Human Malaria: Segregation Analysis of Blood Infection Levels in a Suburban Area and a Rural Area in Burkina Faso

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The genetic control of blood infection levels in human malaria remains unclear. Case control studies have not demonstrated a strong association between candidate genes and blood parasite densities as opposed to surveys that have focused on severe malaria. As an alternative approach, we used segregation analyses to determine the genetic control of blood parasitemia. We surveyed 509 residents (53 pedigrees) in a rural area and 389 residents (41 pedigrees) in an urban area during 18 months. Each family was visited 20 times and 28 times in the urban area and in the rural area; the mean number of parasitemia measurements per subject was 12.1 in the town and 14.9 in the village. The intensity of transmission of Plasmodium falciparum was 8-fold higher in the rural area than in the urban area. Using the class D regressive model for both populations, we found that blood parasite densities were correlated between sibs. We obtained strong evidence for a major effect, but we found that the transmission of this major effect was not compatible with a simple Mendelian model, suggesting a more complex mode of inheritance. Moreover, there was a strong interaction between major effect and age, suggesting that the influence of the putative major gene may be more prominent in children than in adults. Further nonparametric linkage studies, such as sib pair analysis, that focus on children would help us better understand the genetic control of blood infection levels. Genet. Epidemiol. 15:435–450, 1998. © 1998 Wiley-Liss, Inc.
Key words: Plasmodium falciparum; blood parasitemia; complex segregation analysis; Africa

INTRODUCTION

Plasmodium falciparum malaria remains a major cause of morbidity and mortality in many developing countries. Drug-resistant parasite strains are spreading rapidly across the world, and a malaria vaccine is a distant prospect [Nosten and van Vugt, 1996; Nosten et al., 1996]. Vaccine development faces major difficulties in part because of the genetic control of immunity to the parasite [Stevenson et al., 1982; Stevenson and Skamene, 1985; Weiss et al., 1989; Tian et al., 1996]. In particular, HLA class II-associated nonresponsiveness has been reported in humans vaccinated with Spf66 [Pattaroyo et al., 1991]. Furthermore, individuals naturally exposed to malaria can remain persistently seronegative to highly conserved antigens, despite years of repeated reinfections [Riley et al., 1994]. Also, during natural infection, antibody responses to a variety of antigens from P. falciparum have been shown to be more concordant within monozygous pairs than within dizygous pairs [Sjöberg et al., 1992; Jepson et al., 1997], indicating that specific immune responses are genetically controlled. The development of efficient control measures calls for a better knowledge of human genes controlling the infection and the disease.

Case control studies have been carried out to investigate the role of candidate genes in clinical malaria. Several genes have been associated with resistance to severe malaria; these are essentially genes encoding red blood cell proteins [Nagel and Roth, 1989; Ruwende et al., 1995] and MHC genes [Hill et al., 1991; McGuire et al., 1994]. Conversely, no similar associations have been described for mild malaria disease or for parasite densities [Hill et al., 1991; Bennett et al., 1993], although malaria attacks may be regulated by genetic mechanisms [Jepson et al., 1995].

Another approach to analyze host genetic susceptibility to malaria infection is complex segregation analysis. Abel et al. [1992] reported on a recessive major gene controlling parasite densities in families from Cameroon. Such studies should be extended to endemic areas that may differ in parasite strains, host genetic background, or environmental factors. The intensities of transmission are highly variable in Africa [Gazin et al., 1987; Trape and Zoulani, 1987], and thus the genetic control of blood parasitemia in high and low transmission areas may differ.

The aim of the present study was to establish whether a major gene controls malaria parasite densities by investigating families from two differently exposed areas in Burkina Faso. The study was done on 41 pedigrees from an urban community and on 53 pedigrees from a rural community by a regressive model [Bonney, 1984]. We report on the results of the complex segregation analysis.

MATERIAL AND METHODS

Study Area and Population

The study was carried out from April 1994 to December 1995 in an urban district of Bobo-Dioulasso and in Logoforouso, a village southwest of Bobo-Dioulasso (Fig. 1).

