Erythrocyte polymorphisms, including ovalocytosis, have been associated with protection against malaria. This study in the Wosera, a malaria holoendemic region of Papua New Guinea, examined the genetic basis of ovalocytosis and its influence on susceptibility to malaria infection. Whereas previous studies showed significant associations between Southeast Asian ovalocytosis (caused by a 27-base pair deletion in the anion exchanger 1 protein gene) and protection from cerebral malaria, this mutation was observed in only 1 of 1019 individuals in the Wosera. Polymerase chain reaction strategies were developed to genotype individuals for the glycophorin C exon 3 deletion associated with Melanesian Gerbich negativity (GPCex3). This polymorphism was commonly observed in the study population (GPCex3 frequency = 0.465, n = 742). Although GPCex3 was significantly associated with increased ovalocytosis, it was not associated with differences in either Plasmodium falciparum or P vivax infection measured over the 7-month study period. Future case-control studies will determine if GPCex3 reduces susceptibility to malaria morbidity. (Blood. 2001;98:3489-3491)

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Results and discussion

Ovalocytosis in the Wosera

To assess the relationship between ovalocytosis and Melanesian ancestry, 13 North Americans and 199 individuals from the Wosera were compared. The frequency of ovalocytes per 1000 RBCs was significantly higher in residents of the Wosera than North Americans (median, 292 ± 131.4; median, 24 ± 22.6, respectively, Wilcoxon, \( P < .0001 \)). Representative blood smears from a North American Caucasian and 3 Melanesians with different GPC and band 3 genotypes are illustrated in Figure 1A-D.

**AE1Δ27**

Previous studies reported an absence of AE1Δ27 in the Wosera (n = 216).\(^2\) Consistent with these findings, only 1 of 1019 residents from the Wosera had this mutation (Figure 1B). The PCR product from this individual was cloned and sequenced, verifying that this individual carried AE1Δ27 (not shown).\(^3\)

**GPC genotype in the Wosera**

GPC genotyping was performed on 742 individuals (Figure 1E-G).

The first reaction, screening for the presence or absence of the wild-type (wt) GPC allele where exons 2 and 3 are both present (Figure 1E), identified homozygous GPCex3 individuals (lane 3). The second (Figure 1F) and third reactions (Figure 1G) amplified GPC sequence within the wt or GPCex3 alleles, respectively, and allowed homozygous wt individuals (Figure 1F-G, lanes 1 and 4) to be distinguished from heterozygous individuals (Figure 1F-G, lane 2). Allele frequencies for GPC wt and GPCex3 were 0.535 and 0.465, respectively. Genotyping showed 211 (28.4%) of 742 individuals as homozygous wt, 372 (50.1%) of 742 as heterozygotes, and 159 (21.4%) of 742 as GPCex3 homozygotes. This distribution is in Hardy-Weinberg equilibrium, indicating that GPCex3 does not confer a selective disadvantage. This is in contrast to AE1Δ27, a balanced polymorphism, where the disadvantage of lethality in the homozygous form is outweighed by the selective advantage against severe malaria for heterozygotes.\(^5\)

**GPC genotype and ovalocytosis**

The association between ovalocytosis and GPC genotype was evaluated in 134 individuals who did not carry AE1Δ27. The wt individuals (n = 32) had the lowest proportion of ovalocytes per 1000 RBCs (median, 238 ± 115.1). Heterozygous individuals (n = 52) had a higher proportion of ovalocytes (median, 297 ± 103.8), while homozygotes (n = 49) had the highest of all 3 genotypes (median, 312 ± 145.9). In a comparison of all 3 genotypes, the proportion of ovalocytes was significantly associated with GPCex3 (Kruskal-Wallis, \( P = .021 \)). Individual comparisons among the 3 genotypic groups showed significant differences by a one-sided Wilcoxon test (wt/wt vs wt/GPCex3, \( P = .0392 \); wt/wt vs GPCex3/GPCex3, \( P = .0045 \)). These results suggest that GPCex3 contributes to ovalocytosis in the Wosera. When erythrocyte morphology was compared between homozygous wt individuals from the Wosera and North Americans, the former had a significantly increased ovalocyte frequency (Wilcoxon, \( P < .0001 \)). This suggests that altered RBC morphology in the Wosera is a heterogenous condition caused by additional unknown mutations in RBC membrane proteins, such as protein 4.1 and spectrin as well as environmental or nutritional factors.
The prevalence of infection with \textit{P} \textit{falciparum} and \textit{P} \textit{vivax} determined by blood smear has been examined in relation to serologic Ge antigen status in one published study of 266 people.\textsuperscript{11} This study observed a lower combined smear positive rate for \textit{P} \textit{falciparum} and/or \textit{P} \textit{vivax} in Ge-negative individuals, suggesting that Ge antigen negativity protects against infection.\textsuperscript{11} To examine the relationship between GPC genotype and susceptibility to malaria infection more rigorously, we studied a larger group of individuals in the 3 GPC genotypic groups at any time (Table 1). These results parallel findings of other RBC polymorphisms, such as \textit{AE1G27}, where genotypic differences are associated with reduced susceptibility to severe malaria morbidity with no effect on susceptibility to infection.\textsuperscript{3} The relationship of GPC\textit{Δ}3 to malaria morbidity in young children, the age group most susceptible to the clinical phenotype, requires further study.

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The association of the glycophorin C exon 3 deletion with ovalocytosis and malaria susceptibility in the Wosera, Papua New Guinea

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