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GENETIC CONTROL OF BLOOD INFECTION LEVELS IN HUMAN MALARIA: EVIDENCE FOR A COMPLEX GENETIC MODEL

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Abstract. There is now accumulating evidence for the involvement of genetic factors in the control of immune response against malaria. These arguments come from numerous animal models, from population studies showing associations of red blood cell genetic defects as well as HLA antigens with severe malaria, and from familial studies including a recent segregation analysis, which led to detection of a major gene effect predisposing to high infection levels. The heterogeneity and complexity of this genetic control is one of the main findings of these previous studies, and probably a major cause of the difficulty in developing an effective malaria vaccine. A segregation analysis of blood infection levels is performed here in 44 pedigrees living in the tropical rain forest of southern Cameroon and exposed to high vectorial transmission intensity. The results confirm the existence of complex genetic factors controlling blood infection levels in human malaria but are not consistent with the parent-offspring transmission of a single Mendelian gene. This study also shows the dramatic effect of age on infection levels and its interaction with a putative major gene suggesting that genetic related differences are much more important in children than in adults. Further genetic studies focused on children may help to identify the nature of the genetic factors involved in the expression of human malaria, by means of linkage analyses using both familial information and genetic markers.

Malaria is a major cause of morbidity and mortality in Africa. It has been previously reported that host mechanisms of innate resistance, as genetic defects of red blood cells,[1] are likely to be important determinants of the outcome of infection even though parasite strains of *Plasmodium falciparum* may differ in virulence as suggested by in vitro studies.[2] While there is evidence for genetic control of either disease resistance[3] or immune responses to malaria antigens[4] in murine malaria, the nature of this genetic control in human malaria remains unclear. Although it is now admitted that common genetic disorders of red blood cells might confer protection against malaria,[5,6] the molecular and cellular mechanisms involved are still being investigated.[7] Investigation of the genetic susceptibility/resistance to human malaria progressed along two complementary ways: 1) case-control studies, mainly aimed at testing associations between clinical malaria or immune responses to infection and genetic markers, as HLA antigens; and 2) familial studies searching for a major gene effect involved in human susceptibility/resistance to infection. Case-control studies provided important, but sometimes discordant, results. A recent study in Gambia showed that an HLA class I antigen and an HLA class II haplotype were independently associated with protection from severe malaria;[8] in the same population, homozygotes for a variant of the tumor necrosis factor-α gene promoter region were found to have an increased risk of cerebral malaria independently of their HLA alleles.[9] However, conflicting results exist with respect to the direct implication of HLA genes in the genetic regulation of immune responses induced by malaria vaccine antigens,[10–12] strengthening the complexity of this genetic control. Nevertheless, it is preferable that the case-control method should be complemented by familial studies, using specific genetic analyses.[13]

The main feature of family studies is the statistical dependence of family data and the modeling objective in genetic epidemiology is to explain these familial correlations in terms of biologic relationships and shared environment. Among familial studies, segregation analysis is a statistical method that attempts to detect the existence of a single gene, called the major gene, among all other risk factors (individual, behavioral, or environmental) involved in the variability of a complex trait denoted as the phenotype. It is important to notice that a large number of genes, as well as environmental, and cultural factors can contribute to the variability of the trait, although only the effect of one or a few genetic loci may be discernible, at least statistically, in the phenotype. We refer to the gene as a major gene in a biometric sense, i.e., its effect on the variability is large enough to be detected without assuming that this major gene alone accounts for all the dependence among relatives. Such studies have been applied to both mycobacterial and parasitic infections,[14,15] and a recent segregation analysis performed in 42 Cameroonian families provided evidence for a major gene controlling blood infection levels in human malaria.[16] One of the main differences between the two approaches, case-control and/or familial studies, for understanding the genetics of human malaria is the choice of the phenotype under study. Specific clinical phenotypes, such as cerebral malaria or severe anemia, studied in case-control approaches, are relatively too rare to be used efficiently in familial studies.

The aim of the present study is to confirm the result of Abel and others[16] by means of another segregation analysis performed in a different population belonging to another ethnic group, and living in a forest area with higher vectorial transmission. The same quantitative phenotype was used in the present study, i.e., the mean infection level computed from several parasitologic measurements performed during a one-year follow-up.

SUBJECTS AND METHODS

Family data, measures, and phenotype of interest. The study was conducted between April 1992 and April 1993 in

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a southern Cameroon village (region of Mbalmayo) located 70 km from Yaounde, the capital city. The population studied was clearly informed and the protocol of the study, including blood samples, was approved by traditional (chief and village committee), local, and national government authorities (Public Health Ministry). The study area was located in the tropical rain forest with an important rainy season between June and November. The characteristics of the whole population of the village consisting of 667 individuals from the Ewondo ethnic group were described previously.[17] From the whole population, a familial study was carried out in a randomly selected sample consisting of the families living in the village and including at least three members with a known phenotype, i.e., sampled at least twice during the survey. The final sample included 44 pedigrees accounting for first- and second-degree relatives and totaled 315 subjects. No familial relationship closer than third degree has been detected between pedigrees by repeated investigations performed by two Cameroonian sociologists from the Ewondo ethnic group who spent two months in the village with the population. Inhabitants of the village excluded from the family study were elderly people (> 60 years old) living alone, subjects with unreliable familial relationships, or those who refused to participate.

