Familial Aggregation of Cerebral Malaria and Severe Malarial Anemia

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Background. The predominant manifestations of severe malaria in African children are cerebral malaria (CM) and severe malarial anemia (SMA). As a first step toward a family-based approach to identify the environmental and genetic pathways that contribute to severe malaria, we tested whether it aggregates within families.

Methods. Family history of severe malaria was explored during face-to-face interviews with parents. Logistic regression was used to determine whether CM and SMA aggregate within individuals and within families. The pattern of familial aggregation was then expressed as familial odds ratios that were adjusted for relevant risk factors.

Results. This study was of 2811 inhabitants of Bamako, Mali, clustered in 407 nuclear families. The probands were 136 children with severe malaria and 271 healthy children from the community. Within-person association of CM and SMA was significant (odds ratio, 6.15 [95% confidence interval (CI), 2.62–14.41]). Over a lifetime, with each additional affected relative, the odds of a person contracting CM increased by 1.98 times (95% CI, 1.59–2.45), and the odds of having SMA increased by 1.91 times (95% CI, 1.05–3.47). Over a lifetime, for a child whose sibling had a history of CM, the odds of having CM were 2.49 times greater (95% CI, 1.51–4.10) than the odds for a child whose sibling had no such history; for a child whose sibling had a history of SMA, the odds of having SMA were 4.92 times greater (95% CI, 1.21–19.9) than the odds for a child whose sibling had no such history.

Conclusion. Our data suggest strong familial aggregation of CM and SMA.

Plasmodium falciparum infections cause >1 million deaths each year in African children. Most cases of clinical malaria are uncomplicated and are associated with a very low case-fatality rate, but a small number of cases are severe, with a case-fatality rate of 10%–30% in children undergoing treatment [1]. The primary manifestations of severe malarial disease in African children are cerebral malaria (CM) and severe malarial anemia (SMA). Whether a P. falciparum infection ultimately results in uncomplicated or severe malaria depends on a complex combination of host and parasite factors. The immunological, nutritional, and sociological status of the host may play a role in the severity of disease, and there is growing evidence that genetic factors influence susceptibility to malaria [2–6]. Clinical investigations of sickle-cell anemia, thalassemias, and other human diseases that involve hemoglobins provided initial insight into genetic factors implicated in resistance to malaria (reviewed in [7, 8]). Subsequent population-based studies have associated various malaria-related phenotypes, including CM and SMA, with polymorphisms in genes that may be involved in the immune-response pathway and the pathogenesis of P. falciparum infection [9–13]. Population-based studies, however, suffer from potential pitfalls, the most serious of which are confounding factors that are due to population admixture. Conversely, family-based studies are relatively unaffected by population substructure, and, thus, their results can be used in the search of the entire human genome, for loci controlling susceptibility to disease [4, 14]. Previous family-based studies of malaria have been limited to uncomplicated malaria [15–19]. As a first step toward a family-based...
Table 1. Characteristics of the study population.

<table>
<thead>
<tr>
<th>Proband group</th>
<th>No. of probands</th>
<th>No. of relatives*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>65</td>
<td>444</td>
</tr>
<tr>
<td>SMA</td>
<td>32</td>
<td>176</td>
</tr>
<tr>
<td>CM and SMA</td>
<td>39</td>
<td>217</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>271</td>
<td>1567</td>
</tr>
<tr>
<td>Total</td>
<td>407</td>
<td>2404</td>
</tr>
</tbody>
</table>

NOTE. CM, cerebral malaria; SMA, severe malarial anemia.

* Parents and siblings of the proband.

Table 2. Lifetime prevalence of cerebral malaria (CM) and severe malarial anemia (SMA) in relatives, according to proband group.

