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A Major Genetic Mechanism Involved in Resistance to Malaria in Western Amazonia

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KEY WORDS Western Amazon; segregation analysis; Rondônia; malaria; genetic epidemiology; major gene.

ABSTRACT Malaria, the world's most important cause of infectious morbidity and mortality, has a widespread geographical distribution in the tropical region, while its endemicity is very heterogeneous. The Amazonia region, due mainly to its relatively low demographic index, is characterized as an hypo-endemic region. A population of 182 individuals, living in Portuchuelo, State of Rondônia, Brazil, right bank of the Madeira river (8^0 37' S, 63^0 49' W) was surveyed, in order to ascertain data on the epidemiological aspects of malaria and some other infectious diseases. Two main phenotypes involved with *Plasmodium* infection were studied: a) the presence of symptomless infection *i.e.*, presence of *Plasmodium*, diagnosed either by traditional thick smear or by PCR amplification of Plasmodium ribosomal DNA in blood samples; b) the reported number of previous malaria episodes. Segregation analysis was applied to both phenotypes. There were no evidences of any type of familial mechanisms acting on the distribution of the symptomless phenotype in this population, since there was no significant familial aggregation. As for the number of malarial episodes, this phenotype showed clear signs of Mendelian inheritance. The most parsimonious model includes a co-dominant major gene with frequency of $q = 0.15$ and a small multifactorial component ($H = 0.08$). Due to this frequency and the mode of inheritance, this finding seemed to be independent of the Hb, Fy or G-6-PD polymorphisms.

INTRODUCTION

Although the etiological agent of malaria is known for more than a century, the complexity of this disease has been accumulating as the studies on the field progressed. Not only the variability of the infectious agent and the variability of ecological influences, but also the host's genetic variability became increasingly important since Allison's (1954a,b) pioneer studies showing that one of the alleles of a human polymorphism (β chain of hemoglobin) conferred significant resistance to *Plasmodium falciparum* infection.

Several other genetic mechanisms were also shown to provide protection to both the *falciparum* and the *vivax* types of malaria in the ancient world (for reviews *cf.* Hill 1998; Krieger and Feitosa 1999). The used traits to measure this resistance varied from presence of infection, severity of the disease, formation of special erythrocytes complexes, parasitemia, among others (*cf.* Hill 1998).

The present study aims to investigate the distribution of two different traits associated to the infection of *Plasmodium* among families of an hypo-endemic malarial area of the Western Amazonian region.

SUBJECTS AND METHODS

Almost all population of Portuchuelo (8^o37' 44" S, 63^o 49' 28" W), a small hamlet situated at the left bank of the Madeira river, an important tributary of the Amazon river, was ascertained in a epidemiological survey. Data on 185 individuals distributed within 65 complete or incomplete nuclear families were collected and used on the forthcoming analyses. For more details on sampling and for further information on the studied population *cf.* Camargo et al. (1999); Camargo, Alves and Pereira-da-Silva (1999) and Ferreira et al. (2000).

Two main phenotypes involved with *Plasmodium* infection were studied: a) the presence of symptomless infection, *i.e.*, presence of *P. vivax* or *P. falciparum*, diagnosed by the traditional thick smear and/or by PCR amplification of *Plasmodium* ribosomal DNA in blood samples; b) the reported number of previous malaria episodes.

Segregation analysis was conducted using the unified mixed model (Lalouel et al. 1983) and implemented in the computer program POINTER

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(Lalouel and Morton 1981; Morton et al. 1983). The mixed model assumes that a phenotype is composed of independent and additive contribution from a major gene locus, a multifactorial component, and a random environmental component. The major gene effect results from segregation at a single locus having two alleles (*A, a*), where the means and variances are estimated, and up to three distributions in Hardy-Weinberg proportions are fitted.

There are seven parameters in the model: the overall variance (V); the overall mean (m); the allele frequency (*q*), which determines the relative proportion of the component distribution with the highest mean (q^2); the displacement between the two extreme component means (*t*); and the relative position of the mean of the heterozygote, or dominance (*d*); and two parameters representing the multifactorial heritabilities in children (*H*) and parents (*HZ*).

The transmission pattern from parents to offspring can be tested through estimated transmission probabilities (τ_1, τ_2, and τ_3), *i.e.* it is verified if the gene is segregating according to Mendelian expectations. For a single diallelic locus, the three τ_s denote the probabilities of transmitting allele *A* for genotypes *AA, Aa*, and *aa*, with Mendelian expectations of 1, 1/2, and 0, respectively. Under Mendelian transmission τ_1 = 1, τ_2 = 1/2, τ_3 = 0, and no transmission of the major effect is obtained when the three t_s are equal ($\tau_1 = \tau_2 = \tau_3$). Parameters are estimated by maximizing the conditional likelihood of the nuclear family data (*i.e.*, the likelihood of offspring conditional on parental phenotypes). Tests of hypotheses for nested model comparisons are carried out using the likelihood ratio test, where the difference in *–2lnL* of a nested model and a general alternative is asymptotically distributed as a χ^2 with degrees of freedom equal to the number of independent parameter restrictions. For non-nested model comparisons, Akaike's (1974) information criterion (AIC) is used. AIC is computed as minus twice the log likelihood of the model plus twice the number of estimated parameters. The model with the lowest AIC indicates the most parsimonious fit to the observed data.

