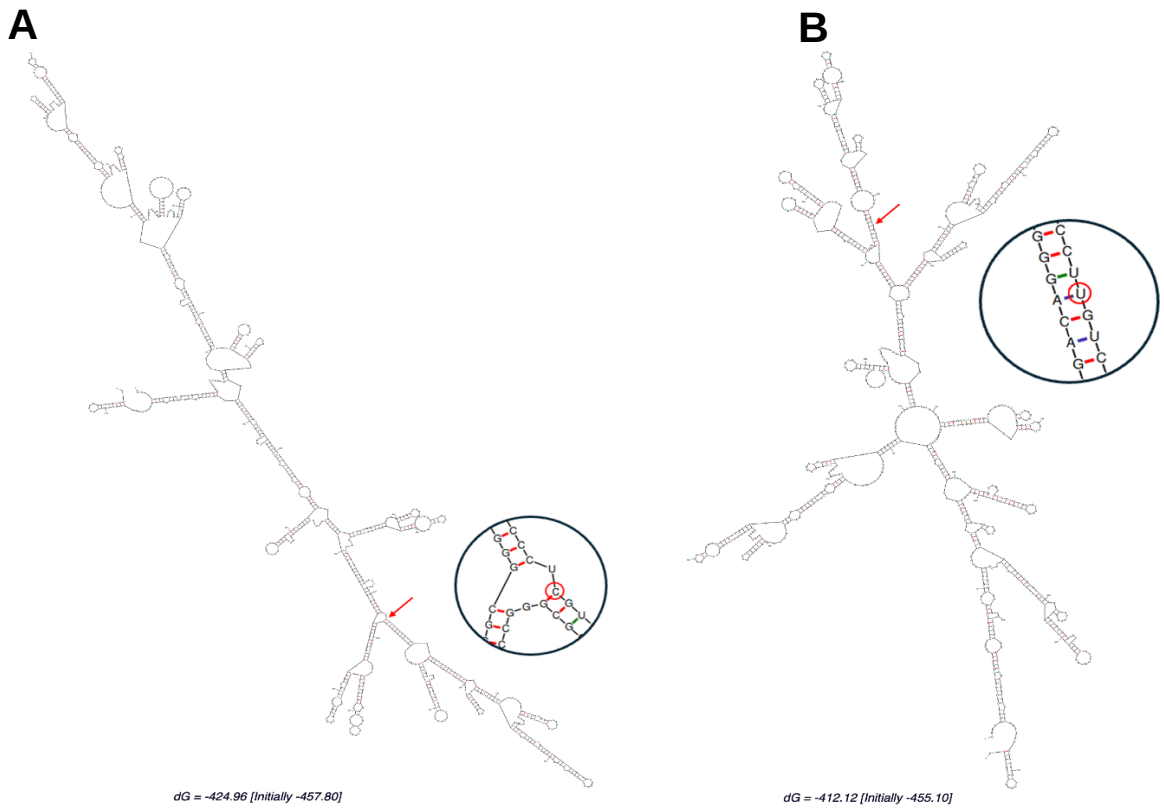
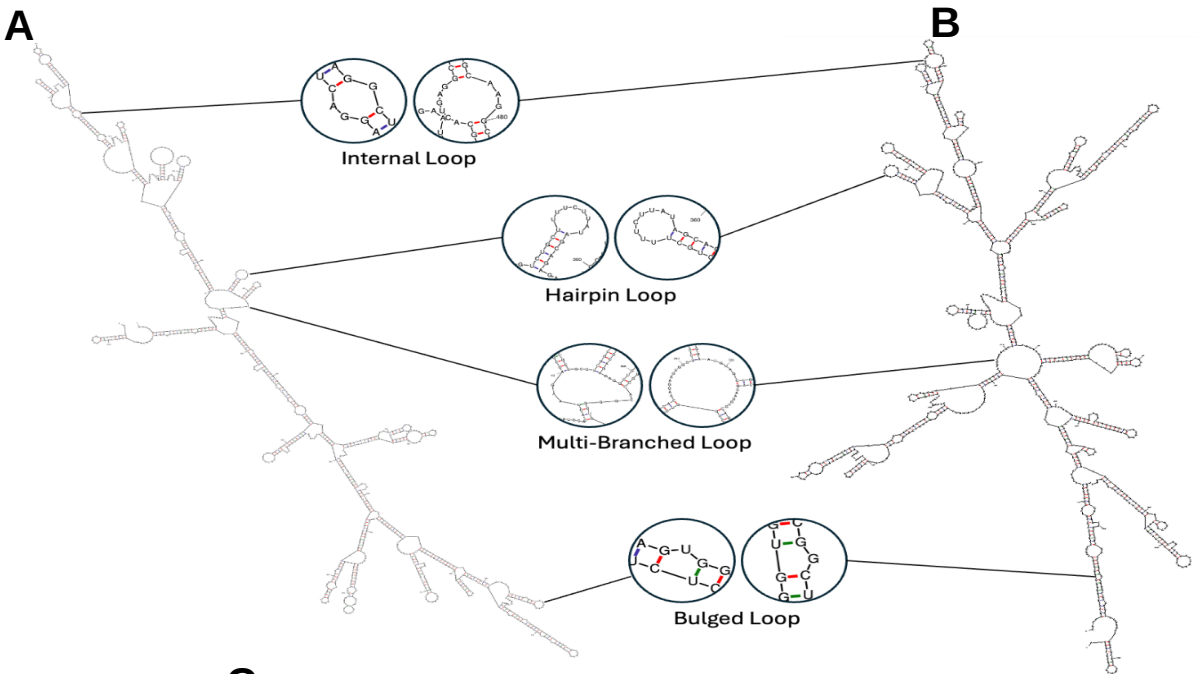