<table>
<thead>
<tr>
<th>Diagnosis in relatives</th>
<th>CM</th>
<th>SMA</th>
<th>CM and SMA</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>% (95% CI)</td>
<td>N</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td>CM</td>
<td>23</td>
<td>5.18 (3.31–7.67)</td>
<td>7</td>
<td>3.98 (1.61–8.02)</td>
</tr>
<tr>
<td>SMA</td>
<td>2</td>
<td>0.45 (0.05–1.62)</td>
<td>1</td>
<td>0.57 (0.01–3.13)</td>
</tr>
<tr>
<td>CM and SMA</td>
<td>1</td>
<td>0.23 (0.01–1.25)</td>
<td>1</td>
<td>0.57 (0.01–3.13)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.

approach to identify the environmental and genetic pathways that contribute to severe malaria, we tested whether CM and SMA aggregate within families and attempted to quantify this familial aggregation.

SUBJECTS AND METHODS

The probands in this family case-control study were either children who had been diagnosed with severe malaria or healthy children from the community who were used as controls. Each child’s parents were informed of the purpose of the investigation and gave informed consent prior to their family’s inclusion in the study. The study was approved by the ethics review board of the University of Mali.

Recruitment and definition of probands. Probands were selected by means of a research-oriented surveillance system that has been in force in the pediatric ward of the Gabriel Touré Hospital in Bamako, Mali, since 1999. In this system, medical data are recorded for all children presenting with CM and/or SMA who are 6 months–14 years old. In a child with a \( P. falciparum \) infection, CM was defined as a Blantyre coma score of \( <3 \) persisting for \( >30 \) min and/or at least 2 seizures within \( 24 \) h, no other diagnosis by laboratory investigations, and no other apparent cause of coma and/or seizures, and SMA was defined as either a hemoglobin level of \( <5 \) g/dL or a packed cell volume of \( <15\% \) and no other apparent cause of anemia. The healthy community probands were children with no history of severe malaria. They were recruited in houses adjacent to those of the probands with severe malaria, provided that they were the same age (± 2 years) and had a duration of residence in their houses that was at least equal to that of the probands with severe malaria [20].

Collection of data. Two groups of trained interviewers, each group including at least 1 physician, conducted face-to-face interviews with the proband’s mother and/or father, using a standardized family-history questionnaire. The 2 groups alternated between interviewing the parents of probands with severe malaria and interviewing those of the healthy controls. Data were collected on each first-degree relative (father, mother, and siblings) of the probands. Information gathered on each family member included history of severe malaria and general characteristics, such as gender, ethnicity, date of birth, date and cause of death (if applicable), and consanguinity between father and mother.

Diagnosis of severe malaria in relatives. Severe malaria was retrospectively diagnosed during the interview with each proband’s parents. When relevant, the age at onset was noted. The retrospective diagnosis, which was determined by the physician conducting the interview, was based primarily on (1) season of occurrence (in Bamako, malaria peaks during the rainy season, whereas meningitis, the most common differential diagnosis for CM in the area, peaks during the dry season); (2) occurrence of seizures (duration and number of episodes per day) and/or coma (duration); (3) consultation with a health practitioner; (4) duration of medical follow-up (>24 h); (5) type and duration of treatment (antimalarial drug[s] and route[s] of administration used); (6) blood transfusion (for SMA); and (7) outcome. The retrospective diagnoses were blinded before the statistical analysis was begun.