The ascertainment scheme was a complete selection of the persons living within a district of Bobo-Dioulasso (suburban area); in the village (rural area), families were first randomly selected from 3,500 inhabitants. Informed consent was then obtained individu-
ally from all participants or their parents. Finally, 898 subjects of different ages (from newborns to adults; see Table I) were included in the study. The families comprised 41 pedigrees in the suburban area and 53 pedigrees in the rural area.

In the suburban area, most of the population belongs to the Mossi ethnic group (50%); the other groups included the Dafing (19%), Guian (5%), Bissa (15%), Samogo (1%), Bobo (6%), and Nounouma (4%). The Mossi and the Bissa are originally from the center of Burkina-Faso, and the others are from the western part of the country. Although each ethnic group has its own language, they usually speak Dioula. The families have been established there for more than 20 years, and their habits are almost the same. Professional situations are various (teacher, artisan, worker, etc.). All the people living in the rural area belong to the Bobo ethnic group. They usually speak Bobo, but they can speak Dioula when necessary. All are farmers.

**Entomological Parameters**

Three and four capture sites were chosen in the suburban area and in the rural area, respectively. In the suburban area, mosquitoes were collected outdoors over 2 days every 2 weeks from March 1994 to December 1995. In the rural area, mosqui-

**TABLE I. Age Distribution in the Two Populations***

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<th>10–14</th>
<th>15–19</th>
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<th>30–39</th>
<th>40–49</th>
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<td>64 (16.4)</td>
<td>64 (16.4)</td>
<td>60 (15.4)</td>
<td>45 (11.6)</td>
<td>44 (11.3)</td>
<td>43 (11.1)</td>
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<tr>
<td>Logoforousso</td>
<td>93 (18.2)</td>
<td>115 (23.0)</td>
<td>78 (15.2)</td>
<td>41 (8.0)</td>
<td>50 (9.8)</td>
<td>49 (9.6)</td>
<td>55 (10.7)</td>
<td>28 (5.5)</td>
</tr>
</tbody>
</table>

*Age recorded in 1995 and expressed in years. Number of subjects (percentage) in each age class.
toes were collected outdoors 4 days each month (during two nights every 2 weeks) from July 1994 to December 1995. During the 2 years, 264 and 288 night/biting/men collections were performed in the suburban and rural areas, respectively. *Plasmodium falciparum* circumsporozoite proteins were detected by ELISA [Burkot et al., 1984], and the number of infected bites per person per year was determined. The malariometric indices show seasonal variations in both areas; the transmission occurred essentially during the rainy season (August to December). In the suburban area, infected mosquitoes were detected only during August, September, and October, and the inoculation rates ranged from 5 to 22 infected bites per person per month. In the rural area, the inoculation rates were less than 2 infected bites per person per month from January to June and ranged from 25 to 75 infected bites per person per month from July to December.

The numbers of infected bites per person per year calculated in the three suburban capture sites were similar, and only slight differences were recorded among the four rural capture sites (Traoré and Coosemans, unpublished data). In contrast, the numbers of infective bites per person per year were less than 30 in the suburban area and more than 230 in the village, showing that the transmission intensities differed greatly in the two areas.

**Parasitological Examinations, Blood Groups, and Hemoglobin Genotypes**

During the 18 months of the study, each family in the suburban area was visited 20 times and those in the rural area were visited 28 times. Blood samples were taken from all individuals present. The mean number of samples per subject was 12.8 ± 5.1 (range = 1–20) in the suburban area and 14.7 ± 8.0 (range = 1–28) in the rural area.

Thin blood films prepared in the field were stained with Giemsa (Fluka, France). Using microscopy, two observers counted the parasitized erythrocytes on 100 fields of thin films, corresponding to almost 0.005 µl. The parasite density (PD) was defined as the number of parasitized erythrocytes in that volume. More than 95% of parasites identified on thin blood smears were *P. falciparum*; the others were *P. malariae*. Only the *P. falciparum* asexual forms served to determine parasitemia. To allow for zero counts, we applied a logarithmic transformation based on Ln(1 + PD) (LPD) to the parasite densities.

