Variants of the *PKLR* gene are associated with rates of hospitalization in Angolan children with sickle cell anemia

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INTRODUCTION

Sickle cell anemia (SCA), caused by the c.20 A>T (p.Glu6Val) mutation in the HBB gene, is one of the most prevalent hereditary diseases in Sub-Saharan Africa [1]. Patients with the HbSS genotype exhibit variable phenotypic expressions and disease severity, often reflected in hospitalization rates. Reduced activity of pyruvate kinase (PKR, gene: *PKLR*), a key enzyme in glycolysis, impacts SCA pathophysiology through 2,3-DPG accumulation and ATP deficit [2]. In a recent study, several *PKLR* variants were found significantly associated with hospitalization rate for acute pain in SCA [3].

This study aims to analyze the association of the three *PKLR* variants rs8177970, rs3020781 and rs1052177 with hospitalization rates and clinical parameters in Angolan children with SCA.

METHODS

Sixty-three SCA children (3–12 years) were monitored in a prospective study (2019–2022) [4]. The single nucleotide polymorphisms (SNPs) rs8177970 and rs3020781 (in intron 3) and rs1052177 (at 3' UTR) in the *PKLR* gene were analyzed by polymerase chain reaction–restriction fragment length polymorphism analysis (PCR-RFLP).

Allele frequencies and Hardy-Weinberg equilibrium (HWE) were assessed using Arlequin 3.5. Association studies were performed comparing continuous variables between genotypes using the Mann-Whitney U test, combining heterozygous and homozygous individuals for the minor allele into a single group, and the Kruskal-Wallis test between the three genotypes. SPSS 27 was used for these statistical analyses.

RESULTS

Minor allele frequencies for the *PKLR* SNPs rs8177970, rs3020781, and rs1052177 were 0.214, 0.221, and 0.377, respectively. All genotype distributions were consistent with HWE ($p > 0.05$) (Table 1).

The polymorphisms rs1052177 and rs8177970 were significantly associated with hospitalization rates ($p = 0.012$ and $p = 0.025$, respectively) using the Mann-Whitney U test (Table 1). Individuals homozygous for the major alleles showed lower values of hospitalizations when compared to those with the minor allele (Fig. 1).

Moreover, when the comparisons were made among the three genotypes using the Kruskal-Wallis test, similar patterns of significance were found (Table 1).

The rs3020781 showed no significant association with hospitalization rates ($p > 0.05$) (Table 1).

No associations were found with hematological parameters for the three analyzed *PKLR* polymorphisms.

Table 1. Allelic and genotypic frequencies of the *PKLR* gene variants in Angolan children with sickle cell anemia and association with rates of hospitalization

Location	dbSNP	Alleles (1:2)	MAF	p-HWE	Genotypes (n)	Mean Nr.hosp (SD)	Median Nr.hosp (IQR)	p*	p**
1:155299985	rs3020781	A:G	0.221	0.4605	G/G (38)	2.63 (2.39)	2.00 (3)		
					A/G (19)	3.21 (2.86)	3.00 (3)	0.566	0.652
					A/A (4)	2.00 (1.83)	2.00 (4)		
1:155295870	rs8177970	C:T	0.214	0.4517	T/T (40)	2.42 (2.68)	1.50 (3)		
					T/C (19)	3.63 (2.34)	3.00 (4)	**0.025**	0.068
					C/C (4)	2.50 (1.00)	2.00 (2)		
1:155290559	rs1052177	T:C	0.377	0.273	C/C (26)	1.96 (2.13)	1 (2)		
					C/T (24)	3.88 (2.98)	3 (5)	**0.012**	**0.019**
					T/T (11)	2.27 (1.27)	2 (2)		

Abbreviations: 1, minor allele; 2, major allele; Nr.hosp, number of hospitalizations; MAF, minor allele frequency; p-HWE, p-value for the Hardy Weinberg Equilibrium.
The p-values were obtained with the Mann-Whitney U (*) and the Kruskal—Wallis (**) tests. For the Mann-Whitney test, homozygous genotypes with the minor allele and heterozygous were combined in a single group. Significant p-values (<0.05) are in bold.

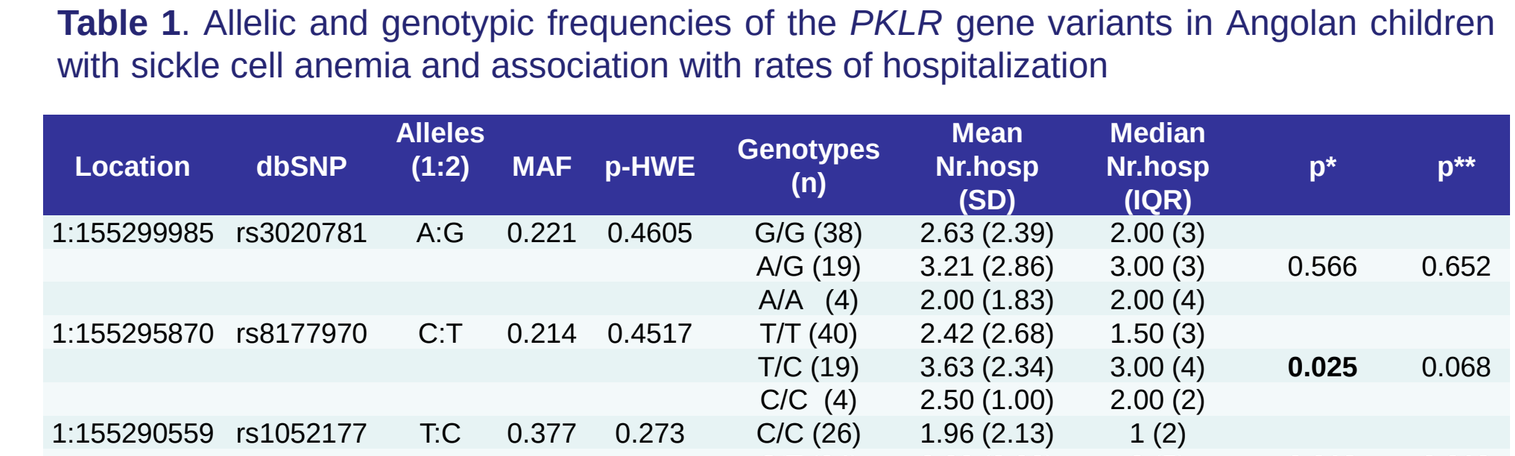

Figure 1. Boxplots showing the distribution of the number of hospitalizations within genotypes of the *PKLR* SNPs rs1052177 and rs8177970 in Angolan children with sickle cell anemia. Each rectangle represents the data between the 25th and 75th quartiles, and the bar within each rectangle is the median value for the number of hospitalizations.

CONCLUSIONS

This study, conducted in SCA children from Angola, found potential links between two *PKLR* SNPs and hospitalization rates. The *PKLR* gene may act as a genetic modifier of the clinical progression of SCA, as these SNPs can affect gene expression levels.

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