During the one year follow-up, each family was visited every two months and parasitologic measurements were performed at each visit from thick blood smears. Thick blood films were stained with Giemsa and asexual parasites (*P. falciparum* and *P. malariae*) and leukocytes were counted in 150 fields. The parasite density (PD) was defined as the number of parasites observed in these fields, and from this PD a classic parasitemia per milliliter of blood can be calculated assuming an average number of 8,000 leukocytes/ml. In the present study, all further analyses were based on PD, i.e., the number of parasites observed in 150 fields without multiplicative factor. Approximately 50% of the thick smears were independently examined by two readers, and, in case of a discrepancy, an experimented reader was asked to review the film. The quantitative differences between the two readers were very limited, and no qualitative discordance (i.e., positive negative thick film) was observed. *Plasmodium falciparum* was present alone in 80% of the positive blood samples and was associated with *P. malariae* (mixed infections) in 10%. Infections with *P. malariae* alone represented the remaining 10%. Since the main objective of our study was to confirm the results of Abel and others,[16] the PD considered for further analyses was the total number of parasites due to both species of *Plasmodium*. However, each species of *Plasmodium* involve different immune mechanisms with a genetic control that can be different. Thus, we also performed segregation analysis on *P. falciparum* parasite density alone, denoted as PFD, by removing from the analysis blood samples that exhibited *P. malariae* (20% of the positive blood samples). Analyses of PD (PFD) were conducted using a logarithmic transformation based on log (PD + 1) to allow for 0 counts; in the following analysis log-transformed PDs (PFDs) are designated as LPD (LPFD).

Each person was not present at each visit and the mean number of measurements per subject was 4.2 (range = 2–6). To deal with a unique variable accounting for the intensity of malaria infection, a mean parasite density (MPD) was

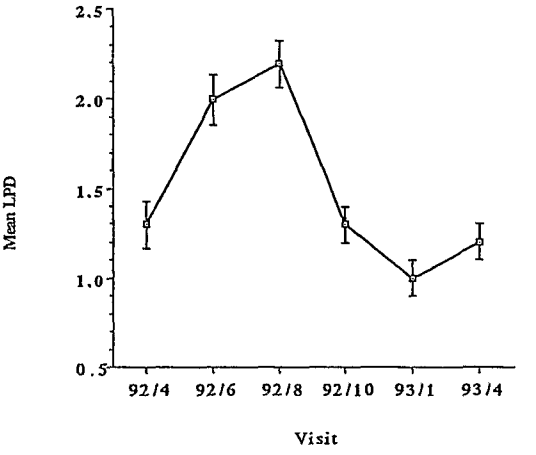


FIGURE 1. Influence of the visits on the log-transformed parasite density (LPD). Results are presented as the mean with the 95% confidence interval of the LPD values observed during the six visits.

determined for each subject. During the one-year follow-up, the LPD values varied significantly across time ($P < 0.0001$) with highest values during the rainy season (Figure 1). Consequently, the individual LPDs were first adjusted for the season effect by subtracting the mean LPD of the corresponding visit from each individual LPD. The MPD was then computed for each subject as the mean of his season-adjusted LPDS, and this represented the individual phenotype of interest. The same adjustment was performed to determine a mean *P. falciparum* density, denoted as MPFD, and representing the mean of season-adjusted LPFDs.

Risk factors influencing the MPD (MPFD). The variability of MPD (MPFD) can be explained by an unobserved genetic factor but also by individual, behavioral, or environmental factors, and these risk factors, which have a significant influence on MPD (MPFD), should be taken into account in segregation analysis. The five measured factors potentially influencing the MPD (MPFD) were 1) sex; 2) prophylaxis intake divided in three groups: a) no intake (64%, n = 203); b) irregular intake (27%, n = 85), and c, regular intake, (9%, n = 27); 3) use of protection against mosquitoes, such as bed nets and mosquito coils, for which six individuals in the village declared they were using a protection against mosquitoes bites; 4) area of residence studied by means of three entomologic surveys that were carried out during the follow-up and assessed the genetic homogeneity of vectorial transmission in the village by measuring the number of infective bites per human and per night in five different areas; 5) age, in years, considered as a quantitative variable.

Statistical methods. *Effect of measured risk factors on MPD (MPFD).* The effect of age was assessed by means of a polynomial regression and analysis of variance was used to study the effects of categorical variables (sex, prophylaxis intake, protection against mosquitoes). These analyses were performed using SAS software (SAS Institute, Cary, NC).

Segregation analysis. A regressive approach, classically used in epidemiology, has been proposed by Bonney[18] to construct comprehensive models by the direct use of the biologic relationships among relatives in defining simple patterns of familial dependence. These models specify a re-

TABLE 1
Parameters used in regressive models for segregation analysis

Effect	Parameters	Significance
Major effect	q	Frequency of allele A predisposing to high mean parasite density values
	μ_{AA}, μ_{Aa}, μ_{aa}	Phenotypic mean for individuals AA, Aa, and aa respectively
	$\tau_{AA\to A}$, $\tau_{Aa\to A}$, $\tau_{aa\to A}$	Transmission probabilities*
Residual covariation from major effect	ρFM, ρPO, ρSS	Phenotypic correlations between father–mother, parent–offspring, and sib–sib, respectively
	σ^2R	Phenotypic variance residual from the major effect
Covariates	β_g†	Regression coefficient

* When the transmission probabilities are fixed to their Mendelian values ($\tau_{AA\to A}$ = 1, $\tau_{Aa\to A}$ = 0.5, and $\tau_{aa\to A}$ = 0), the major effect is a major gene.
† May vary with genotype in case of interaction between genotype and covariate (β_{AA}, β_{Aa}, β_{aa}).

gressive relationship between each individual's phenotype (i.e., the MPD in the present study) and explanatory variables including 1) a major gene effect resulting from the segregation of two alleles (A and a), 2) the phenotypes, i.e., the MPDs, of preceding relatives, and 3) measured covariates (individual, behavioral, or environmental). The parameters of the regressive model corresponding to these three types of explanatory variables are detailed in the three following paragraphs and summarized in Table 1.