Statistical analysis. Logistic-regression analysis was used to determine whether host-related risk factors influence the occurrence of either CM or SMA. All analyses were adjusted for age (age was coded as the square root of age in years, the overall best-fitting age function for both CM and SMA data), and included a dummy binary variable indicating whether the subject belonged to a family in which the proband had severe malaria. A multivariate logistic model (“the family predictive model”) was then used to investigate the existence of familial aggregation for CM and SMA and of familial coaggregation for both diseases [21]. Briefly, in the family predictive model, the
outcome is the bivariate disease status for each of the 2 diseases and for each family member, with each subject’s responses being modeled as a function of both the disease status of all the other members of the family and the subject’s covariates. With this model, the following parameters could be estimated: (1) the within-person association of the 2 diseases, expressed as an odds ratio measuring the increase in the odds that a person who had 1 of the 2 diseases would also have the other disease compared with the odds for a person who did not have 1 of the 2 diseases; (2) the aggregation of CM, measuring the increase in the odds that a person who had \( k + 1 \) relatives with CM would have CM, compared with the odds for a person who had \( k \) relatives with CM; (3) the aggregation of SMA, measuring the increase in the odds that a person who had \( k + 1 \) relatives with SMA would have SMA, compared with the odds for a person who had \( k \) relatives with SMA; and (4) the coaggregation of CM and SMA within different family members, measuring the increase in the odds that a person who had \( k + 1 \) relatives with CM (or SMA) would have CM (or SMA) compared with the odds for a person who had \( k \) relatives with CM (or SMA).

Regression analyses were performed with the Proc Genmod tool of SAS software (version 8.2; SAS) by use of estimating equations (EE1) with an independent working correlation structure [22]. To test for the sample’s family-size heterogeneity, we performed a naive analysis (i.e., not accounting for within-person and within-family correlations), by use of the family predictive model, on the total sample as well as on 1 subsample of 253 families with <8 members each and on 1 subsample of 154 families with >7 members each. Under the family-size homogeneity hypothesis, twice the difference between the likelihood of the total sample and the summed likelihood of the 2 subsamples is asymptotically distributed as a \( \chi^2 \), with 2 degrees of freedom.

When the above analyses suggested familial aggregation, the pattern of familial dependency was determined. For this purpose, 4 types of familial dependency were studied: father-mother, father-child, mother-child, and sibling-sibling. These dependencies were expressed in terms of odds ratios. To take into account nonindependence between the pairs of relatives within a family, familial odds ratios were estimated by use of EE1 extended to the estimation of correlation parameters (EE2) [23]. The EE2 approach estimates marginal odds ratios (adjusted, if possible, for relevant risk factors) and their standard errors (SEs). These estimations are robust even in the case of misspecification of dependency between the various pairs of relatives within a family. The correlations between marginal odds ratios were modeled by use of a Gaussian structure [23–25] corresponding to that of a multivariate normal distribution. EE2 analysis was performed by use of a binary version of GEESE software [26].

The status of the probands with severe malaria was considered “fixed by design” because, unlike the other study participants, they were prospectively recruited. Thus, (1) probands with CM were excluded from multivariate family-based aggregation analysis as well as from analysis of familial dependencies for CM but were included in the analysis of familial dependencies for SMA; (2) probands with SMA were excluded from multivariate family-based aggregation analysis as well as from analysis of familial dependencies for SMA but were included in the analysis of familial dependencies for CM; and (3) probands with CM and SMA were excluded from multivariate family-based aggregation analysis as well as from analysis of familial dependencies for both CM and SMA.

RESULTS

**Characteristics of the study population.** We collected information on 2811 individuals belonging to 407 nuclear families. The median age of the participants was 11 years (interquartile

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**Table 3. Distribution of history of cerebral malaria (CM) and severe malarial anemia (SMA).**

<table>
<thead>
<tr>
<th>Subject</th>
<th>CM (N = 2707)</th>
<th>SMA (N = 2740)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Mother</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Child</td>
<td>89</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>17</td>
</tr>
</tbody>
</table>

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**Table 4. Odds ratios and 95% confidence intervals (CIs) for parameters of familial aggregation of cerebral malaria (CM) and severe malarial anemia (SMA), in the multivariate family predictive model.**