In 150 slides that were selected randomly, parasitemia was also evaluated by counting the infected erythrocytes in thick films fields containing 800 leukocytes, and parasite densities were calculated by assuming a leukocyte count of 8,000/µl [Trape, 1985]. Comparison of the techniques showed a strong correlation (r = 0.85; P < 10⁻⁴).

The hemoglobin genotypes were identified by electrophoresis on acetate sheets. The blood groups ABO and Rh were determined by agglutination with two different sets of antibodies. The analyses were performed in samples taken from venous blood donors aged 1–80 years who resided in the suburban area (n = 281) and in the rural area (n = 249). The median ages of the donors were 15 years in the rural area and 16 years in the suburban area. The median ages of the Mossi, the Dafing, and the Bissa donors (the most represented ethnic groups of the suburban population) were 16, 15, and 19 years, respectively. The hemoglobin genotypes AA, AS, AC, SC, and CC were identified, and the most frequent genotype was AA (see Table II). In the rural area, the frequencies of AS and AC genotypes were 4.8% and 28.5%, respectively.
In the suburban area, the frequencies of AS by ethnic group were 12.2% (Mossi), 3.7% (Dafing), and 2.2% (Bissa); and the frequencies of AC by ethnic group were 21.4% (Mossi), 35.2% (Dafing), and 17.8% (Bissa).

**Data Adjustment and Phenotype of Interest**

Malaria seasonally transmitted in the suburban area and in nearby villages has been well documented [Gazin et al., 1987, 1988]. To take into account the seasonality of the transmission, the influence of the date of the visits on LPD was evaluated by one-way analysis of variance. The mean LPD observed during each visit was calculated. The individual LPD was then corrected for the visit effect by subtracting from each individual LPD the mean LPD of the corresponding visit. The mean of adjusted parasite densities (MAPD) was calculated for each subject. Subjects who were present less than two times made up 8% of both study populations and were classified as unknown phenotype.

**Risk Factors Influencing the MAPD**

Before segregation analysis, the influence of the covariates on MAPD phenotypes was assessed by analysis of variance for categorical variables and by polynomial regression for age. The factors recorded were sex, area of exposure (three and four subdistricts were defined on the basis of the mosquito capture sites in the suburban area and in the rural area, respectively), ethnic group in the suburban area classified as four major groups (Mossi, Dafing, Bissa, and others), and age expressed in years and considered to be a quantitative variable. Covariates with a significant effect on MAPD were retained for segregation analysis of this phenotype. We also evaluated the influence of blood group, ABO and Rh, and hemoglobin genotype. All calculations were done with the SAS software (SAS Institute, Cary, NC).

**Familial Correlation and Segregation Analysis**

Segregation analyses were done with the regressive model for continuous traits developed by Bonney [1984]. These models are constructed by specifying a regression relation between the phenotype of an individual (i.e., MAPD) and a major gene effect, the phenotype of the preceding relatives, and covariates. Under the hypothesis of one major gene, the major effect results from the segregation of two alleles (A, B) at a single locus. The parameters of the major gene are the frequency, \( q \), of B, which represents the allele predisposing to high values of MAPD and the means \( \mu_{AA} \), \( \mu_{AB} \), and \( \mu_{BB} \) corresponding to the genotypes AA, AB, and BB. For a given genotype, the phenotype distribution is assumed to be normal, with residual variance \( \sigma^2 \); in some analyses, the residual variance of the parents’ phenotype was assumed to differ.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>AA</th>
<th>AS</th>
<th>AC</th>
<th>SC</th>
<th>CC</th>
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<tr>
<td>Suburban</td>
<td>–0.03 ± 0.02</td>
<td>–0.07 ± 0.06</td>
<td>0.06 ± 0.05</td>
<td>–0.26 ± 0.12</td>
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<td></td>
<td>193</td>
<td>22</td>
<td>62</td>
<td>0</td>
<td>4</td>
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<tr>
<td>Rural</td>
<td>–0.22 ± 0.06</td>
<td>–0.27 ± 0.13</td>
<td>–0.20 ± 0.08</td>
<td>–0.45 ± 0.22</td>
<td>–0.51 ± 0.18</td>
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<td></td>
<td>156</td>
<td>12</td>
<td>71</td>
<td>3</td>
<td>7</td>
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</table>