Figure 2. Predicted mRNA secondary structure and Gibbs free energy. **(A)** mRNA with allele C: ΔG, - 424.96 kcal/mol; **(B)** mRNA with allele U: ΔG, - 412.12 kcal/mol.



C

Type of Loops	Allele T	Allele C
Hairpin Loops	19 loops	15 loops
Multi-branched Loops	16 loops	13 loops
Bulged Loops	16 loops	14 loops
Internal Loops	6 loops	11 loops

Figure 3. Loop structures in the mRNA secondary structure. **(A)** mRNA with allele C; **(B)** mRNA with allele U; **(C)** Changes in loop types between alleles T and C.

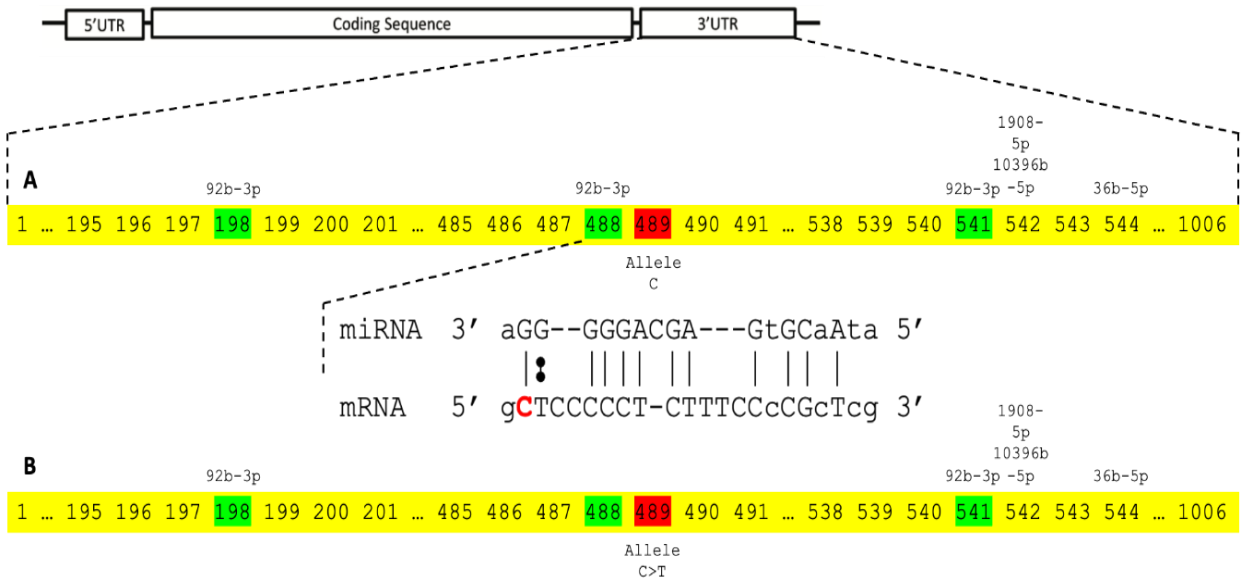


Figure 4. hsa-miR-92b-3p binding site in the G6PD mRNA**. (A)** mRNA with allele C, showing three binding sites (198, 488, and 541); **(B)** mRNA with allele T, showing two binding sites (198 and 541). G6PD, glucose 6-phosphate dehydrogenase.

