PREVENTION OF CEREBRAL MALARIA IN CHILDREN IN PAPUA NEW GUINEA BY SOUTHEAST ASIAN OVALOCYTOSIS BAND 3

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Abstract. Southeast Asian ovalocytosis (SAO) occurs at high frequency in malarious regions of the western Pacific and may afford a survival advantage against malaria. It is caused by a deletion of the erythrocyte membrane band 3 gene and the band 3 protein mediates the cytoadherence of parasitized erythrocytes in vitro. The SAO band 3 variant may prevent cerebral malaria but it exacerbates malaria anemia and may also increase acidosis, a major determinant of mortality in malaria. We undertook a case-control study of children admitted to hospital in a malarious region of Papua New Guinea. The SAO band 3, detected by the polymerase chain reaction, was present in 0 of 68 children with cerebral malaria compared with six (8.8%) of 68 matched community controls (odds ratio = 0.95, 95% confidence interval = 0.035) but acidosis was not affected. The remarkable protection that SAO band 3 affords against cerebral malaria may offer a valuable approach to a better understanding of the mechanisms of adherence of parasitized erythrocytes to vascular endothelium, and thus of the pathogenesis of cerebral malaria.

Malaria resistance genes provide the best example of natural selection occurring in human populations. Although the mechanisms by which malaria is prevented are not known, recent case-control studies have shown that genetic variants may affect susceptibility to specific severe manifestations of malaria. For example, in African children, HLA-B53 and glucose-6-phosphate dehydrogenase deficiency are associated with protection against both cerebral malaria and severe malarial anemia, whereas a polymorphism of the tumor necrosis factor-α promoter gene increases the risk of cerebral malaria but not malarial anemia. Recently, we have reported that homozygous α-thalassemia affords significant protection against malaria complicated by severe anemia, acidosis and hyperlactatemia in children in Papua New Guinea.

Southeast Asian ovalocytosis (SAO) occurs in up to 35% of the population of malarious regions of the western Pacific and is caused by a deletion of 27 basepairs of the erythrocyte membrane band 3 gene on chromosome 17. Band 3 is the major transmembrane protein of red blood cells and has two main functions: the cytoplasmic domain maintains cell shape by attaching the cell membrane to the cell cytoskeleton and the transmembrane domain increases the capacity of the blood to carry carbon dioxide by exchanging intracellular bicarbonate for chloride. An isoform of band 3 is present in the distal nephron, in which its anion exchange function contributes to urine acidification. The SAO band 3 protein has a deletion of amino acids 400–408 in the boundary between the cytoplasmic and the first transmembrane domains. The SAO red blood cells have altered morphology and anion transport is reduced to about 40% of normal.

Homozygotes for SAO band 3 have not been identified and this condition is assumed to be lethal in utero. The high frequency of SAO in malarious regions might reflect heterozygote protection against malaria. The SAO band 3 could modify disease caused by malaria in several ways. Protection by SAO band 3 against cerebral malaria was suggested in a clinical study of children from rural areas of Madang, although the possibility of confounding by other factors that reduce malarial disease was not considered in this study. We have reported that malaria anemia is exacerbated in children with SAO. Also, the decreased anion transport of the variant band 3 protein may worsen acidosis which is an important risk factor for mortality in acute malaria.

We undertook a prospective case-control study of children admitted to Madang hospital with malaria to assess the magnitude of the protection by SAO band 3 against cerebral malaria and to investigate the relationship between the variant and other common severe manifestations of malaria. The frequency of the variant in severe malaria cases was compared with that in controls living in the community. Factors other than SAO band 3 that may contribute to resistance to malaria and may differ between cases and controls include other genetic factors, especially α-thalassemia, and acquired immunity as a result of the degree of previous exposure to malaria. To minimize confounding by these factors, and local geographic variations in the prevalence of SAO, a community control child was individually-matched to each severe malaria case for age, sex, ethnicity, village, and season.

MATERIALS AND METHODS

Study site and population. The study was undertaken between October 1993 and February 1996 and details of the study site and population have been published previously.2 The study was based in the pediatric ward of Madang Hospital, which is situated on the north coast of Papua New Guinea, a region hyperendemic for Plasmodium falciparum.12 Only children who had lived for at least 12 months in Madang were included in the study. Ethnicity was categorized according to the region of origin of the languages spoken by children’s parents. The study was approved by the Medical Research Advisory Committee of Papua New Guinea and consent for inclusion was obtained from accompanying parents.

The relationship between band 3 status and the risk of death caused by malaria was assessed in two ways. First, in children admitted to hospital with malaria, indices of the severity of disease were compared according to band 3 status. Second, cases with one or more severe manifestations
of malaria, defined according to World Health Organization criteria, were selected as surrogates for children who would be expected to die of malaria. Band 3 status in these index cases was compared with that in individually matched community controls.

