Brief report

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

Sheral S. Patel, Rajeev K. Mehlotra, William Kastens, Charles S. Mugone, James W. Kazura, and Peter A. Zimmerman

**Introduction**

The geographic overlap between malaria and red blood cell (RBC) disorders led Haldane to hypothesize that many polymorphisms in the human genome have arisen by natural selection to protect from severe malaria infection and thereby increase reproductive fitness of populations living in malaria endemic regions. In Papua New Guinea, Southeast Asian ovalocytosis, caused by a 27–base pair (bp) deletion in the anion exchanger 1 protein gene (AE1Δ27), is observed in many coastal malaria holoendemic areas and is associated with protection from cerebral malaria. While AE1Δ27 has not been reported in residents of the malaria holoendemic Wosera region of Papua New Guinea, ovalocytic RBCs are common.

Additional polymorphisms characterizing the human population in the Wosera include an exon 3 deletion of the integral membrane sialoglycoprotein glycoporphin C (GPC). This deletion (GPCΔex3) changes serologic phenotypes of the Gerbich (Ge) blood group system in Melanesians. Because GPC is involved in maintaining the integral RBC membrane lattice, the association between GPCΔex3 and ovalocytosis was tested. Furthermore, because GPCΔex3 is distributed within a malaria holoendemic region, we tested the association between GPCΔex3 and susceptibility to malaria infection.

**Study population and malaria**

The study was conducted in the Wosera region of Papua New Guinea, where all 4 human *Plasmodium* species are transmitted year-round. Blood samples were collected monthly from permanent residents (median age, 17 years; range, 1–86 years) of 6 villages within the Wosera from July 1998 to January 1999. 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.

Malaria and red blood cell morphology

Thick and thin films stained with 4% Giemsa were prepared at the time of blood collection (Figure 1A-D). Malaria parasites were identified by light microscopy. Parasite densities were recorded as the number of parasites per 200 leukocytes (average 8000 leukocytes per microliter). Blood smears from North Americans were prepared using a modified Wright stain (Diff-Quick Stain Set, Dade-Behring, Newark, DE). One reader reviewed all of the blood smears. Two additional observers independently reviewed a subset of smears.

Genotyping for AE1 and GPC polymorphisms

Blood was collected in ethylenediaminetetraacetic acid 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 was performed as previously described. New polymerase chain reaction (PCR) genotyping strategies for GPC are described in Figure 1E-G.

Statistical analysis

Categorical variables were analyzed by the χ² test and continuous variables by the Wilcoxon or Kruskal-Wallis test. Statistical Analysis Systems version 8.1 software package (Cary, NC) was used.
were performed as previously described.12 (E) To assess the presence of exons 2 and 3, homologous, 2 reactions are needed to delineate all 3 genotypes. All PCR amplifications
North American, wt/wt genotype. Because exon 2 and exon 3 of the GPC gene are highly

cycling program: 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 90 seconds

agarose gel. (G) To identify the GPC

(GenBank M24628)5 (GPC349up and GPC1680dn 5

51-bp intron 2 insert (GenBank AF342984) and downstream intron 2 polymorphism
exon 2 and 3 amplicons. (F) To identify individuals with the wt allele, primers annealing to a

The heteroduplex product is created by hybridization between complementary strands of
exon 2 and 3 amplions. (F) To identify individuals with the wt allele, primers annealing to a
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-

results suggest that GPCαx3 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). These results suggest 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.
GLYCOPHORIN C, OVALOCYTOSIS, AND MALARIA INFECTION

Table 1. Glycophorin C genotype and infections over time

<table>
<thead>
<tr>
<th>Month</th>
<th>No.§</th>
<th>Prevalence (%)</th>
<th>Parasitemia (95% CI)</th>
<th>Prevalence (%)</th>
<th>Parasitemia (95% CI)</th>
<th>Prevalence (%)</th>
<th>Parasitemia (95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Glycophorin C genotype and P falciparum</td>
<td></td>
<td>Glycophorin C genotype and P vivax</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>July</td>
<td>696</td>
<td>34/200 (17.0)**</td>
<td>2.21 (2.16-2.62)</td>
<td>72/345 (20.9)</td>
<td>2.42 (2.28-2.55)</td>
<td>34/151 (22.5)</td>
<td>2.38 (2.24-2.71)</td>
<td>.392 .880</td>
</tr>
<tr>
<td>Aug</td>
<td>528</td>
<td>27/139 (19.4)**</td>
<td>2.30 (2.27-2.70)</td>
<td>60/264 (22.7)</td>
<td>2.38 (2.24-2.54)</td>
<td>30/125 (24.0)</td>
<td>2.38 (2.21-2.72)</td>
<td>.638 .739</td>
</tr>
<tr>
<td>Sept</td>
<td>415</td>
<td>23/121 (19.0)**</td>
<td>2.30 (2.16-2.57)</td>
<td>56/199 (28.1)</td>
<td>2.15 (2.14-2.49)</td>
<td>19/95 (20.0)</td>
<td>2.08 (2.02-2.47)</td>
<td>.112 .627</td>
</tr>
<tr>
<td>Oct</td>
<td>466</td>
<td>23/136 (16.9)**</td>
<td>2.21 (2.06-2.56)</td>
<td>41/235 (17.5)</td>
<td>2.30 (2.29-2.70)</td>
<td>24/95 (25.3)</td>
<td>2.08 (2.04-2.76)</td>
<td>.203 .315</td>
</tr>
<tr>
<td>Nov</td>
<td>495</td>
<td>35/130 (26.9)**</td>
<td>2.68 (2.44-3.00)</td>
<td>75/248 (30.2)</td>
<td>2.30 (2.31-2.64)</td>
<td>36/117 (30.8)</td>
<td>2.34 (2.21-2.72)</td>
<td>.752 .211</td>
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<tr>
<td>Dec</td>
<td>331</td>
<td>25/85 (29.4)**</td>
<td>2.45 (2.29-3.03)</td>
<td>43/171 (25.2)</td>
<td>2.56 (2.47-2.97)</td>
<td>18/75 (24.0)</td>
<td>2.85 (2.46-3.25)</td>
<td>.692 .586</td>
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<tr>
<td>Jan</td>
<td>325</td>
<td>25/93 (26.9)**</td>
<td>2.07 (2.33-2.82)</td>
<td>37/162 (22.8)</td>
<td>2.60 (2.67-3.23)</td>
<td>17/70 (24.3)</td>
<td>2.38 (2.12-2.92)</td>
<td>.769 .049</td>
</tr>
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GPC genotype and infection status

The prevalence of infection with P falciparum or P vivax determined by blood smear has been examined in relation to serologic Ge antigen status in one published study of 266 people.11 This study observed a lower combined smear positive rate for P falciparum and/or P vivax infection in Ge-negative individuals, suggesting that Ge antigen negativity protects against infection.11 To examine the relationship between GPC genotype and susceptibility to malaria infection more rigorously, we studied a larger population over 7 months. Results for genotype and infection status were available for 325 to 696 individuals at each of 7 monthly intervals. This analysis showed that the prevalence or density of P falciparum and P vivax infection was not significantly different for individuals in the 3 GPC genotypic groups at any time (Table 1). These results parallel findings of other RBC polymorphisms, such as AE1Δ27, where genotypic differences are associated with reduced susceptibility to severe malaria morbidity with no effect on susceptibility to infection.3 The relationship of GPCΔex3 to malaria morbidity in young children, the age group most susceptible to the clinical phenotype, requires further study.

Acknowledgments

We thank the residents of the Wosera for participating in this study. We thank Chloe Hill for reviewing blood smears.

References

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