Published in final edited form as:
Am J Hematol. 2004 January ; 75(1): 1–5. doi:10.1002/ajh.10448.

Glycophorin C (Gerbich Antigen Blood Group) and Band 3 Polymorphisms in Two Malaria Holoendemic Regions of Papua New Guinea

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Abstract

The geographic overlap between the prevalence of erythrocyte polymorphisms and malaria endemicity is thought to be an example of natural selection on human populations. In Papua New Guinea (PNG), the Gerbich-negative phenotype is caused by an exon 3 deletion in the glycophorin C gene (*GYPC*Δ*ex3*) while heterozygosity for a 27-base pair deletion in the *SLC4A1* gene (anion exchanger 1 or erythrocyte membrane protein, band 3), *SLC4A1*Δ*27*, results in Southeast Asian ovalocytosis. Two geographically and ethnically distinct malaria endemic regions of PNG (the Wosera [East Sepik Province] and Liksul [Madang Province]) were studied to illustrate the distribution of two prominent deletion polymorphisms (*GYPC*Δ*ex3* and *SLC4A1*Δ*27*) and to determine if the genetic load associated with *SLC4A1*Δ*27* would constrain independent assortment of *GYPC*Δ*ex3* heterozygous and homozygous genotypes. The frequency of the *GYPC*Δ*ex3* allele was higher in the Wosera (0.463) than Liksul (0.176) (χ^2; $P < 0.0001$). Conversely, the frequency of the *SLC4A1*Δ*27* allele was higher in Liksul (0.0740) than the Wosera (0.0005) (χ^2; $P < 0.0001$). No individuals were homozygous for *SLC4A1*Δ*27*. In 355 Liksul residents, independent assortment of these two deletion polymorphisms resulted in 14 *SLC4A1*Δ*27* carriers heterozygous for *GYPC*Δ*ex3* and one *SLC4A1*Δ*27* carrier homozygous for *GYPC*Δ*ex3* (Fisher's exact test; $P = 0.8040$). While homozygosity for *SLC4A1*Δ*27* appears to be nonviable, the *GYPC*Δ*ex3* allele is not lethal when combined with *SLC4A1*Δ*27*. Neither mutation was associated with altered susceptibility to asymptomatic *Plasmodium falciparum* or *P. vivax* infection. While these erythrocyte polymorphisms apparently have no effect on blood-stage malaria infection, their contribution to susceptibility to clinical malaria morbidity requires further study.

Keywords

glycophorin C; Gerbich; band 3; Southeast Asian ovalocytosis; malaria; Papua New Guinea

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INTRODUCTION

Malaria has been one of the most important environmental influences of natural selection in human populations [1]. The protective effect of various erythrocyte polymorphisms against severe disease caused by *Plasmodium falciparum* infection has been suggested by the epidemiologic association of these mutations in populations where malaria currently or historically has high endemicity [1–5]. Since children who are susceptible to severe malaria die before reproductive age, alleles that offer protection from severe disease would be expected to rise in frequency in these populations. Identifying these alleles could determine which children are at high risk for malaria morbidity while expanding knowledge of the mechanisms of disease and protection from malaria.

The erythrocyte polymorphism referred to as Southeast Asian ovalocytosis (SAO) is found in malaria endemic regions of Papua New Guinea (PNG) [6]. It is strongly associated with heterozygosity for a 27-base pair deletion of the anion exchanger 1 or erythrocyte membrane protein, band 3 gene (*SLC4A1*Δ*27*) on chromosome 17 [7,8]. Band 3 is an integral red blood cell membrane protein that plays an important role in maintaining the erythrocyte cytoskeleton and shape, and functions as the principal exchanger of bicarbonate for chloride in the process of removing carbon dioxide from tissues [9–11]. While associations between heterozygosity for *SLC4A1*Δ*27* and prevalence and parasitemia of asymptomatic blood-stage *P. falciparum* and *P. vivax* infections may be equivocal, the selective advantage of this mutation is demonstrated by its powerful protection against cerebral malaria [12,13]. This advantage is balanced by presumed nonviability in the homozygous state [6,13].

Another integral red blood cell membrane protein polymorphism found in malaria holoendemic regions of Papua New Guinea is the Gerbich-negative phenotype caused by an exon 3 deletion in the glycophorin C gene (*GYPC*Δ*ex3*) on chromosome 2 [14–19]. Our recent studies in the Wosera region of PNG have shown that these residents have ovalocytic red blood cells but do not possess the *SLC4A1*Δ*27* mutation [20]. In this population, the proportion of ovalocytes on blood smear progressively increases with the presence of *GYPC*Δ*ex3* so that individuals who are homozygous for the deletion have a greater percentage of ovalocytes than heterozygotes [20]. When malaria infection status was examined at monthly intervals over a 7-month period, we found that blood-stage *P. falciparum* or *P. vivax* infection was not affected by *GYPC*Δ*ex3* [20]. These findings parallel those for *SLC4A1*Δ*27* and susceptibility to blood-stage malaria infection.

