The Association of Genetic Markers and Malaria Infection in the Brazilian Western Amazonian Region


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Almost all individuals (182) belonging to an Amazonian riverine population (Portuchuelo, RO, Brazil) were investigated for ascertaining data on epidemiological aspects of malaria. Thirteen genetic blood polymorphisms were investigated (ABO, MNS, Rh, Kell, and Duffy systems, haptoglobins, hemoglobins, and the enzymes glucose-6-phosphate dehydrogenase, glyoxalase, phosphoglucomutase, carbonic anhydrase, red cell acid phosphatase, and esterase D). The results indicated that the Duffy system is associated with susceptibility to malaria, as observed in other endemic areas. Moreover, suggestions also arose indicating that the EsD and Rh loci may be significantly associated with resistance to malaria. If statistical type II errors and sample stratification could be ruled out, hypotheses on the existence of a causal mechanism or an unknown closely linked locus involved in susceptibility to malaria infection may explain the present findings.

Key words: malaria - genetic markers - association studies - Western Amazonia - Rondônia - Brazil

Malaria, an infectious disease caused by an intra-cellular erythrocyte parasite (Plasmodium sp.), has significant associations with several red cell polymorphisms (hemoglobin, glucose-6-phosphate dehydrogenase, ABO and Duffy systems) detected in populations who are or were living under hyper-endemic conditions in the Old World (Allison 1954a,b, Vandepitte & Delaisse 1957, Motulsky 1960, 1964, Siniscalco et al. 1961, Miller et al. 1976, Santos et al. 1983, Udomsangpetch et al. 1989, 1993, Carlson & Wahlgren 1992, Barragan et al. 2000). The Amazon region, characterized by an hypo-endemic pattern of infection, due mainly to its low demographic index, provides an excellent field to test hypotheses on the generalization of these associations, as well as to investigate the existence of other associations due to either different mechanisms or to linkage disequilibria between genetic markers and genes involved with susceptibility/resistance to Plasmodium infection.

The present study is part of a large scientific project aimed to investigate the biological characteristics of some infectious diseases in the Western Amazon region of Brazil (Camargo et al. 1994, 1996, 1999) in which the association of some blood polymorphisms with two traits involved with the malaria infection is tested. Exception made to haptoglobin, which has an important role as hemoglobin carrier, the investigated genetic markers are red blood cell polymorphisms, since erythrocytes are the ultimate target cells of the malarial parasite.

MATERIALS AND METHODS

A blood sample was collected from each of 182 individuals, practically the whole population living in Portuchuelo, a riverine settlement in the state of Rondônia, Brazil (Figure), right bank of the Madeira river (8°37'S, 63°49'W) that can be reached by boat throughout the year, and also by road during the dry season (from May to September). All individuals were clinically examined, malaria being diagnosed by light microscopy and by nested polymerase chain reaction (PCR) assay. The clinical examination included a detailed anamnesis with emphasis on past exposure to malaria. The same information was given by the parents of children unable to answer satisfactorily. This research was approved by the “Medical Board of the State of Rondônia, Brazil”, and an informed consent was obtained from all adults, as well as from the parents or legal guardians of minors who participated in the present study.

The community of Portuchuelo is composed by descendants of the 19th and beginning of the 20th century European settlers, with a heavy admixture with the Amerindians who lived in this part of the Madeira region. During the “rubber boom” and the construction of the Madeira-Mamoré railroad, an African contribution to the gene pool of Portuchuelo’s population was brought by both the Northeastern Brazilian and Caribbean workers, particularly from Granada and Barbados, who migrated to this area. Now, this tri-hybrid population seems to be stable, and the contribution of each of its components is estimated as Amerindians = 0.44 ± 0.64; Caucasoids = 0.35 ± 0.069; Africans = 0.21 ± 0.046 (Ferreira et al. 2002).
Infections were studied: a) the presence of symptomless infections in agricultural activities. While the mothers are dedicated to both domestic and subsistence agriculture, (Beiguelman et al. 1995). That is usually found in other Brazilian populations elsewhere (Camargo et al. 1999, Alves et al. 2002).