*The mean of MAPD and the SEM are shown.
from the residual variance of the offspring’s phenotype. Parent–offspring transmission at the major locus was parametrized in terms of $\tau_{AAA}$, $\tau_{ABA}$, and $\tau_{BBA}$, which denote the probability of transmitting A for AA, AB, and BB individuals, respectively. Mendelian transmission corresponds to $\tau_{AAA} = 1$, $\tau_{ABA} = 0.5$, and $\tau_{BBA} = 0$; no parent–offspring transmission of the major effect is represented by $\tau_{AAA} = \tau_{ABA} = \tau_{BBA}$. General transmission corresponds to free $\tau$ parameters between 0 and 1. Besides the major effect, one may consider different patterns of dependence between an individual and preceding relatives in terms of phenotypic correlations. In the class D model [Bonney, 1984] used, these correlations were parametrized in terms of $\rho_{FM}$ (the father–mother correlation), $\rho_{FO}$ (the father–offspring correlation), $\rho_{MO}$ (the mother–offspring correlation), and $\rho_{SS}$ (the sib–sib correlation). No significant differences between $\rho_{FO}$ and $\rho_{MO}$ were found; hence, the parameters were fixed as $\rho_{FO} = \rho_{MO} = \rho_{PO}$ for a global parent–offspring correlation. The class D regressive model has been shown to include several patterns of dependence as particular cases, for example, the pure polygenic model when $\rho_{FM} = 0$, $\rho_{FO} = \rho_{MO} = \rho_{SS}$ [Demenais and Bonney, 1989]. The effects of covariates were parametrized in terms of regression coefficients that can be genotype dependent. Age was introduced into this model as a covariate, so that the parameters of the linear or quadratic regression in age were estimated simultaneously with the genetic and familial correlation parameters. Furthermore, an interaction between genotypes and age was taken into account. The genotype-specific regression coefficients were calculated; they were denoted as $\beta_{AA}$, $\beta_{AB}$, and $\beta_{BB}$ for age, $\gamma_{AA}$, $\gamma_{AB}$, and $\gamma_{BB}$ for age$^2$. Under the hypothesis of no interaction, the regression coefficients are not genotype dependent ($\beta_{AA} = \beta_{AB} = \beta_{BB}$ and $\gamma_{AA} = \gamma_{AB} = \gamma_{BB}$).

The likelihood of the hypotheses was calculated with the REGRESS program [Demenais and Lathrop, 1994], which incorporates the regressive approach into the LINKAGE package [Lathrop et al., 1984], as proposed by Bonney et al. [1988]. Calculations were done under different models by using the approximation proposed by Demenais et al. [1990]. Nested models were compared through the likelihood ratio criterion. Evidence for a major gene is obtained by rejecting the null hypothesis of familial correlations without a major gene against a model including a major gene and residual familial correlations. Two additional criteria concerning the parent–offspring transmission of the major effect are required to confirm the presence of a major gene: (1) failure to reject mendelian transmission and (2) rejection of the no parent–offspring transmission model when compared with the general transmission model.

**RESULTS**

**Presentation of the Raw Data and Calculation of MAPD**

Figure 2 shows the mean of LPD for each visit in the rural area and in the suburban area. The parasite densities were 5.5- to 10-fold higher in the rural area than in the suburban area during the dry season (January to July). During the rainy season (August to December), these densities were only 1.5- to 5-fold higher in the rural area than in the suburban area.