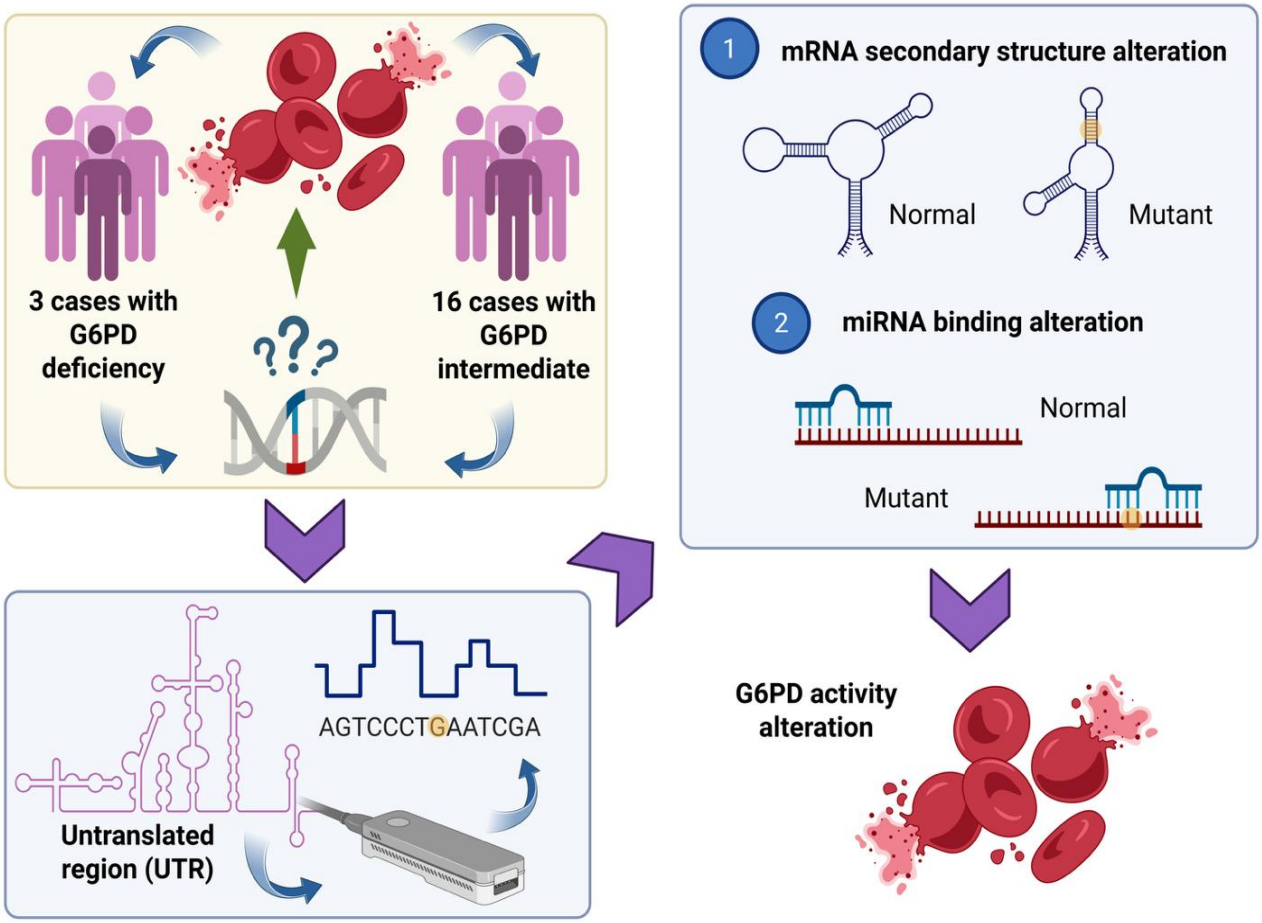


Figure 5. Graphical abstract illustrating the main concept and results of this study. G6PD, glucose 6-phosphate dehydrogenase.

Discussion

G6PD deficiency is a widespread genetic disorder with considerable variability across populations, particularly in malaria-endemic regions such as Thailand.[1] Although this disorder has been extensively studied, most studies have primarily focused on mutations in the coding region of the *G6PD* gene. However, the absence of coding mutations in some individuals with G6PD deficiency highlights the potential role of regulatory regions, such as the promoter, 5' UTR, and 3' UTR, in modulating the expression and enzymatic activity of G6PD.[21] This study investigated the noncoding regions, their allele frequencies, and their contribution to the phenotypic variability in G6PD deficiency.

In this study, no genetic mutations were detected in the promoter or 5' UTR regions of the *G6PD* gene. This finding is consistent with previous studies, which suggest that mutations in these regions are relatively rare in clinical samples compared to those of experimental models.[22, 23] While the absence of mutations could reflect the specific genetic composition of the studied cohort, it also emphasizes the need to consider additional regulatory mechanisms, such as DNA methylation, which may influence gene expression in these regions.[24, 25] Studies on other genes have shown that methylation can regulate transcription; however, its role in *G6PD* expression remains poorly elucidated.[24, 25] Integrating methylation assessments with genetic analyses in future studies could provide a more comprehensive understanding of *G6PD* regulation.