The mean age of this community was 24.9 years. Since this population was represented by nuclear families the very large standard deviation observed (19.75 years) is understandable. As expected, when parents and offspring were analyzed separately, the standard deviations narrowed, notwithstanding continuing broad. Thus, the mean age of the parental generation was 47.6 ± 16.0 for males and 41.5 ± 15.0 for females, whereas for the offspring generation it was 15.5 ± 13.6 for males and 13.4 ± 12.0 for females. The sex ratio of the population was 1.04, a value that is usually found in other Brazilian populations (Beiguelman et al. 1995).

The main occupation of the great majority of fathers is concerned with both fishing and subsistence agriculture, while the mothers are dedicated to both domestic and agricultural activities.

Two main phenotypes involved with Plasmodium infection were studied: a) the presence of symptomless infection, i.e., presence of Plasmodium vivax or Plasmodium falciparum in blood, diagnosed by the traditional thick smear and/or by PCR amplification of Plasmodium ribosomal DNA, but without malaria symptoms during a 60 days follow-up after diagnosis, regardless of the onset of the infection; b) the reported number of previous malaria episodes for life.

During the first year of this study 45 patients presented symptomatic infection. All of them were treated according to the National Health Foundation standard protocols. The annual parasite index (API) in this settlement was 385 per 1,000 inhabitants in 1999, a much higher value than the API estimated for the whole state of Rondônia in the same year (49.6 per 1,000 inhabitants).

Table I shows the results of the independence chi-square ($\chi^2$) tests between the ABO, MNSs, Rh, Kell, Duffy, Hb, Hp, G-6-PD, GLO, PGM1, ACP1, and EsD systems and the symptomless infection with P. vivax, P. falciparum, and the pooled infection, as well as results of the Kruskal-Wallis independence test (H) for investigating the association between the same genetic systems and the number of malarial episodes.

In this Table it seems clear that when the independence tests were applied to the distribution of the ABO blood groups no significant associations could be detected. In contrast, a significant association between individuals with A or/and B antigens and the number of malaria episodes was observed.

In the population of Portuchuelo eight Rh phenotypes have been recognized with five Rh anti-sera (CCDee, CCDeE, CcDee, ccDeE, ccDeE, and ccddde). No significant associations could be observed either when all these phenotypes were taken into account or when the reactions with anti-D or with anti-C and anti-E sera were analyzed separately. However, when the EE, Ee, and ee phenotypes were considered, a tendency for association with the number of malarial episodes was observed. This prompted us to retest this association by analyzing only two phenotypic classes (E_ and ee phenotypes). This time a significant association could be observed ($H = 4.499; 1 \text{ d.f.}; P = 0.034$), ee phenotype individuals exhibiting a higher number of malaria episodes than phenotype E_ persons.

Similarly, no significant association was observed when the phenotypes Fy(a+b-), Fy(a+b+), Fy(a-b+), and Fy(a-b-) of the Duffy system were tested. However, when these phenotypes except Fy(a-b-) were pooled together for the independence tests, a significant association with the
number of malarial episodes emerged. In Table I it is also seen that the number of malarial episodes was significantly associated with the esterase D system, this association being due to a lesser number of these episodes manifested by the heterozygous phenotype (1-2) for EsD. Concerning the independence tests between the different genetic polymorphisms and the symptomless infections, only G-6PD system among females has shown a significant association with symptomless *P. vivax* infection.

As expected, age was significantly associated with the number of malarial episodes (Table II), the lower age groups exhibiting lower mean ranks of the number of malarial episodes than the higher age groups (H = 31.418; D.F. = 9; P < 0.001). Since the observed associations with the number of malarial episodes could be an effect of age, the mean rank of ages of the sample grouped as: either O or with A or/and B antigens; E_ and ee phenotypes; Fy(a-b-) and non-Fy(a-b-) groups; as well as EsD phenotypes (1-1, 1-2, 2-2) were compared by applying a Kruskal-Wallis test to all cases. The results obtained enabled to reject the hypothesis that age could have influenced the observed association between the number of malarial episodes with E_ and ee phenotypes (H = 0.299; D.F. = 1; P = 0.585), the Duffy system (H = 3.362; D.F. = 1; P = 0.067) or with EsD (H = 0.926; D.F. = 2; P = 0.629). The same was not true for the ABO system, since the mean rank of age of the group O subjects was significantly lower than that of individuals with A or/and B antigens (H = 8.001; D.F. = 1; P = 0.005). Curiously, this phenomenon was confirmed in the offspring generation (H = 5.657; D.F. = 1; P < 0.017) but not among the parents (H = 0.446; D.F. = 1; P = 0.504).