One-way analysis of variance showed that the influence of the date of the visits was, as expected, highly significant ($P < 10^{-4}$) in both areas; the highest infection levels were essentially during the rainy season, when malaria transmission was high.
The entomological inoculation rates and the mean of LPD were correlated in the rural area ($r = 0.62, P = 0.017$) and in the suburban area ($r = 0.64, P = 0.018$) except at the end of the rainy season (December to January); at this time, the entomological inoculation rates dramatically decreased, whereas the mean of LPD was maintained. Nevertheless, the entomological inoculation rates and the mean of LPD showed seasonal variations in both areas. Therefore, the individual LPD were adjusted for the visit effect before computing the MAPD.
Influence of Risk Factors on MAPD

In the rural and suburban areas, no significant effect of sex (\(P > 0.75\)), subdistrict of exposure (\(P > 0.5\)), and ethnic group (\(P > 0.2\)) was found on MAPD values. In contrast, age had a significant effect on MAPD in the suburban and rural areas (Fig. 3). Polynomial regression analysis showed that the best fitting functions were linear in the suburban area and quadratic in the rural area; the regression lines accounted for 17% (\(P < 10^{-4}\)) and 50% (\(P < 10^{-4}\)) of the variance of MAPD in the suburban area and in the rural area, respectively. Hence, age was retained for segregation analyses of MAPD.

In each area, MAPD values were not associated with ABO and Rh blood groups (\(P > 0.2\)) or with hemoglobin genotypes (\(P > 0.5\)). In particular, MAPD in AA subjects and in AS individuals were similar in each area (Table II). Furthermore, MAPD in AA subjects from the rural and urban areas did not differ significantly (\(P > 0.5\)) from MAPD observed in AC individuals from these two areas (Table II). Similar results were obtained when MAPD were adjusted for the age effect (\(P > 0.1\)) and when the subjects were matched for ethnic group (\(P > 0.2\)).

Segregation Analyses of MAPD

Results of segregation analysis obtained in the suburban area with the class D regressive model are shown in Table III. We found a significant sib–sib correlation (\(\rho_{SS} = 0.24\)), as shown by the comparison of models I and II.2 (\(\chi^2 = 19.2, \text{df} = 1, P < 10^{-4}\)); the parent–offspring correlation (model II.1 vs model II.2) was not significant. The father–mother correlation coefficient was never significant (data not shown).

The hypothesis of no major gene (model II.2) was highly rejected when compared with the codominant major gene model III.1a (\(\chi^2 = 55.2, \text{df} = 1, P < 10^{-10}\)). Moreover, interaction between age and gene was found to be highly significant (model III.1a vs. III.1b; \(\chi^2 = 49.3, \text{df} = 2, P < 10^{-10}\)); there was no residual sib–sib correlation when the interaction was taken into account. With the gene–age interaction, both the recessive (model III.2a vs. III.2c; \(\chi^2 = 58.2, \text{df} = 2, P < 10^{-10}\)) and the dominant (model III.2a vs. III.2b; \(\chi^2 = 46.7, \text{df} = 2, P < 10^{-10}\)) hypotheses for a single major gene were rejected. Hence, at this step, the best fitting model was a codominant gene model with a strong gene–age interaction and without residual familial correlation. However, when compared with the general transmission model (model IV), the codominant gene model (model III.2a) was rejected (\(\chi^2 = 25.2, \text{df} = 3, P < 10^{-4}\)). The nontransmission model that assumes equality of transmission probabilities (\(\tau_{AAA} = \tau_{ABA} = \tau_{BBA}\)) was also rejected (\(\chi^2 = 19.8, \text{df} = 2, P < 10^{-4}\)) (not shown). The results were similar when the variances of parents and children were considered different (data not shown).