The rs1050757 C > T variant in the 3' UTR was identified as a key regulatory variant in this cohort, with the T allele predominating in the Thai population, which is consistent with its prevalence in Southeast Asia. Furthermore, computational analyses using an integrated multi-tool bioinformatics approach revealed that the T allele altered the secondary structure of G6PD mRNA, which resulted in reduced stability, as indicated by the increased ΔG and MFE values. These structural changes included alterations in the loop patterns, such as an increase in hairpin and bulge loops and a reduction in internal loops, which may influence the mRNA stability and its translational efficiency (**Figure 5)**. Similar effects have been observed in studies on other genes, where changes in the mRNA secondary structure affected protein production and enzymatic activity.[26-32]

In addition, the rs1050757 variant disrupted the hsa-miR-92b-3p seed region, thereby reducing the miRNA binding efficiency. Although the total number of miRNA binding sites was minimally affected, this variant likely altered the post-transcriptional regulation of G6PD expression. This finding is supported by previous studies, which have shown that regulatory mutations can disrupt miRNA interactions, thus influencing gene expression and protein levels.[26, 27] Despite these molecular effects, no significant differences were observed in G6PD activity or hematological parameters between individuals with T and C alleles. Although the MCV differed significantly between the two groups, the values of both groups remained within the normal range. This variation might not result directly from the rs1050757 allele itself but could reflect underlying factors such as undetected thalassemia traits or individual variability in erythropoiesis. Given the small sample size and absence of thalassemia screening, further studies are warranted to verify the clinical relevance of this finding.

According to the updated WHO classification of genetic variations, rs1050757 is categorized within the G6PD variant as class U.[33] This class encompasses variants associated with moderate enzyme activity, meaning that individuals who carry these variants may exhibit mild G6PD deficiency but generally experience fewer complications compared to those with more severe variants.[34] Identifying mutations such as rs1050757 within the U class is essential to refine clinical management strategies, prevent complications, and advance personalized medicine. This classification framework enhances our understanding of G6PD variants with intermediate enzyme activity and uncertain clinical importance, thereby addressing gaps in patient care and public health initiatives.[33] The clinical relevance of U-class mutations lies in their potential to influence the treatment approaches. Unlike severe G6PD-deficient variants, individuals with U-class mutations maintain moderate enzyme activity, thus necessitating tailored clinical interventions.[33, 34] Recognizing these mutations enables healthcare providers to develop personalized management plans that mitigate the patients' hemolytic risk while also avoiding unnecessary lifestyle restrictions, thereby ultimately improving patient outcomes.

The population-specific analysis further supports the relevance of the rs1050757 variant. Its enrichment in Southeast Asia may reflect evolutionary pressures,

such as malaria selection, that shaped G6PD variant distributions. Although the rs1050757 variant alone may not significantly affect enzyme activity, its interactions with other genetic factors likely contribute to the observed phenotypic variability. Computational models predict that this variant affects the mRNA secondary structure and miRNA binding, and effect size analysis reinforces the role of noncoding variants in G6PD regulation. The limitations of this study include the small sample size, particularly in the neonatal subgroup, the absence of G6PD-normal controls, and incomplete genotyping of noncoding regions. Consequently, the statistical power is limited, and the findings should be interpreted with caution. Larger and more diverse cohorts along with experimental validation of the effects of the rs1050757 variant on mRNA stability, miRNA binding, and protein expression are required to confirm and extend these observations.

By integrating computational and thermodynamic analyses, this study provides insights into the regulatory role of the rs1050757 variant in the 3' UTR of the *G6PD* gene. These findings highlight the need to investigate noncoding variants to improve the understanding of G6PD deficiency. Future research should focus on comprehensive genotyping and experimental validation to enhance diagnostic accuracy and inform treatment strategies, particularly in malaria-endemic regions where a patient's G6PD status substantially influences therapeutic decisions.

Conclusion

The rs1050757 C > T variant in the 3' UTR of *G6PD* exhibits minor computationally predicted effects on mRNA stability and miRNA binding but no significant effect on enzyme activity. These findings highlight the role of noncoding mutations in G6PD deficiency and support the classification of rs1050757 as a U-class variant under the World Health Organization guidelines. Further studies with larger cohorts and functional assays are required to further refine its clinical importance.

Acknowledgments

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Conflicts of interest

All authors have completed and submitted the International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors have any conflicts of interest to disclose.

Data sharing statement

All data generated or analyzed in the present study are included in the published article. Further details are available from the corresponding author for non-commercial purposes upon reasonable request.

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