Taking into account these results, the influence of several independent variables [age, squared age, sex (coded as zero for females and 1 for males), age x sex, squared age x sex, and ABO system grouped as O (coded as zero) and with A or/and B antigens (coded as 1)] on the

### TABLE I

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Degrees of freedom</th>
<th>Symptomless infection</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Plasmodium vivax</em></td>
<td>P</td>
<td><em>P. falciparum</em></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>χ²; P</td>
<td>χ²; P</td>
<td>χ²; P</td>
<td>H ; P</td>
<td></td>
</tr>
<tr>
<td>ABO</td>
<td>3</td>
<td>0.352; 0.996</td>
<td>0.400; 0.940</td>
<td>0.523; 0.914</td>
<td>4.589; 0.204</td>
</tr>
<tr>
<td>A+B+AB</td>
<td>1</td>
<td>0.003; 0.953</td>
<td>0.003; 0.953</td>
<td>0.044; 0.833</td>
<td>4.054; 0.044</td>
</tr>
<tr>
<td>B + AB</td>
<td>1</td>
<td>0.001; 0.980</td>
<td>0.261; 0.609</td>
<td>0.065; 0.800</td>
<td>2.933; 0.087</td>
</tr>
<tr>
<td>MNss</td>
<td>8</td>
<td>8.583; 0.379</td>
<td>5.813; 0.668</td>
<td>5.048; 0.752</td>
<td>5.684; 0.683</td>
</tr>
<tr>
<td>MM, MN, NN</td>
<td>2</td>
<td>0.655; 0.721</td>
<td>0.012; 0.994</td>
<td>0.348; 0.840</td>
<td>1.036; 0.596</td>
</tr>
<tr>
<td>SS, Ss, ss</td>
<td>2</td>
<td>1.248; 0.536</td>
<td>4.233; 0.120</td>
<td>1.187; 0.552</td>
<td>2.836; 0.242</td>
</tr>
<tr>
<td>Rh</td>
<td>7</td>
<td>5.616; 0.585</td>
<td>8.943; 0.257</td>
<td>3.151; 0.871</td>
<td>7.273; 0.401</td>
</tr>
<tr>
<td>CC, Cc, cc</td>
<td>2</td>
<td>0.719; 0.698</td>
<td>1.050; 0.592</td>
<td>0.216; 0.698</td>
<td>1.843; 0.398</td>
</tr>
<tr>
<td>D_, dd</td>
<td>1</td>
<td>1.341; 0.247</td>
<td>0.355; 0.551</td>
<td>0.310; 0.578</td>
<td>0.472; 0.492</td>
</tr>
<tr>
<td>EE, Ee, ee</td>
<td>2</td>
<td>1.625; 0.444</td>
<td>0.988; 0.610</td>
<td>1.953; 0.377</td>
<td>5.713; 0.057</td>
</tr>
<tr>
<td>Kell</td>
<td>1</td>
<td>1.518; 0.218</td>
<td>0.219; 0.640</td>
<td>0.488; 0.485</td>
<td>0.866; 0.347</td>
</tr>
<tr>
<td>Duffy</td>
<td>1</td>
<td>1.255; 0.740</td>
<td>0.800; 0.849</td>
<td>1.719; 0.633</td>
<td>5.043; 0.169</td>
</tr>
<tr>
<td>Fy(a-b-)</td>
<td>1</td>
<td>0.001; 0.970</td>
<td>0.551; 0.456</td>
<td>0.315; 0.575</td>
<td>4.632; 0.031</td>
</tr>
<tr>
<td>Hp</td>
<td>3</td>
<td>2.120; 0.548</td>
<td>2.141; 0.544</td>
<td>0.365; 0.947</td>
<td>1.104; 0.776</td>
</tr>
<tr>
<td>Hp1-1</td>
<td>1</td>
<td>0.726; 0.394</td>
<td>1.526; 0.217</td>
<td>0.090; 0.764</td>
<td>0.499; 0.480</td>
</tr>
<tr>
<td>Hb</td>
<td>1</td>
<td>0.040; 0.841</td>
<td>0.483; 0.487</td>
<td>0.052; 0.820</td>
<td>0.202; 0.653</td>
</tr>
<tr>
<td>G-6-PD, Males</td>
<td>2</td>
<td>4.195; 0.123</td>
<td>1.029; 0.598</td>
<td>1.240; 0.538</td>
<td>1.182; 0.554</td>
</tr>
<tr>
<td>G-6-PD, Females</td>
<td>1</td>
<td>4.353; 0.037</td>
<td>0.064; 0.801</td>
<td>2.721; 0.099</td>
<td>0.323; 0.570</td>
</tr>
<tr>
<td>GLC</td>
<td>2</td>
<td>4.263; 0.119</td>
<td>2.018; 0.365</td>
<td>3.530; 0.171</td>
<td>0.825; 0.662</td>
</tr>
<tr>
<td>PGM1</td>
<td>2</td>
<td>3.944; 0.139</td>
<td>0.029; 0.985</td>
<td>4.002; 0.135</td>
<td>0.473; 0.790</td>
</tr>
<tr>
<td>ACP1</td>
<td>4</td>
<td>4.849; 0.303</td>
<td>7.123; 0.129</td>
<td>8.617; 0.071</td>
<td>4.342; 0.362</td>
</tr>
<tr>
<td>EsD</td>
<td>2</td>
<td>0.660; 0.719</td>
<td>4.240; 0.120</td>
<td>0.784; 0.676</td>
<td>6.840; 0.033</td>
</tr>
</tbody>
</table>