Definitions of severe malaria. Cerebral malaria was defined as a Blantyre coma score \( \leq 2 \) in children with asexual \( P. falciparum \) parasitemia and no evidence of bacterial or viral meningoencephalitis on examination of cerebrospinal fluid. The level of consciousness was determined \( \geq 30 \) min after a generalized convolution and \( \geq 6 \) hr after anticonvulsant treatment. In children with asexual \( P. falciparum \) parasitemia, severe malarial anemia was defined as a febrile illness with a hemoglobin level \(< 5\, \text{g/dL};^2\) acidosis as a plasma bicarbonate concentration \(< 15\, \text{mmol/L};^2\) and hyperlactateemia as a plasma lactate concentration \( \geq 5\, \text{mmol/L};^2\).

Recruitment of community controls. The house of the index case was visited soon after the child’s admission and a neighboring house of an unrelated family was selected randomly by spinning a pencil. One child matched as closely as possible to the index case for ethnicity, age (less than one year difference), and sex was selected. If no suitable child was available, the next house in a clockwise direction was visited.

Other clinical groups. Protection against mild malarial disease was assessed by comparing band 3 status in the community controls with that in children treated for malaria (fever and \( P. falciparum \) parasitemia \( \geq 10,000/\mu\text{l} \)) as out-patients in six local clinics. The relationship between the variant and the prevalence of malaria in the community was assessed by comparing malarriometric indices in the community control children according to band 3 status. To assess the specificity of any protection against malaria detected in the preceding analyses, the frequency of SAO band 3 in the community controls was compared with that in non-malaria controls with severe illness (hospital cases with mostly acute infections but without parasitemia) and mild illness (febrile children without parasitemia treated as out-patients).

Laboratory methods. Blood count (MD8; Coulter Electronics, Luton, United Kingdom), biochemical indices (dry slide chemistry, Ektachem; Eastman Kodak, Rochester, NY) and glucose-6-phosphate dehydrogenase activity (procedure no. 400; Sigma, St. Louis, MO) were measured in venous blood. The \( P. falciparum \) density was calculated from the number of parasites per 200 white blood cells in thick blood films stained with Giemsa and the measured white blood cell count. A DNA lysate was prepared from heparinized blood and SAO band 3 was detected by the polymerase chain reaction and the genotype for \( \alpha^1 \)-thalassemia by Southern blotting. In hospital cases, urine was collected into a sterile container, adhesive bags (Urogard; Terumo Corporation, Tokyo, Japan) or post-mortem by supra-pubic aspiration, and tested with Combur7 Test strips (Boehringer Mannheim, Lewes, United Kingdom). The determination of severe manifestations of malaria and the categorization of patients into the various clinical groups was completed before results of the genetic analyses were known.

Statistical analysis. Categorical variables were analyzed by the chi-square test and continuous variables by the Kruskal-Wallis test. Odds ratios were derived from the index case–community control pairs who were discordant for band 3 status and 95% confidence intervals for the odds ratios were based on the exact confidence interval for a binomial proportion. Possible confounding caused by differences between cases and controls in ethnicity and age, which occurred despite matching, and genotype for \( \alpha^1 \)-thalassemia, was assessed by including these variables in a conditional logistic regression analysis in which case-control status was the dependent variable and band 3 status the predictor variable. Comparison of band 3 status in the community controls with that in other clinical groups was by logistic regression analysis. EGRET software (Statistics and Epidemiology Research Corp., Seattle, WA) was used for all regression analyses.

RESULTS

Sufficient DNA was available from 1,224 (81.4%) of 1,503 children to determine SAO band 3 status. The frequency of SAO was 6.8% (70 of 1,023) in children with one or both parents of Madang ethnicity, 1.4% (2 of 142) in Sepiks, 3.0% (1 of 33) in individuals from other coastal regions, and 0% in 26 Highlanders. The frequency was 8.1% (27 of 334) in \( \alpha^1 \)-thalassemia homozygotes, 8.0% (23 of 288) in heterozygotes and 3.5% (5 of 142) in children without \( \alpha^1 \)-thalassemia \((P = 0.17)\). Among boys, glucose-6-phosphate dehydrogenase deficiency was detected in 0 of 30 children with the variant and in 15 (2.9%) of 522 children with a normal band 3 \((P = 1.00\), by Fisher’s exact test). Frequencies of SAO band 3 in the clinical groups. The SAO band 3 was present in 5.9% of 307 community controls, and a similar frequency was observed in all children admitted to the hospital with malaria, in children treated for malaria as out-patients, and in controls with severe and mild non-malarial illnesses (Table 1). Logistic regression analysis showed that band 3 status was not significantly associated with the risk of clinical disease, including when differences in the frequency of demographic variables and genotype for \( \alpha^1 \)-thalassemia between some of the clinical groups and the community controls were included in the model.

Cerebral malaria. Among the hospital malaria cases, cerebral malaria was diagnosed in 17.6% of the children with a normal band 3 but in none of 27 children with an SAO band 3 \((P = 0.013; Table 2)\). There were 284 severe malaria–community control pairs (Table 3). The SAO band 3 was present in six of 68 community controls selected for the cerebral malaria cases and conditional logistic regression
Coma score no. (%)
5

interval
Clinical subgroups
(Table 4).

