

*Polymorphism in the *pklr* gene, and malaria risk in the Brazilian Amazon*

Evelyn Gonçalves Macedo, Carlos Augusto Prete Junior, Winni Alves Ladeia, Marcelo Urbano Ferreira

Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil (FAPESP; 2022/11963-3 and 2024/21891-5) and Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil (CNPq; 303039/2024-8)

Pyruvate kinase deficiency (PKD) is an autosomal recessive hereditary enzymopathy caused by mutations in the *pklr* gene, which may lead to hemolytic anemia of variable severity. Although *pklr* variants are common in areas endemic for *Plasmodium falciparum*, little is known about their impact in populations exposed to *P. vivax*, such as those in the Amazon. This study investigated the association between PKD and malaria infection in a cohort exposed to *P. vivax* and *P. falciparum* in Mâncio Lima, Acre, Brazil. From 2,037 blood samples, molecular diagnosis of *P. vivax* and *P. falciparum* was performed using TaqMan qPCR, and three *pklr* SNPs (rs1052176, rs4971072, rs11264359) were genotyped by TaqMan allelic discrimination. Episodes per individual were modeled with mixed-effects negative binomial regression, adjusted for age, sex, household, wealth index, and *pklr* genotype, with FY genotyping for *P. vivax*. A total of 1,591 samples with known *pklr* and FY genotypes were analyzed after excluding FY-negative individuals. Mutant allele frequency ranged from 46-55%. Homozygous mutants showed more *P. falciparum* episodes than wild-type and heterozygotes (IRR: 1.34-1.38; 95% CI: 1.01-1.81; p : 0.015-0.042). Males showed increased risk compared to females (IRR: 1.59; 95% CI: 1.24-2.06; p <0.001). In both species, incidence increased with age. Compared to individuals younger than 17 years, both adult age groups (17-40 and >40) showed a significantly higher incidence of infection, with IRRs above 3 for *P. falciparum* (p <0.001) and above 1 for *P. vivax* (p ≤0.049). No SNPs were associated with *P. vivax* incidence. Individuals in the highest wealth tertile had fewer episodes than those in the lowest (IRR: 0.74; 95% CI: 0.59-0.92; p ≤0.008). Overall, *P. falciparum* was influenced by *pklr* variants independently of global ancestry, PKD phenotype, and other polymorphisms, suggesting these tag SNPs may reflect factors linked to susceptibility to *P. falciparum*, but not *P. vivax*, in Amazonian populations.

Keywords: *malaria; Plasmodium vivax; pyruvate kinase l/r gene; human polymorphism; Amazon.*