In segregation analysis for the rural area, the conclusions were similar (Table IV). A significant sib–sib correlation was found (\(\rho_{SS} = 0.09\); model I vs. II.2: \(\chi^2 = 4.8, \text{df} = 1, P < 0.03\)). The hypothesis of no major gene was rejected (model II.2 vs. III.1a: \(\chi^2 = 65.7, \text{df} = 1, P < 10^{-10}\)), and there was evidence for a strong interaction between age and gene (model III.1a vs. III.1b: \(\chi^2 = 78.1, \text{df} = 4, P < 10^{-10}\)). The codominant major gene model (model III2.a) fitted better with the data than did the dominant and the recessive models (models III2.b and III2.c). The Mendelian model, however, was rejected when compared with the general transmission model (model IV vs. III2a: \(\chi^2 = 71.9, \text{df} = 3, P < 10^{-10}\)).
**DISCUSSION**

The present study evaluated the blood infection levels in a rural and a suburban population during almost 2 years. The aim was to investigate the presence of a major gene controlling blood infection levels in two populations that were differently exposed to *P. falciparum*. To detect the effect of such a gene, we evaluated the influence of various covariates on the MAPD, and we also took into account the significant risk factors.
<table>
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<td>0.19</td>
<td>1.70</td>
<td>5.10</td>
<td>0.32</td>
<td>0.155</td>
<td>(1)</td>
<td>(.5)</td>
<td>(0)</td>
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<tr>
<td>codominant</td>
<td>0.19</td>
<td>1.70</td>
<td>5.10</td>
<td>0.32</td>
<td>0.155</td>
<td>(1)</td>
<td>(.5)</td>
<td>(0)</td>
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<td>major effect</td>
<td>0.19</td>
<td>1.70</td>
<td>5.10</td>
<td>0.32</td>
<td>0.155</td>
<td>(1)</td>
<td>(.5)</td>
<td>(0)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.09</td>
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*Parameters in parentheses are fixed at the values shown. FC, familial correlation; SS, sib–sib; PO, parent–offspring.

*Phenotypic variance residual from the major effect.

bRegression coefficient that can be genotype dependent.

*The values are scaled by the transformed likelihood of the general model IV; $c = 2\ln L$ of the general model.
**TABLE IV. Segregation Analysis of 53 Pedigrees From the Rural Population***

<table>
<thead>
<tr>
<th>Model and hypothesis</th>
<th>Parameters</th>
<th>µ&lt;sub&gt;AA&lt;/sub&gt;</th>
<th>µ&lt;sub&gt;AB&lt;/sub&gt;</th>
<th>µ&lt;sub&gt;BB&lt;/sub&gt;</th>
<th>σ&lt;sup&gt;2&lt;/sup&gt;</th>
<th>q</th>
<th>τ&lt;sub&gt;AAA&lt;/sub&gt;</th>
<th>τ&lt;sub&gt;ABA&lt;/sub&gt;</th>
<th>τ&lt;sub&gt;BBA&lt;/sub&gt;</th>
<th>p&lt;sub&gt;PO&lt;/sub&gt;</th>
<th>p&lt;sub&gt;SS&lt;/sub&gt;</th>
<th>β&lt;sub&gt;AA&lt;/sub&gt;</th>
<th>β&lt;sub&gt;AB&lt;/sub&gt;</th>
<th>β&lt;sub&gt;BB&lt;/sub&gt;</th>
<th>γ&lt;sub&gt;AA&lt;/sub&gt;</th>
<th>γ&lt;sub&gt;AB&lt;/sub&gt;</th>
<th>γ&lt;sub&gt;BB&lt;/sub&gt;</th>
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<td>II. FC</td>
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<tr>
<td>a. Codominant</td>
<td>0.65 0.64 2.33 0.24 0.316 (1) (.5) (0) (0) 0.04</td>
<td>-0.072 (≠β&lt;sub&gt;AA&lt;/sub&gt;) (≠β&lt;sub&gt;AA&lt;/sub&gt;)</td>
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<td>0.0008 (≠γ&lt;sub&gt;AA&lt;/sub&gt;) (≠γ&lt;sub&gt;AA&lt;/sub&gt;)</td>
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<td>b. Codominant,</td>
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<tr>
<td>Interaction</td>
<td>-0.12 0.91 2.65 0.17 0.466 (1) (.5) (0) (0) 0.00</td>
<td>-0.033 -0.085 -0.182 0.0003 0.0009 0.0023</td>
<td>71.9</td>
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<tr>
<td>a. Codominant</td>
<td>-0.12 0.91 2.65 0.17 0.466 (1) (.5) (0) (0) 0.04</td>
<td>-0.033 -0.085 -0.182 0.0003 0.0009 0.0023</td>
<td>71.9</td>
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<td>b. Dominant</td>
<td>0.38 2.27 (≠µ&lt;sub&gt;AB&lt;/sub&gt;)</td>
<td>0.23 0.198 (1) (.5) (0) (0) (0)</td>
<td>-0.058 -0.168 (≠β&lt;sub&gt;AB&lt;/sub&gt;) 0.0006 0.0021 (≠γ&lt;sub&gt;AB&lt;/sub&gt;)</td>
<td>99.1</td>
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<td>c. Recessive</td>
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<td>2.32 0.22 0.567 (1) (.5) (0) (0) (0)</td>
<td>-0.058 (≠β&lt;sub&gt;AA&lt;/sub&gt;) -0.071 0.0006 (≠γ&lt;sub&gt;AA&lt;/sub&gt;)</td>
<td>92.4</td>
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<tr>
<td>transmitted</td>
<td>-0.16 1.16 2.81 0.12 0.057 0.47 0.88 0.21 (0) (0)</td>
<td>-0.030 -0.131 -0.234 0.0003 0.0019 0.0054</td>
<td>0</td>
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</table>