<table>
<thead>
<tr>
<th>Interpretation of parameters</th>
<th>Adjusted odds ratio(^a) (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-person association of CM and SMA</td>
<td>6.15 (2.62–14.41)</td>
<td>.0001</td>
</tr>
<tr>
<td>Aggregation of CM within families</td>
<td>1.98 (1.59–2.45)</td>
<td>.00001</td>
</tr>
<tr>
<td>Aggregation of SMA within families</td>
<td>1.91 (1.05–3.47)</td>
<td>.03</td>
</tr>
<tr>
<td>Coaggregation of CM and SMA within families</td>
<td>1.22 (0.95–1.57)</td>
<td>.13</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted for age and proband type. Estimating equations were used to adjust for within-family correlations.
range, 4–40 years). Families contained 3–15 members, and there was no significant difference between the number of family members in the severe malaria group and that in the control group ($\chi^2 = 7.44; df = 12; P = .82$). The proband group comprised 136 children with severe malaria and 271 healthy children. At the time of recruitment into the study, of the children with severe malaria, 47.79% had CM, 23.53% had SMA, and 28.68% had both CM and SMA (table 1). The lifetime prevalence of clinical forms of severe malaria in probands' relatives is detailed in table 2, and the distribution of lifetime history of CM and SMA in study participants is detailed in table 3. It is noteworthy that the proportion of deaths recorded in relatives was similar for probands with severe malaria (13.6 [95% confidence interval (CI), 11.3–16.1]) and for healthy probands (13.8 [95% CI, 12.1–15.6]).

**Within-person association between CM and SMA.** The family predictive model revealed a very significant within-person association between CM and SMA (table 4). Over a lifetime, for a person with a history of 1 clinical form of severe malaria, the odds of having the other clinical form of severe malaria were $>6$ times greater than the odds for a person with no history of the other clinical form.

**Familial aggregation of CM and SMA.** The familial aggregation of CM was highly significant (table 4): over a lifetime, for a person who had $k+1$ relatives with a history of CM, the odds of having CM were 1.98 times greater than the odds for a person who had $k$ such relatives. The familial aggregation of SMA was also significant (table 4): over a lifetime, for a person who had $k+1$ relatives with a history of SMA, the odds of having SMA were 1.91 times greater than the odds for a person who had $k$ such relatives. The coaggregation of CM and SMA (table 4) was modest but nonsignificant over a lifetime, for a person who had $k+1$ relatives with a history of CM (or SMA), the odds of having CM (or SMA) were 1.22 times greater than the odds for a person who had $k$ such relatives. Finally, the family-size homogeneity of the sample, was not statistically rejected ($\chi^2 = 7.44$, by the family predictive model; $df = 2; P = .31$), indicating that variation in family size is unlikely to have affected these findings substantially.

**Familial dependencies for CM and SMA.** The results of analysis of familial dependencies for CM are summarized in table 5. By use of the EE2 analysis, the odds ratios were adjusted for age and proband type, with the dependency among pairs being accounted for by 95% CIs calculated from SEs. The father-mother correlations could not be estimated because both parents were affected in only 1 family. Over a lifetime, for a child whose father had a history of CM, the odds of having CM were 2.53 times greater (95% CI, 1.07–5.97) than the odds for a child whose father had no such history. Over a lifetime, for a child whose mother had a history of CM, the odds of having CM were 2.10 times greater (95% CI, 0.82–5.34) than the odds for a child whose mother had no such history, although this difference was not significant ($z = 1.56; P = .12$). Over a lifetime, for a child whose sibling had a history of CM, the odds of having CM were 2.49 times greater (95% CI, 1.51–4.10) than the odds for a child whose sibling had no such history.

A history of SMA was noted for 1 mother, 16 children, and 0 fathers (table 3). Thus, only sibling-sibling dependency for SMA could be estimated, and the EE2 analysis could not be adjusted for covariates. The sibling-sibling correlation was significant over a lifetime, for a child whose sibling had a history of SMA, the odds of having SMA were 4.92 times greater (95% CI, 1.21–19.92; $z = 2.23; P < .03$) than the odds for a child whose sibling had no such history.