The parameters of the major gene effect are q, the frequency of A, the allele assumed to predispose to high MPD values, and the three phenotypic means (MPD) adjusted on covariates denoted as μ_{AA}, μ_{Aa}, and μ_{aa}, for individuals with genotypes AA, Aa, and aa, respectively; given the genotype, the distribution of the phenotype is assumed to be normal with residual variance σ^2_R (Figure 2). Dominance of allele A is defined through constraints on the genotype specific means, e.g., $\mu_{AA} = \mu_{Aa}$ (allele A is dominant), $\mu_{Aa} = \mu_{aa}$ (allele A is recessive), with in the general case of codominance, $\mu_{aa} < \mu_{Aa} < \mu_{AA}$. When the parent-offspring transmission of allele A is assumed to follow Mendelian laws, no additional parameters are necessary, and the major effect is actually due to the segregation of a major gene. However, different studies have shown that the Mendelian transmission hypothesis should be tested against alternative hypotheses of parent-offspring transmission to avoid false conclusions for the presence of a major gene.[19] To perform these tests, three parameters, called transmission probabilities and noted as

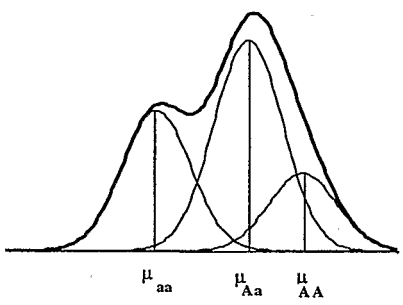


FIGURE 2. Distribution of a quantitative trait in the general population under a model including a major effect. Under the Mendelian hypothesis, the population is composed of three categories of individuals aa, Aa and AA with respective proportions $(1 - q)^2$, $2q(1 - q)$, and q^2, where q is the frequency of A predisposing to high mean parasite density values in our study. Within each category, the distribution is assumed to be normal with a mean μ_g (g = aa, Aa, or AA) and a residual variance σ^2_R.

$\tau_{AA\to A}$, $\tau_{Aa\to A}$, and $\tau_{aa\to A}$, have been defined; [20] these parameters represent the probability of transmitting A to an offspring for individuals AA, Aa, and aa, respectively. Under the Mendelian hypothesis, these probabilities are fixed to the following values: $\tau_{AA\to A} = 1$, $\tau_{Aa\to A} = 0.5$, and $\tau_{aa\to A} = 0$, whereas under a general parent-offspring transmission model these probabilities are free parameters estimated between 0 and 1. This latter case corresponds to a parent-offspring transmission more complex than a single major gene, and is generally denoted as a major effect model. Under this model, the population is composed of three categories of persons with a certain degree of familial resemblance and different infection levels (Figure 2), but the distribution of these three categories within families does not fit Mendelian expectations.

Conditional on this major gene, the dependence of an individual's phenotype on relatives' phenotypes is expressed in terms of phenotypic correlations that account for the observed phenotypic resemblance between relatives residual from the major gene effect, without assuming its behavioral, environmental, or genetic origin. Different patterns of familial dependencies can be considered, and in the class D model used in this study, four familial correlations are specified: ρ_{FM}, the father-mother (or spouse) correlation; ρ_{FO}, the father-offspring correlation; ρ_{MO}, the mother-offspring correlation; and ρ_{SS}, the sib-sib correlation.[18] In the present analysis, ρ_{FO} was never significantly different from ρ_{MO}, and these parameters were fixed equal to a parameter ρ_{PO} for a global parent-offspring correlation.

The effect of measured covariates are parametrized in terms of regression coefficients (βs), as usually done in linear regression. An interesting feature of the regressive models is that they allow to test for an interaction between a covariate and the major gene effect. For each covariate the genotype specific regression coefficients are denoted β_g, with g = AA, Aa, or aa. Under the hypothesis of no interaction, we have $\beta_{AA} = \beta_{Aa} = \beta_{aa}$, whereas the three β_g parameters are estimated in presence of interaction.

The estimation of relevant parameters and the strategy of tests are based on maximum likelihood theory. The likelihood of the sample of families is computed under different hypotheses (or models), and nested hypotheses are tested by means of the likelihood ratio criterion: if the null hypothesis is true, minus twice the natural logarithm of the likelihood ratio is distributed as a χ^2, the degrees of freedom (df) being equal to the difference between the number of independent parameters estimated under the two hypotheses. For example, evidence for a major gene effect is obtained by rejecting

TABLE 2

Distribution of individuals according to their number of parasite measurements in the whole population and in the population of subjects younger than 15 years

	Number of measurements					
	2	3	4	5	6	Total
All subjects	45	44	64	86	76	315
(%)	(14.3)	(14)	(20.3)	(27.3)	(24.1)	(100)
0–15 years old	25	23	26	39	49	162
(%)	(15.5)	(14.2)	(16)	(24.1)	(30.2)	(400)