*Parameters in parentheses are fixed at the values shown.

* Regression coefficients β (age) and γ (age<sup>2</sup>); all models include a quadratic regression on age.
As expected, our results show that the transmission intensity and the blood parasitemia were higher in the rural area than in the urban area [Gazin et al., 1987, 1988; Trape, 1987]. This result is consistent with studies showing the influence of transmission force on parasite density [Robert et al., 1986; Trape and Zoulani, 1987]. Within each study area, however, the transmission intensities and the blood infection levels were homogeneous. In particular, we found no significant difference between blood infection levels in the subdistricts. Hence, neither in the suburban area nor in the rural area does the transmission intensity explain the MAPD variability.

Interethnic differences in MAPD were analyzed in the urban area, where seven ethnic groups were recorded; all subjects living in the village belonged to the Bobo ethnic group. Comparative analysis of MAPD in the ethnic groups showed no significant difference; in particular, the MAPD for the most represented group (the Mossi) were very similar to those for other ethnic groups. A recent study has shown that parasitemias are strikingly similar among several ethnic groups including the Mossi, whereas the Fulani living in the same area are clearly less parasitized [Modiano et al., 1996].

The heterozygosity for hemoglobin S was not found to be associated with lower MAPD, although it is known to provide protection against severe malaria [Nagel and Roth, 1989; Hill et al., 1991]. This finding is consistent with the findings of previous studies focused on blood parasitemia [Carnevale et al., 1981; Cot et al., 1993] that showed that individuals with sickle trait are not resistant to *P. falciparum* infection but are less likely to die from their infection than individuals with normal hemoglobin.

In contrast, we observed a strong effect of age on MAPD. We cannot rule out the possibility that the age effect could be due in part to differential mortality or morbidity at earlier ages; however, such age effect on parasitemia is also observed in people recently exposed to malaria, indicating that the age effect depends on the development of naturally acquired immunity [Baird et al., 1991]. As proposed by Baird [1995], intrinsic immune factors that change with age independently of the cumulative effects of repeated exposure may govern the degree of naturally acquired immunity. However, the effect of age is currently viewed as a cumulative product of repeated exposure either to strain-specific epitopes or to conserved epitopes from different *P. falciparum* strains [Gupta and Day, 1994]. The relative contributions of conserved and strain-specific immune responses are still being debated. Nevertheless, modeling studies have indicated that the slow accumulation of immune responses against poorly immunogenic conserved determinants better explains the effect of age on infection [Gupta and Day, 1994].