While the prevalence of *SLC4A1*Δ*27* and *GYPC*Δ*ex3* has been correlated with malaria endemicity, the distribution of, and genotype–genotype interactions between these two mutations in the Wosera and Liksul regions of PNG has not been investigated [6,14].

Both SLC4A1 and GYPC are integral red blood cell membrane proteins. While it has been suggested that homozygosity for *SLC4A1*Δ*27* is lethal, we were interested in learning whether the combination of *SLC4A1*Δ*27* and *GYPC*Δ*ex3* alleles would have a similar deleterious effect. In order to test this, we first determined the distribution of *SLC4A1*Δ*27* and *GYPC*Δ*ex3* in two distinct populations residing in different malaria endemic areas of PNG. Second, we determined whether the presence of one polymorphism influenced the distribution of the other. Would the apparent genetic load of *SLC4A1*Δ*27* constrain independent assortment of *GYPC*Δ*ex3* heterozygous and homozygous genotypes? Finally, we determined the relationship of *SLC4A1*Δ*27* and *GYPC*Δ*ex3* genotypes to asymptomatic malaria infection status.

MATERIALS AND METHODS

Study Population

Cross-sectional studies were conducted in villages within the Wosera (East Sepik Province) and Liksul (Madang Province) areas of Papua New Guinea. These are historically isolated populations from two different linguistic groups that are separated by 350 km of rugged terrain [21–23]. There is little migration into and out of either region. Intra-village and intervillage marriages are equally common. Marriages within patrilineal clans are considered taboo. Nearly all of the residents of the study villages volunteered to participate. All four human *Plasmodium* species are transmitted year round in both areas [21–23]. Blood samples were collected from Wosera residents in July 1998 and from Liksul residents in May through August 2000. A subset of the samples included in our previous study in the Wosera was used [20]. The Human Investigations Institutional Review Boards of Case Western Reserve University, University Hospitals of Cleveland, and the Papua New Guinea Medical Research Advisory Committee approved all protocols. Informed consent was obtained from study participants.

Malaria Infection Status

Thick and thin blood films were prepared with 4% Giemsa stain and read by light microscopy [21]. Parasite densities were recorded as the number of parasites per 200 leukocytes (average 8,000 leukocytes/μl) [21].

Genotyping for Band 3 and Glycophorin C Polymorphisms

Blood was collected in EDTA Vacutainer tubes and stored at −70°C until DNA extraction was performed with the QIAmp96 DNA blood kit (Qiagen, Valencia, CA). Genotyping of band 3 and glycophorin C was performed as previously described [7,20]. All Wosera genotypes are a subset of data presented in our previous study in the area [20].

Statistical Analysis

Categorical variables were analyzed by the chi-square or Fisher's exact test. Continuous variables were analyzed by the Wilcoxon or Kruskal–Wallis test. The Statistical Analysis Systems version 8.1 (SAS, Inc., Cary, NC) software package was used.

RESULTS

Frequency of the *SLC4A1Δ27* and *GYPCΔex3* in the Wosera (East Sepik Province) and Liksul (Madang Province)

Only one person of 976 (0.1%) tested from the Wosera carried *SLC4A1Δ27* (Table I). In Liksul, 54 of 365 (14.8%) residents carried the mutation. Consistent with previous studies, no homozygous *SLC4A1Δ27* individual was identified [6,20]. The allele frequency of *SLC4A1Δ27* was significantly higher in Liksul than the Wosera (0.0740 vs. 0.0005, respectively; Fisher's exact, $P < 0.0001$). In the Wosera, 152 of 705 (21.6%) participants were homozygous for *GYPCΔex3*. In Liksul, 11 of 357 (3.1%) individuals were *GYPCΔex3* homozygotes (Table II). The allele frequency for *GYPCΔex3* was significantly higher in the Wosera than Liksul (0.463 versus 0.176, respectively; $\chi^2 = 161$; $P<0.0001$). In both the Wosera and Liksul, these frequencies were in Hardy–Weinberg proportions ($\chi^2 = 0.0151$, $P = 1$ and $\chi^2 = 0.0018$, $P = 1$, respectively).