TABLE 3

Number of measurements, number (proportion) of infected individuals, and mean log-transformed parasite density (LPD) according to the visit time

Visit	Number of measurements	Number infected (%)	Mean LPD (SD)
4/92	160	93 (58%)	1.3 (1.5)
6/92	211	146 (69%)	2.0 (1.9)
8/92	241	158 (66%)	2.2 (1.9)
10/92	265	145 (55%)	1.3 (1.5)
1/93	250	145 (58%)	1.0 (1.5)
4/93	186	72 (39%)	1.2 (1.4)

the null hypothesis of familial correlations without a major gene against a model including both a major gene and residual familial correlations. As mentioned in a previous paragraph, tests concerning the parent-offspring transmission of the major effect should be performed. To conclude to the presence of a major gene instead of a major effect, the null hypothesis of Mendelian transmission ($\tau_{AA\to A} = 1$, $\tau_{Aa\to A} = 0.5$, and $\tau_{aa\to A} = 0$) should not be rejected when compared with the general transmission hypothesis (free τs). All computations were performed using the computer program REGRESS, which incorporates the regressive approach into the LINKAGE[21] package as proposed by Bonney and others.[22]

RESULTS

Descriptive results. Segregation analysis was carried out in 44 pedigrees totaling 315 subjects with a mean age of 23.4 years (range = 1–80). As indicated previously, each person was not present at each visit, but no effect of the number of measurements per individual on the MPD was detected ($P > 0.8$). The distribution of subjects according to their number of measurements is shown in Table 2. Over the one-year follow-up, more than 70% of the population was sampled at least four times, giving a total of 1,313 measurements, of which 759 (0.578) were parasite positive. From the subjects sampled four, five, and six times, the proportions of individuals having all their PDs positive were 20.3% (13 of 64), 12.8% (11 of 86), and 7.9 (6 of 76), respectively. These same proportions expected under the hypothesis that a positive PD occurs at random are much lower and equal to 0.112 (0.578^4), 0.064 (0.578^5), and 0.037 (0.578^6), respectively, indicating a stability over time of the individual infection status. The proportion of infected persons at each visit significantly varied across time ($P < 0.0001$) as well as the mean LPD per visit (Table 3) as described previously in the Subjects and Methods (season effect), and to take into account this variability, each individual LPD was adjusted for the season effect before computing the MPD. An illustration of the computation of MPD from LPD values is provided for two families in Figure 3. Similar results were obtained for the MPFD with respect to the number of measurements per individual as well as the season effect. The distribution of standardized MPD (MPFD) is shown in Figure 4 and is slightly skewed on the right by individuals having high MPD (MPFD) values.

The variations of MPD and MPFD with age were very similar, and are shown in Figure 5. As usually observed, the MPDs decreased rapidly during the first years of life and then showed a plateau. The detailed effect of age on MPD and MPFD is described later.

Influence of measured risk factors on MPD (MPFD) values. There was no difference in the MPD values according to sex ($P > 0.8$). Prophylaxis intake had no significant effect on MPDs ($P > 0.4$). The low doses of chloroquine taken by the subjects reporting regular or irregular intake may explain this result. To confirm this hypothesis, we performed a urine chloroquine assay using the Haskins and Mount method[23] in 120 randomly selected subjects. The results were consistent with the absence of chloroquine excretion in 93% of the tested subjects and with a very low excretion corresponding to infratherapeutic intake in the remaining 7%. The use of a protection against mosquitoes was reported by only 2% of the population (n = 6), but during the one-year follow-up, neither the presence of bed nets nor the use of other protections had been reported by physicians frequently present in the village.

The effect of the area of residence on MPDs was assessed by means of entomologic surveys aimed mainly at detecting a geographic heterogeneity in the intensity of vectorial transmission. The village was divided in five areas and three surveys of five nights each were performed in November 1992, April 1993, and July 1993. In each of these areas, routine collections of mosquitoes were carried out in five houses by four men per house, between 6:00 PM and 6:00 AM. The results of these surveys were consistent with the absence of geographic differences in vectorial transmission conditions within the study area.[24]

The effect of age on MPDs was assessed by polynomial regression. The best fitting equation was a function of age ($P < 0.001$) and age^2 ($P < 0.001$) with regression coefficients of -0.0689 and 0.0006, respectively, and explained 45% of the variance of MPDS. In particular, a model with age alone was highly rejected when compared with a model including age and age^2 ($\chi^2 = 25.58$; df =1; $P < 0.001$), indicating that a quadratic function was necessary to account for the age effect shown in Figure 5.

The effect of measured risk factors on MPFD was similar, and the only relevant factor was age. As for the MPD, both age ($P < 0.001$) and age^2 ($P < 0.001$) significantly influenced MPFD values with regression coefficients of -0.0636 and 0.00055, respectively. This quadratic function in age explained 38% of the variance of the MPFDs.