The segregation analyses that took into account the influence of age showed that sib-sib correlations were significant in the two populations. Furthermore, we obtained evidence for a highly significant codominant major effect that explains 62% and 65% of the residual parasitemia variance in the rural and the urban populations, respectively. However, the transmission of alleles at the putative major locus was not consistent with a single Mendelian model, suggesting a more complex mode of inheritance. In addition, a strong age-gene interaction indicates that the influence of genetic factors depends on age. This influence suggests that such genetic factors may control the development of naturally acquired immunity.
Our results do not confirm those that showed a major gene controlling blood infection levels in Cameroon [Abel et al., 1992]. Several explanations can be proposed. First, the rejection of the Mendelian hypothesis (and the absence of parent–offspring correlations) may be explained by the low adult MAPD variance, which probably led to wrongly inferring the parents' genotype from the phenotype (MAPD). The MAPD variance of the parents ($\sigma^2 = 0.088$ in the urban population, $\sigma^2 = 0.037$ in the rural population) was much lower than the MAPD variance of the offspring ($\sigma^2 = 0.324$ in the urban population, $\sigma^2 = 0.364$ in the rural population). Therefore, the blood infection levels appeared similar in adults; alternatively, the technique for measuring blood parasitemias may be insufficiently sensitive to detect significant differences between adults. This result clearly indicates that putative genetic differences should be easier to detect in children than in adults.

Second, the genetic control of malaria infection may differ in Central Africa and in West Africa: blood parasitemia may be controlled by more than one single major gene in Burkina Faso, and the genes involved in the resistance may differ between the Cameroon population and the Burkina Faso population. Such a complex genetic control of malaria has been described: in Africa, resistance has been associated with sickle hemoglobin [Nagel and Roth, 1989; Hill et al., 1991], and in Melanesia, resistance has been associated with a mutation in erythrocyte band 3 [Schosfield et al., 1992; Miller, 1994]; a recent segregation analysis also has supported the existence of complex genetic factors controlling blood infection levels [Garcia et al., 1998]. Moreover, in mice, H2-linked major gene [Stevenson et al., 1982; Weiss et al., 1989] and non-H2-linked genetic factors [Wunderlich et al., 1988; Weiss et al., 1989] have been shown to control resistance to \textit{P. chabaudi} and \textit{P. yoelii}.

Third, parasite factors may influence peripheral blood parasitemia and may be confounding factors in our study. Parasite strains are likely to differ in their growth properties [Gupta et al., 1994]. Moreover, sequestration is thought to depend on variation in the expression of erythrocyte surface molecules mediating adhesion to endothelia [Marsh et al., 1988; Borst et al., 1995] and to depend on variation in the production of endotoxins eliciting a tumor necrosis factor-$\alpha$ response by macrophages [Allan et al., 1993]. Parasite strains thus may be differently sequestered in internal organs. However, the proportion of sequestered parasitized erythrocytes is likely low in subjects without cerebral malaria [Miller, 1969; Pongpontratn et al., 1991] and should not influence the peripheral blood parasitemia of such subjects.

In summary, for two differently exposed populations, we obtained evidence for sib–sib correlations and for a major effect that accounts for the global blood infection level distribution. Nevertheless, the rejection of a single Mendelian model suggests that several major genes control the resistance to \textit{P. falciparum} or that the genetic effect can be detected only in children. In both cases, further linkage analyses that do not require knowing the genetic model and that focus on children may help in determining the genetic control of human malaria.

ACKNOWLEDGEMENTS

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Dioulasso, for its contribution. Y.T. was a fellow of ORSTOM and the Ministère de la Coopération et du Développement, Paris, France. We are grateful to the Ministère de la Santé, Ouagadougou, Burkina Faso and the Direction provinciale de la Santé, Bobo-Dioulasso for their encouragement.

REFERENCES