| χ²: results of the independence chi-square test; H: results of the independence Kruskal-Wallis test; P: probability |
logarithm of the number of malarial episodes (dependent variable) were analyzed by stepwise multiple regression. This analysis enabled to see that, exception made to the product of squared age × sex, all other variables have not influenced significantly the number of malarial episodes. Since the females were coded as zero and the partial regression coefficient of squared age × sex on the dependent variable was positive (3.702 with a standard error of 0.834; t = 4.437; P < 0.001), this result also indicates that the influence of age is restricted to the males.

**DISCUSSION**

It is known that parasitized erythrocytes form rosettes more readily with red blood cells of either A, B or AB groups than with those belonging to blood group O (Udomsangpetch et al. 1989, 1993, Carlson & Wahlgren 1992, Barragan et al. 2000), and that, in Zimbabwe, blood group A was associated with both lower hemoglobin levels and severe central nervous system malaria with coma (Fisher & Boone 1998). In spite of the fact that these phenomena might provoke an association of ABO blood groups and the number of malarial episodes, the results presented above do not enable to reject the null hypothesis that this polymorphic system is not associated with the number of malarial episodes. The absence of association between ABO system and malaria infection was also observed in other populations (Osisanya 1983, Singh et al. 1986, 1995, Bayoumi et al. 1986, Montoya et al. 1994) but Santos et al. (1983) detected in Brazil a significant association between the presence of B antigen and the number of malarial episodes. Although our results pointed to the same direction, we were not able to confirm this observation by combining the frequency of B and AB individuals. However, it should be mentioned that, agreeing with Santos et al. (1983) data, performing a preliminary analysis on another sample (Camargo et al., in preparation) we were able to detect an association between the number of malarial episodes and the presence of B antigen among 873 individuals living in Monte Negro, a rural area of the same State of Rondônia (10°15′S, 63°18′W), in New Guinea, homozygotes for Hp gene are more likely to show signs of hepatomegaly and splenomegaly than Hp, heterozygotes. Santos et al. (1983) and Singh et al. (1986) found a significant excess of individuals with the Hp1-1 phenotype among malaria patients in Brazil and in India, respectively. In our data haptoglobin did not show significant association with the phenotypes involved in *Plasmodium* infection neither when all detected phenotypes (Hp1-1, Hp1-2, Hp 2-2, Hp1-2M) were considered nor when all of them except Hp1-1 were pooled together. Supporting the present results, no association between haptoglobin phenotypes and *P. falciparum* infection was also observed by Bayoumi et al. (1986) in Sudan.