Segregation analysis. With respect to previous results, all analyses on MPD and MPFD were performed including a quadratic regression on age. Furthermore, a spouse correla-

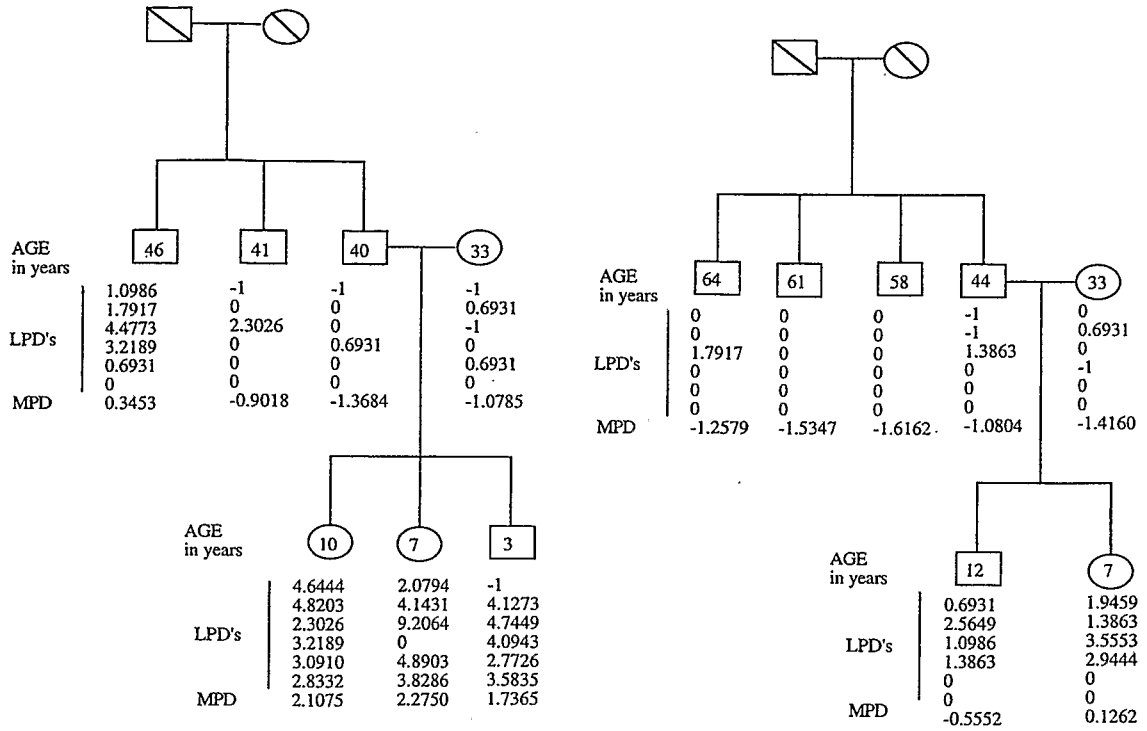


FIGURE 3. Example of two families, indicating for each person age in years, log-transformed parasite density (LPD) values before adjustment on season effect (-1 indicates that the subject was not present for the corresponding visit), and standardized mean parasite density (MPD) values, which represents the phenotype of interest used for segregation analysis.

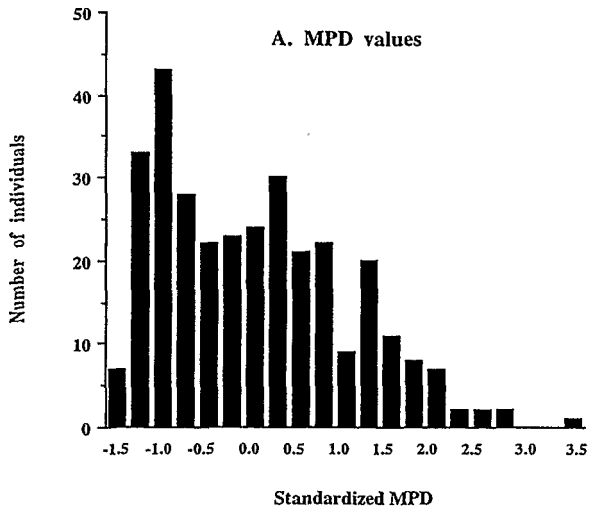
tion was never significant and this parameter (ρ_{FM}) was fixed to 0 for all models presented in Tables 4 and 5.

Results of segregation analysis on the MPD are presented in Table 4. There was evidence for familial correlations since the sporadic model with no familial correlation (model I) was significantly rejected when compared with a model including both parent-offspring ($\rho_{PO} = 0.17$) and sib-sib correlations ($\rho_{SS} = 0.18$) (model I versus II; $\chi^2 = 14.14$, df 2; $P < 0.001$). The presence of a codominant major gene with residual familial correlations and interaction between age and genotype (GxA interaction) was highly significant (model II versus III-2a; $\chi^2 = 58.41$, df = 7; $P < 0.001$). Both the hypotheses of no GxA interaction (model III-1 versus III-2a; $\chi^2 = 55.43$, df = 4; $P < 0.001$) and of no residual familial correlations (model III-3 versus III-2a; $\chi^2 = 6.99$, df = 2; $P < 0.05$) were rejected. In presence of GxA interaction and residual familial correlations, the recessive and the dominant hypotheses for the major gene were rejected when compared with the codominant one (model III-2b versus III-2a; $\chi^2 = 29.59$, df = 3; $P < 0.001$; and model III2c versus III-2a; $\chi^2 = 31.24$, df = 3; $P < 0.001$, respectively). At this step, the best fitting model accounting for the familial distributions of MPDs was a codominant major gene with residual familial correlations and a strong GxA interaction, meaning that the effect of age on infections levels differs according to genotype (Figure 6); this major gene explained about 60% of the MPD variance after accounting for the age effect. However, the Mendelian transmission hypothesis of this codominant major gene ($\tau_{AA\to A} = 1$, $\tau_{Aa\to A} = 0.5$, and $\tau_{aa\to A} = 0$) was rejected when compared with a general transmission hypothesis where the transmission probabilities are estimated (model III-2a versus IV; $\chi^2 = 11.55$, df = 3; $P < 0.01$).