Distribution of Band 3 and Glycophorin C Genotypes in Liksul

Because the *SLC4A1Δ27* and *GYPCΔex3* allele frequencies were both elevated in Liksul, we were able to investigate the interactions between these two erythrocyte membrane

protein deletion polymorphisms. For this analysis the distribution of *SLC4A1* and *GYPC* alleles was examined in 355 Liksul residents (Table III). Based on *SLC4A1*Δ*27* and *GYPC*Δ*ex3* allele frequencies and assuming independent assortment, the expected number of individuals calculated for each genotype combination was similar to the number observed in the study population (Fisher's exact, $P = 0.8040$). In addition, as predicted by allele frequencies, 15 individuals carried both the *SLC4A1*Δ*27* and *GYPC*Δ*ex3* polymorphisms.

Association of Band 3 and Glycophorin C Genotypes to Malaria Infection Status

Next, we determined whether the presence of both SLC4A1 and GYPC polymorphisms would influence susceptibility to asymptomatic blood-stage malaria infection. Baseline characteristics of the two study populations were similar with respect to age, gender, and hemoglobin levels. In the Wosera ($n = 1,690$), the median age was 17.5 years, with 53% females and median hemoglobin 10.7 g/dl. In Liksul ($n = 1,024$), the median age was 18 years, with 48% females and median hemoglobin 11.1 g/dl. The prevalence of *P. falciparum* infection by blood smear was also similar (20.2% in the Wosera vs. 21.8% in Liksul). The prevalence of *P. vivax* infection was twice as high in the Wosera (17.7%) versus Liksul (8.8%).

The prevalence and density of *P. falciparum* and *P. vivax* infections was examined in relation to different SLC4A1 and GYPC genotypes in Liksul (Table IV). For both *P. falciparum* and *P. vivax*, different genotypic combinations did not influence the mean $\log_{10}$ transformed parasitemia or prevalence rates in the population. In both populations, glycophorin C genotype alone did not influence *P. falciparum* and *P. vivax* parasitemia and prevalence rates.

DISCUSSION

Epidemiologic evidence suggests that erythrocyte polymorphisms have arisen in malaria endemic regions to protect an individual from severe malaria [1,2,4]. We studied genetic polymorphisms of two integral red blood cell membrane proteins that have reached a high frequency in malaria endemic regions of Papua New Guinea. Our results show that the allele frequencies for both *SLC4A1*Δ*27* and *GYPC*Δ*ex3* polymorphisms differed in two ethnically and geographically distinct populations. Consistent with previous reports, *SLC4A1*Δ*27* was rare in the Wosera and no *SLC4A1*Δ*27* homozygous individuals were identified in either population [6,20]. *SLC4A1*Δ*27* is considered to be a balanced polymorphism, where the selective advantage against severe malaria for heterozygotes outweighs the disadvantage of lethality in homozygous persons at the population level [12,13]. In contrast, while allele frequencies for *GYPC*Δ*ex3* were higher in the Wosera than Liksul, the distribution of these alleles was in Hardy–Weinberg proportions in both populations and suggests that *GYPC*Δ*ex3* alone does not confer a selective disadvantage.

While both SLC4A1 and GYPC play an important role in red blood cell morphology and function, the genes that encode these proteins are located on different chromosomes [7,19,20]. This would suggest that the *SLC4A1*Δ*27* and *GYPC*Δ*ex3* alleles should assort independently, if the apparent genetic load represented by *SLC4A1*Δ*27* is not increased past a viable threshold by the addition of another significant erythrocyte membrane protein polymorphism.

In Liksul, the *SLC4A1*Δ*27* and *GYPC*Δ*ex3* allele frequencies were high, thus allowing us to examine the interactions of both band 3 and glycophorin C genotypes. The observed and expected frequencies of these genotypes were similar, indicating that *SLC4A1*Δ*27* and *GYPC*Δ*ex3* are independently distributed as would be expected under Mendel's law of independent assortment. This finding suggests that, despite the fact homozygosity for

*SLC4A1*Δ*27* appears to represent a genetic load that is not viable, neither heterozygosity nor homozygosity for *GYPC*Δ*ex3* leads to a readily apparent deleterious effect. It is interesting to note that similar findings with regard to *SLC4A1*Δ*27* and α-globin deletions (located on chromosome 16 and associated with α-thalassemia) have been reported in earlier studies in Madang [24].

The relationship of glycophorin C or band 3 mutations to malaria susceptibility has been investigated [13,20,24]. Neither mutation alone has been shown to significantly influence asymptomatic asexual parasitemia by *P. falciparum* or *P. vivax* [13,20,24]. Our preliminary analysis of the relationship of both *GYPC*Δ*ex3* and *SLC4A1*Δ*27* to malaria susceptibility in Liksul revealed no association with the prevalence of *P. falciparum* or *P. vivax* prevalence or parasite density. These results parallel findings of other red blood cell polymorphisms where genotypic differences are observed at the level of severe malaria morbidity with no discernable effect on susceptibility to blood-stage infection [13].