Since long it is known that sickle cell trait affords protection against *P. falciparum* infection (Allison 1954a,b, Vanderpitté & Delaisse 1957). In our data no significant association between AS genotype and the phenotypes involved in *Plasmodium* infection could be detected due to both a relatively low proportion of *P. falciparum* infection (30%) among the malarial cases and a small frequency of AS individuals in Portuchuelo (3%).

The relative protection of G-6-PD deficiency against *P. falciparum* postulated by Motulsky (1960) is manifested by the heterozygous females but not by the males who are hemizygous for the deficient gene (Bienzle et al. 1972, Usanga & Luzzato 1985). Therefore, the absence of association between G-6-PD among males and the phenotypes among the Negroids studied by Montoya et al. (1994) was conspicuously high (7.8%). Concerning the Rh system, it is curious that in another population of the state of Rondônia, living in Monte Negro (Camargo et al., in preparation), it was observed that the E_ phenotype individuals exhibited a higher number of malarial episodes than ee phenotype persons, the Kruskal-Wallis test resulting in a highly significant value (H = 12.324 ; 1 d.f.; P = 0). Since this result is completely opposite to that observed in the present population, the association of the number of malarial episodes with E_ or ee phenotypes might be taken as fortuitous, although it is attractive to speculate whether the Rh locus might be closely linked to a gene involved in susceptibility to malaria infection, being the observed significant deviations due to different phases of linkage disequilibrium.
involved in *Plasmodium* infection seen in Table I, as well as the significant association of the females symptomless for *P. vivax* with homozygosis for the gene *GdB* has no practical meaning.

The distribution of GLO phenotypes (1-1, 1-2, and 2-2) was independent from the phenotypes involved with *Plasmodium* infection, a result that agrees with that observed by Bayoumi et al. (1986) in Sudan. Regarding PGM1 system, these authors found a significant excess of the 1-1 phenotype among malaria patients, but such association could not be demonstrated in Portuchuelo. No statistical tests were performed concerning C2A, since all individuals except one (phenotype 1-2) exhibited phenotype 1-1.

Bottini et al. (1972) have suggested a possible disadvantage of the allele ACP1*C in areas characterized in the past by high malaria endemicity (Mediterranean area), while other authors indicated that the ACP1*R allele could have this disadvantage in Africa (Spedini et al. 1980). In Portuchuelo, as in African populations where malaria is endemic (Destro-Bisol et al. 1992), the ACP1*R allele was not found while the five ACP1 observed phenotypes (A, AB, AC, B, and BC) showed no association with the phenotypes involved with *Plasmodium* infection.

A result that should be confirmed in other populations, since it is here referred for the first time, is the observed significant association between the number of malarial episodes and EsD. The caution to associate a particular genetic system with a disease stems from the fact that statistic type II error, sample non-randomness, and stratification are common factors that mimic genetic associations. Also it should be stressed that the authors could not find an easy explanation for the correlation of the number of malarial episodes with age being restricted to males.

Finally, it should be emphasized that the authors are aware on the difficulty that people living in an endemic area have to remind the exact number of malarial episodes. The caution to associate a particular genetic system with a disease stems from the fact that statistic type II error, sample non-randomness, and stratification are common factors that mimic genetic associations. Also it should be stressed that the authors could not find an easy explanation for the correlation of the number of malarial episodes with age being restricted to males.

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Mäkelä O, Cantell K 1958. Destruction of M and N blood cell types involved in *Plasmodium vivax* infection seen in Table I, as well as the significant association of the females symptomless for *P. vivax* with homozygosis for the gene *GdB* has no practical meaning.