Table 5 presents the results of segregation analysis on MPFD, which are similar to those obtained for MPD. There was evidence for familial correlations including parent-offspring ($\rho_{PO} = 0.11$) and sib-sib correlations ($\rho_{SS} = 0.20$) (model I versus II; $\chi^2 = 8.45$, df = 2; $P < 0.02$). The presence of a codominant major gene with GxA interaction was highly significant (model II versus III-2; $\chi 2=63.75$; 7 df; p < 0.001). In presence of this major gene, residual familial correlations were not significant (model III-3a versus III-2; $\chi^2= 2.45$, df = 2; $P > 0.3$). Both the recessive and the dominant hypothesis for the major gene were rejected (model III3b versus III-3a; $\chi^2 = 37.3$, df = 1; $P < 0.001$; and model III-3c versus III3a; $\chi^2 = 37.65$, df = 1; $P < 0.001$, respectively). Therefore, the best fitting model that accounts for MPFD familial distribution was a codominant major gene with GxA interaction and no residual familial correlation. However, as for MPD, the Mendelian transmission hypothesis of this major gene was rejected when compared with a more general transmission hypothesis (free τs) (model III-3a versus IV; $\chi^2 = 9.35$, df = 3; $P < 0.05$).

DISCUSSION

The present segregation analyses provided clear evidence for a codominant major effect involved in the control of blood infection levels in human malaria, but the transmission of this major effect was not compatible with a simple Mendelian hypothesis. This result means that the study popula-



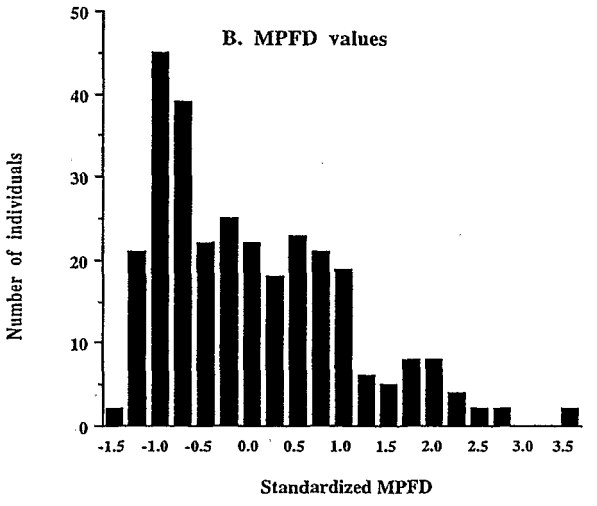


FIGURE 4. Histogram of the standardized mean parasite density (MPD) (**A**) and mean *Plasmodium falciparum* density (MPFD) (**B**) values in 315 persons.

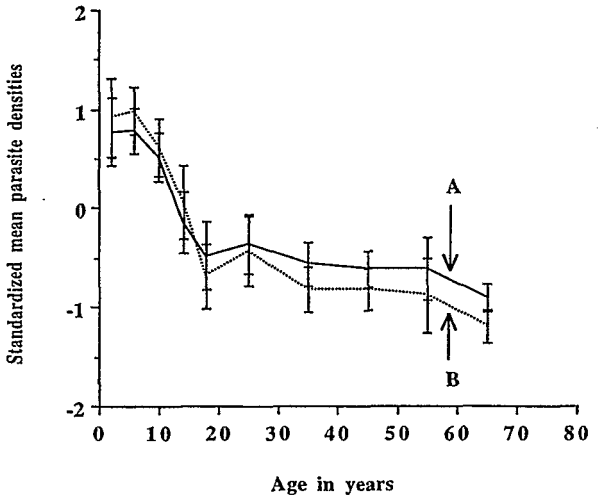


FIGURE 5. Influence of age (in years) on the mean parasite density (MPD) (**solid line A**) and mean Plasmodium falciparum parasite density (MPFD) (**dotted line B**). Plots represent the mean with the 95% confidence interval of MPD and MPFD values observed within 10 age groups (i.e., 0–4, 5–8, 9–12, 13–16, 17–20, 21–30, 31–40, 41–50, 51–60, and >60 years).

tion may be composed of three categories of persons with a certain degree of familial resemblance and different infection levels (Figure 2), but the distribution of these three categories within families does not fit Mendelian expectations. The absence of significant effect of measured risk factors as well as the absence of important geographic differences in vectorial transmission[24] argue against the hypothesis that this familial resemblance could be due only to shared familial environment. The presence of genetic factors involved in the control of malaria infection intensities is strongly supported by both findings in mice that demonstrated the role of host genetic factors in the outcome of experimental malaria infections,[4,25] and a recent segregation analysis performed on families from Cameroon.[16] However, conflicting results are found in experimental studies with respect to the genetic control of susceptibility to malaria depending on various species of *Plasmodium* in mice,[26,27] strengthening the complexity of mechanisms involved. In the present study, the same results were obtained for parasite densities combining both *P. falciparum* and *P. malariae* (MPD), as well as for *P. falciparum* densities alone (MPFD). However, the proportion of *P. malariae* infections was too low to test for a true effect due to Plasmodium species, and this sample did not allow to assess whether or not the genetic control of infection levels due to both species could be different from that due to *P. falciparum* alone.