Children were similar in those with and without SAO band 3
levels and malarial indices in community control children
line statistical significance (Table 3). Median hemoglobin
level in the cases compared with their controls was of bor-
tcase had severe anemia, the increased frequency of the var-
iant in the cases compared with their controls was of bor-

acidic urine (pH 5 or 6) was present in a similar proportion
SAO did not prevent admission with acidosis in the case-

Children with variant band 3 and 117 (87%) of 135 children
with SAO band 3 relative to normals. CI = confidence interval.

All severe malaria
(total no. of pairs, n = 284)
15
16
1.19 (0.58–2.47)
0.61

Clinical subgroups
Severe anemia (n = 173)
15
7
2.14 (0.82–6.21)
0.084
Cerebral malaria (n = 68)
0
6
0 (0–0.85)
0.031
Acidosis (n = 52)
3
1
3.00 (0.24–157)
0.31
Hyperlactatemia (n = 66)
4
1
4.00 (0.40–197)
0.17

with a normal band 3 had acidic urine (P = 0.60, by Fisher’s exact test).

The SAO band 3 has now been shown to protect against
cerebral malaria in two prospective case-control studies,
each using different controls. Previously, the variant band 3
was absent in 35 cerebral malaria cases but was present in
15 of 103 population controls (P = 0.01). 9 In the present
study, community controls were matched individually to in-
dex cases to account for local geographic variations in the
prevalence of SAO and to minimize differences between
cases and controls in other factors that mediate immunity to
malaria. The recruitment of cerebral malaria cases in the
present study overlapped with that in the previous study such
that one case was common to both studies. When the data
from the two studies are combined (ignoring the case-control
matching in the present study), SAO band 3 appears to con-
fer remarkable protection against cerebral malaria (Mantel-
Haenszel summary χ² = 9.79, P = 0.0018, odds ratio = 0,
exact upper 95% confidence limit 9 = 0.35), indicating a

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>SAO deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>404 6.0 (4.3–9.1)</td>
<td>27 4.8 (3.9–7.0)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>384 4.9 (4.1–5.6)</td>
<td>25 4.9 (4.5–5.6)</td>
</tr>
<tr>
<td>Bicarbonate (mmol/l)</td>
<td>377 21 (17–24)</td>
<td>25 20 (17–22)</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>375 2.9 (2.0–4.6)</td>
<td>25 3.0 (2.4–4.2)</td>
</tr>
<tr>
<td>Plasmodium falciparum density (log 10 parasites/µl)</td>
<td>388 4.3 (3.4–4.9)</td>
<td>25 4.0 (3.7–4.8)</td>
</tr>
<tr>
<td>Urine pH 5 or 6 no. (%)</td>
<td>279/322 (86.7)</td>
<td>17/23 (73.9)</td>
</tr>
<tr>
<td>Coma score no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 (cerebral malaria)</td>
<td>70/398 (17.6)</td>
<td>0/27 (0)</td>
</tr>
<tr>
<td>≤4 (impaired consciousness)</td>
<td>86/398 (21.6)</td>
<td>0/27 (0)</td>
</tr>
<tr>
<td>Outcome no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died or neurologic sequelae</td>
<td>20/404 (5.0)</td>
<td>0/27 (0)</td>
</tr>
</tbody>
</table>

* Data are number and median value (inter-quartile range [IQR]) unless otherwise noted. Numbers vary because of missing data. Continuous variables were analyzed by the Kruskal-Wallis test and categorical variables by the chi-square test. SAO = Southeast Asian ovalocytosis.
† By Fisher’s exact test.

### Table 3

<table>
<thead>
<tr>
<th>Clinical group</th>
<th>Number of discordant pairs</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All severe malaria (total no. of pairs, n = 284)</td>
<td>15 7</td>
<td>2.14 (0.82–6.21)</td>
<td>0.084</td>
</tr>
<tr>
<td>Severe anemia (n = 173)</td>
<td>15 7</td>
<td>2.14 (0.82–6.21)</td>
<td>0.084</td>
</tr>
<tr>
<td>Cerebral malaria (n = 68)</td>
<td>0 6</td>
<td>0 (0–0.85)</td>
<td>0.031</td>
</tr>
<tr>
<td>Acidosis (n = 52)</td>
<td>3 1</td>
<td>3.00 (0.24–157)</td>
<td>0.31</td>
</tr>
<tr>
<td>Hyperlactatemia (n = 66)</td>
<td>4 1</td>
<td>4.00 (0.40–197)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* Both members were of Madang ethnicity in 226 pairs. In the majority of pairs, both members did not have Southeast Asian ovalocytosis (SAO) band 3 and there were no pairs in which both members had the variant. Case-control pairs that were discordant for SAO band 3 are shown in the table. The odds ratios represent the risk of developing severe malaria for children with SAO band 3 relative to normals. CI = confidence interval.
Southeast Asian ovalocytosis is a common red blood cell variant in malarious regions of the western Pacific. Its selective advantage appears to be conferred by its powerful protection against cerebral malaria. Investigation of the adhesion properties of parasitized red blood cells with the variant may shed light on the molecular pathogenesis of this severe manifestation of malaria. The selective advantage of SAO is balanced by an exacerbation of malaria anemia in heterozygotes and nonviability of the homozygote state.

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REFERENCES