In summary, we found that the frequency of *GYPC*Δ*ex3* and *SLC4A1*Δ*27* differs in the two study populations. These mutations are independently distributed and heterozygosity for *SLC4A1*Δ*27* does not influence heterozygosity or homozygosity for *GYPC*Δ*ex3*. In addition, we did not observe an association between these erythrocyte membrane protein deletion mutations and alterations in the prevalence or density of asymptomatic blood-stage malaria infection. A prospective longitudinal study examining both parasitemia and malaria morbidity in children would better elucidate a potential protective role of *GYPC*Δ*ex3* and *SLC4A1*Δ*27* in PNG and other Melanesian populations.

Acknowledgments

Contract grant sponsor: National Institutes of Health; Contract grant numbers: K23 AI49390-01, AI-36478-07, AI-07024, AI 46919-01A2

We thank the residents of the Wosera and Liksul for participating in this study. We also thank Will Kastens, Moses Baisor, Moses Bockarie, and John Reeder for their assistance in implementation of the study in Papua New Guinea.

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TABLE I

Band 3 Genotype in the Wosera and Liksul Areas *

	Wosera (*n* = 976)	Liksul (*n* = 365)
Band 3 genotype	***n* (%)**	***n* (%)**
wt/wt[a]	975 (99.9)	311 (85.2)
wt/Δ27[b,c]	1 (0.1)	54 (14.8)

* All Wosera genotypes are a subset of data presented in Patel et al. [20].

[a] Homozygous wild type individuals.

[b] Heterozygotes for band 3 deletion.

[c] No homozygote for the band 3 deletion was identified.

TABLE II

Glycophorin C Genotype in the Wosera and Liksul Areas *

	Wosera (*n* = 705)	Liksul (*n* = 357)
Glycophorin C genotype	***n* (%)**	***n* (%)**
wt/wt[a]	204 (28.9)	242 (67.8)
wt/Δex3[b]	349 (49.5)	104 (29.1)
Δex3/Δex3[c]	152 (21.6)	11 (3.1)

* All Wosera genotypes are a subset of data presented in Patel et al. [20].

[a] Homozygous wild type individuals.

[b] Heterozygotes for glycophorin C exon 3 deletion.

[c] Homozygotes for glycophorin C exon 3 deletion.

TABLE III

Observed and Expected Distribution of Glycophorin C and Band 3 Genotypes in 355 Liksul Residents

	Band 3 genotype	
	wt/wt[a]	wt/Δ27[b]
Glycophorin C genotype	Observed[c] (expected)[d]	Observed (expected)
wt/wt[e]	203 (207)	39 (34)
wt/Δex3[f]	88 (88)	14 (14)
Δex3/Δex3[g]	10 (9)	1 (1)

[a] Homozygous wild type individuals.

[b] Heterozygotes for band 3 deletion.

[c] Number of individuals observed with genotype.

[d] Number of individuals predicted to have genotype.

[e] Homozygous wild type individuals.

[f] Heterozygotes for glycophorin C exon 3 deletion.

[g] Homozygotes for glycophorin C exon 3 deletion.

TABLE IV

Glycophorin C, Band 3, and Susceptibility to Malaria Infection in Liksul, Papua New Guinea

Genotype			*P. falciparum*		*P. vivax*	
Glycophorin C	**Band 3**	***n***	**Mean parasitemia[a]**	**Prevalence (%)**	**Mean parasitemia[a]**	**Prevalence (%)**
wt/wt[b]	wt/wt[b]	203	0.5619 ± 1.0914	46 (22.7)	0.2687 ± 0.7702	24 (11.8)
wt/wt	wt/Δ27[c]	39	0.3652 ± 1.0086	5 (12.8)	0.2227 ± 0.7168	4 (10.3)
wt/Δex3[d]	wt/wt	88	0.7803 ± 1.3428	24 (27.3)	0.2396 ± 0.6944	10 (11.4)
wt/Δex3	wt/Δ27	14	1.1840 ± 1.4470	6 (42.9)	0.1152 ± 0.4310	1 (7.1)
Δex3/Δex3[e]	wt/wt	10	0.3226 ± 0.6800	2 (20.0)	0.2807 ± 0.8876	1 (10.0)
Δex3/Δex3	wt/Δ27	1	0	0 (0)	0	0 (0)
		P value	0.1880[f]	0.2352[g]	0.9894[f]	1.0000[g]

[a] Log_{10} parasites/μl blood.

[b] Homozygous wild type individuals.

[c] Heterozygotes for band 3 deletion.

[d] Heterozygotes for glycophorin C exon 3 deletion.

[e] Homozygotes for glycophorin C exon 3 deletion.

[f] Kruskal–Wallis test.

[g] χ^2 analysis.