Our analysis showing a strong interaction between the major effect and age indicates that the effect of age in the evolution of MPDs (MPFDS) differs according to the genetic background of individuals, and emphasizes the complex effect of this (these) putative gene(s). Lower density of parasitaemia in adults compared with children usually observed in endemic areas can be considered as a progressive process in the development of naturally acquired immunity. Although the differing cumulative experiences with the antigenic repertoire of wild parasites can be an essential difference between the immune systems of children and adults, the constitutional changes in the immune system that occur with aging probably exert an important influence.[28] Thus, genetic-related differences in the development of malarial immunity are certainly easier to detect during childhood than later when effector mechanisms of the immune response are already acquired, as described for other parasitic diseases.[15,29] This smaller genetic effect in adults can explain, at least in part, the much lower MPD (MPFD) variance observed in subjects more than 20 years of age (0.38 and 0.41 for MPD and MPFD, respectively) compared with younger subjects (0.83 and 0.91 for MPD and MPFD, respectively). In segregation analysis, genotypes of parents are inferred mainly from their observed phenotypes, i.e., their MPD (MPFD) values, and the risk of misspecification of this genotype increases as the genetic effect decreases. In these conditions, it is likely that this misspecification occurred in a large number of parents and could lead to the rejection of the Mendelian hypothesis. Another source of misspecification could come from the difficulty to define accurately the

TABLE 4
Segregation analysis of the mean parasite densities using a regressive model*

Model	q	μ_{aa}	μ_{Aa}	μ_{AA}	$\tau_{AA\to A}$	$\tau_{Aa\to A}$	$\tau_{aa\to A}$	σ^2R	ρPO	ρSS	No. of estimated parameters†	−2ln L‡
I. Sporadic	(0)§	1.06	(= μ_{aa})	(= μ_{aa})	–	–	–	0.55	(0)	(0)	4	129.32
II. Familial correlations (FC)	(0)	1.08	(= μ_{aa})	(= μ_{aa})	–	–	–	0.56	0.17	0.18	6	115.18
III. Mendelian major gene (MG)												
III-1. Codominant MG with residual FC and no interaction	0.36	0.71	1.01	2.10	(1)	(0.5)	(0.0)	0.38	0.13	0.18	9	112.20
III-2. MG with residual FC and interaction												
a → Codominant	0.43	−0.23	1.38	2.34	(1)	(0.5)	(0.0)	0.21	0.24	0.06	13	56.77
b → Recessive	0.84	−0.05	(= μ_{aa})	1.54	(1)	(0.5)	(0.0)	0.38	0.08	0.0	10	86.31
c → Dominant	0.41	0.04	1.56	(= μ_{Aa})	(1)	(0.5)	(0.0)	0.37	0.10	0.0	10	88.01
III-3. Codominant MG alone (no residual FC) with interaction	0.48	−0.36	1.22	2.16	(1)	(0.5)	(0.0)	0.21	(0.0)	(0.0)	11	63.76
IV. General transmission (free τs) with residual FC and interaction	0.30	−0.42	1.12	2.10	0.17	0.77	0.56	0.18	0.31	0.20	16	45.22

* – = not relevant.
† All models include a quadratic regression on age representing two additional parameters [β(age) and β(age²)] when no age interaction is considered, four additional parameters when an age interaction with either recessive or a dominant MG is considered, and six additional parameters when an age interaction with a codominant MG is considered [β_{aa}(age), β_{Aa}(age), β_{AA}(age), β_{aa}(age²), β_{Aa}(age²), β_{AA}(age²)].
‡ Minus twice the natural logarithm of the likelihood.
§ () indicates that the parameter is fixed at the corresponding value.

phenotype used in this study, i.e. the MPD (MPFD). Parasite densities present an important variability over time, which can lead to imprecision for the determination of this phenotype. The use of a mean parasite density value based on six measurements over a one-year follow-up took into account a part of this variability, but we cannot rule out that the phenotype of some individuals, especially those having very few measurements, were imprecise. An additional explanation for the rejection of the Mendelian hypothesis might be a mechanism of selection of adults genetically able to acquire natural immunity to *P. falciparum* and predisposed to low levels of infection since such genetic differences may lead to higher mortality rates for children with an inefficient immune response. The impact of malaria on the selection of multiple genes that give the population a selective advantage have been already put forward,[30] including natural selection of genetic red blood cell defects,[6,31] as well as genes of the major histocompatibility complex (MHC).[8]

Although the role of genetic red cell blood defects against severe forms of malaria have been well documented,[6,8] discordant results exist concerning their influence on the levels of infection intensities.[32,33] The distribution of MPD values according to hemoglobin genotypes observed in our data is consistent with previous results that failed to demonstrate lower parasite rates in heterozygous (AS) individuals.[33] The

TABLE 5
Segregation analysis of the mean *Plasmodium falciparum* densities using a regressive model*