**DISCUSSION**

This first study of the familial aggregation of severe malaria has several strengths: (1) it is population based; (2) its study population is large; (3) its design incorporates the fact that family members tend to share certain environmental and cultural factors; and (4) it uses multiple and rigorous analytical methods to account for intrafamilial phenotypic correlation, for differences in family size, and for several risk factors that often conceal the genetic component of severe malaria. This study supports the hypothesis of a significant familial aggregation for both CM and SMA. The odds ratios generated by the family predictive model measure the odds that a family
member will contract a disease if a given number of relatives have that disease. If aggregation of disease exists within families, then the odds ratios reflect a reduced effect of that aggregation, by conditioning on all but 1 relative [21]. In our sample, variation in family size is unlikely to substantially affect the finding of the family predictive model because the family-size homogeneity of the sample was not statistically rejected. This finding contrasts with those of Laird et al. [27], but is in keeping with those of other researchers [28, 29]. One important assumption of the family predictive model is that family members are interchangeable in the model. In our study, interchangeability is plausible, but a proband with a disease might have had a form of illness different than that in a relative. It was therefore reassuring that the EE2 analysis, as well as an analysis based on a proband predictive model [21] (data not shown) that did not assume interchangeability of probands and relatives, produced similar results.

Results of epidemiological studies of familial aggregation require careful interpretation, and we must introduce some words of caution here. In the present study, the diagnosis of CM and SMA in relatives was retrospective and could rarely be verified through medical sources or health records; thus, there was a potential risk of recall bias and misclassification of disease by parents, even though information on family medical history was collected by standardized procedures and, when they were available, from multiple informants. The interviewers were not blinded to the health status of the proband, which could possibly affect the retrospective diagnosis of CM or SMA in a relative. This drawback has been taken into account by adjustment of the analysis according to the proband’s health status.

SMA is more likely than CM to be misdiagnosed, because its clinical picture is less straightforward than that of CM. One of the biggest problems in the present study is that the retrospective diagnosis of SMA relied on a history of blood transfusion, meaning that it was not possible to diagnose SMA in children who died either before or immediately after being hospitalized and therefore did not receive blood transfusions. This inaccuracy in the retrospective diagnosis of SMA is the most likely explanation for the low number of cases of SMA diagnosed among the probands’ relatives. Consequently, the present study lacked the power to detect the coaggregation of CM and SMA within families and to investigate parent-child dependency for SMA. Conversely, children with febrile disease and severe neurological symptoms might have been spuriously diagnosed as having CM. We can only speculate about the presence and extent of such misdiagnoses. It is possible that the parents of a child who met the stringent criteria for inclusion in our research-oriented survey of severe malaria were more likely to misclassify severe malaria as being mild malaria than the inverse. To allow for this, analyses were adjusted according to the proband’s health status. Recall bias is probably more prominent for disease in parents than for disease in children, because severe malaria usually occurs in young children. In the present study, severe malaria was probably underdiagnosed in the parents, which means that the overall extent of parent-child dependency was probably underestimated.

Data from the family-based studies and from studies of diseases that involve hemoglobins, together with the growing body of evidence that polymorphisms in several genes may be associated with either CM or SMA, argue against the possibility that our finding are primarily the consequence of environmental rather than genetic factors [9–13]. Furthermore, both the frequency of exposure and the relative risks of the environmental factors associated with severe malaria have been considered in one of our earlier studies of this population [20], and they suggest that simple familial clustering of environmental factors is unlikely to account substantially for this familial aggregation [30].

Although misdiagnosis and recall bias cannot be excluded, our data suggest that there is strong familial aggregation of severe malaria. This information should encourage researchers to use a family-based approach to continue their investigations of the genetic susceptibility to severe malaria and to elucidate the biomedical, social, and environmental pathways that contribute to its familial aggregation. Identification of these pathways may elucidate the causes of severe malaria and lead to the development of better strategies for its treatment and prevention.

Acknowledgments

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References