Model	q	μ_{aa}	μ_{Aa}	μ_{AA}	$\tau_{AA\to A}$	$\tau_{Aa\to A}$	$\tau_{aa\to A}$	σ^2R	ρPO	ρSS	No. of estimated parameters†	−2ln L‡
I. Sporadic	(0)§	1.16	(= μ_{aa})	(= μ_{aa})	–	–	–	0.61	(0)	(0)	4	225.27
II. Familial correlations (FC)	(0)	1.17	(= μ_{aa})	(= μ_{aa})	–	–	–	0.61	0.11	0.20	6	216.82
III. Mendelian major gene (MG)												
III-1. Codominant MG with residual FC and no interaction	0.34	0.92	0.92	2.79	(1)	(0.5)	(0.0)	0.37	0.14	0.25	9	192.56
III-2. Codominant MG with residual FC and interaction	0.40	−0.23	1.52	2.86	(1)	(0.5)	(0.0)	0.21	0.0	0.23	13	153.07
III-3. MG alone (no residual FC) with interaction												
a → Codominant	0.40	−0.25	1.50	2.79	(1)	(0.5)	(0.0)	0.21	(0.0)	(0.0)	11	155.52
b → Recessive	0.60	0.49	(= μ_{aa})	2.28	(1)	(0.5)	(0.0)	0.38	(0.0)	(0.0)	10	192.82
c → Dominant	0.24	0.41	2.15	(= μ_{Aa})	(1)	(0.5)	(0.0)	0.40	(0.0)	(0.0)	10	193.17
IV. General transmission (free τs) with interaction	0.30	−0.23	1.51	2.88	0.64	0.65	0.10	0.18	(0.0)	(0.0)	14	146.17

* – = not relevant.
† All models include a quadratic regression on age representing two additional parameters [β(age) and β(age²)] when no age interaction is considered, four additional parameters when an age interaction with either recessive or a dominant MG is considered, and six additional parameters when an age interaction with a codominant MG is considered [β_{aa}(age), β_{Aa}(age), β_{AA}(age), β_{aa}(age²), β_{Aa}(age²), β_{AA}(age²)].
‡ Minus twice the natural logarithm of the likelihood.
§ () indicates that the parameter is fixed at the corresponding value.

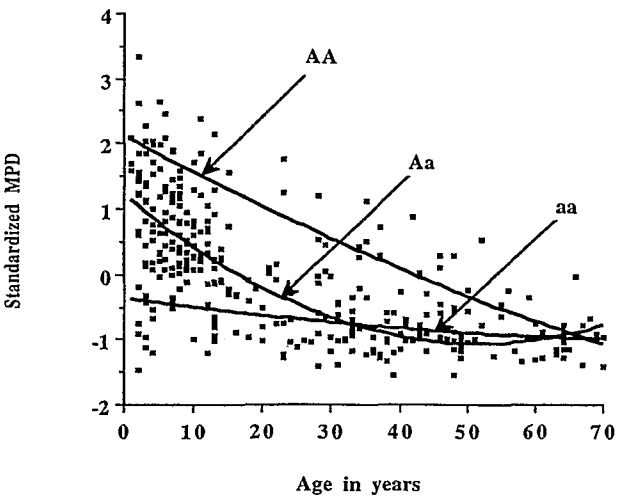


FIGURE 6. Evolution of mean parasite density (MPD) with age. Observed values (points) and predicted curves for each category of individuals under a model including a codominant major gene with an interaction between age and genotype (model III-3 in Table 4).

mean MPD values (± SEM) observed in heterozygous (AS) individuals (0.285 ± 0.112) were not significantly different ($P = 0.27$) from those observed in homozygous (AA) individuals (0.06 ± 0.163). Furthermore, HLA-B53 class I antigen was also shown to provide protection against severe malaria in an association study in the Gambia,[8] but a recent study showed no association between HLA class II alleles and malarial mild attack.[11] Therefore, the apparent discrepancy between these results may be related to the fact that most suspected malaria resistance alleles, both within the MHC region as well as those related to red blood cell genetic disorders,[7] confer more protection against severe forms than against mild ones,[6,9] and do not seem to have a major influence on blood infection levels. Nevertheless, the genetic control of parasite infection levels have been recently confirmed for *Schistosoma mansoni* infection by means of linkage analysis. A locus controlling the intensity of infection have been localized on chromosome 5q31-q33, a chromosomal region containing several candidate genes encoding immunologic molecules.[34]

Our results did not confirm the first segregation analysis performed in Edea (southern Cameroon) that provided clear evidence for the presence of a recessive major gene involved in the control of the degree of infection in human malaria.[16] In this study, parameter estimates showed a frequency of approximately 0.44 for the deleterious allele, indicating that approximately 21% of the population was predisposed to high infection levels. However, important differences exist between the two studies concerning vectorial-transmission as well as behavioral or environmental factors. Edea is an industrial city and nuclear families involved in the study originated from southern Cameroon villages, were employed by a factory, and settled in the city by the factory more than 10 years ago. Family subjects had free access to well-developed and efficient medical services of the factory ensuring a follow-up in some extent comparable with that observed in industrialized countries. Vectorial-transmission features were different in terms of geographic homogeneity and individuals were certainly less exposed to mosquitoes bites than the subjects of our study who were living in the forest. One of the main consequences of these differences might be that the selective force of malaria was less important, or at least reduced by the efficient health service, even for young children predisposed to high levels of infection. This hypothesis is supported by the evolution of infection levels with age observed in Edea (Figure 3 in the report by Abel and others[16]), where parasitemias were highest among children 9–12 years old and decreased with age after 13–16 years of age. This evolution is in agreement with the hypothesis that young children predisposed to high levels of infection can live long enough to ensure an effective maturing of their immunity, have children, and then transmit the deleterious allele.

In conclusion, our results emphasize the complexity of entomologic, behavioral, immunologic, and genetic relationships involved in vectorial-transmitted parasitic diseases. Consequently, identification of the genetic factors requires better knowledge of all other determinants and further genetic analyses focused on children, and accounting for genetic markers, will be important steps towards the understanding of the genetic control of human malaria.

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