RESULTS AND DISCUSSION

The characteristic symptomless positive PCR for either *P. vivax or P. falciparum* turned out to be distributed randomly in this population, with no signs of familial aggregation. This result is not surprising since it is a pin-point observation in a temporal scale and may not reflect familial profiles.

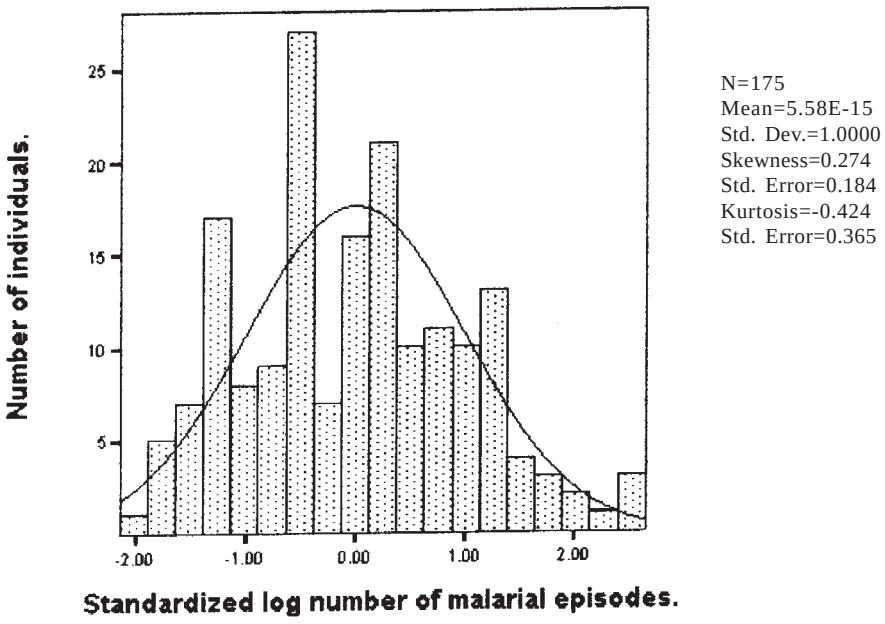

Fig. 1. Distribution of the standardized log number of malarial episodes

Table 1: Segregation analysis of the number of malarial episodes in Portuchuelo, Rondonia State, Brazil

Model	*V*	*μ*	*d*	*t*	*q*	*H*	τ_1	τ_2	τ_3	*-2lnL*	χ^2	*P*	*test*	*AIC*
1. Mixed	0.93	0.02	0.44	3.27	0.15	0.08	[1]	[0.5]	[0]	297.77				309.77
2. Saporadic	0.87	0.05	[0]	[0]	[0]	[0]	-	-	-	329.13	31.36	<001	2 vs. 1	323.13
3. No major effect	0.97	0.07	[0]	[0]	[0]	0.71	[1]	[0.5]	[0]	314.30	31.36	<001	3 vs. 1	320.30
4. No multifactorial	0.88	0.00	0.44	3.13	0.15	[0]	[1]	[0.5]	[0]	298.74	0.97	0.325	4 vs. 1	308.74
5. Recessive (d=0)	0.83	-.01	[0]	1.41	0.51	[0]	[1]	[0.5]	[0]	314.08	15.34	<001	5 vs. 4	322.08
6. Additive (d=0.5)	0.86	0.00	[0.5]	2.82	0.15	[0]	[1]	[0.5]	[0]	299.22	0.48	0.488	6 vs. 4	307.22
7. Dominant (d=1)	0.81	-.05	[1]	1.49	0.13	[0]	[1]	[0.5]	[0]	304.59	5.85	0.016	7 vs. 4	312.59
8. Free τ_S (d=0.5)	0.70	-.17	[0.5]	2.72	0.10	[0]	1.0*	0.28	0.0*	296.76	2.46	0.483	8 vs. 5	310.76
9. Equal τ_S (D=0.5)	0.89	-.05	[0.5]	2.47	0.23	[0]	0.77	0.77	0.77	324.53	27.77	<001	9 vs. 8	332.53

* Estimated parameter reached the bound. Parameters in square brackets were fixed at the value indicated.
V = variance; μ = mean: d = dominance; t = displacement; q = allele frequency; H = multifactorial heritability; τ_S = transmission probabilities parameters (#8 is under the free τ_S model, while #9 is under equal τ_S model); -2 ln L = minus twice the log likelihood.

The number of malarial episodes showed to be a highly variable trait. Therefore, a logarithmic transformation was applied to the data, as well as correction for age and sex. The distribution of the standardized values of this final variable is shown in figure 1. As can be seen, its fit to the normal distribution is rather good, with neither significant skewness nor kurtosis.

The segregation analyses results are shown in Table 1. Compared to the mixed Mendelian model [1] the sporadic model [2] was rejected (models: 2 vs. 1; $\chi^2_4 = 31.36$, $P < 0.001$). The hypothesis of no major effect [3] was rejected (models: 3 vs. 1; $\chi^2_3 = 16.53$, $P < 0.001$), whereas the hypothesis of no multifactorial component [4] was not rejected (models: 4 vs. 1; $\chi^2_1 = 0.97$, $P = 0.325$). In absence of multifactorial component, the recessive and dominant Mendelian modes of inheritance were rejected (models: 5 vs. 4; $\chi^2_1 = 15.34$, $P < 0.001$; models: 7 vs. 4; $\chi^2_1 = 5.85$, $P = 0.016$, respectively), while the additive was not rejected (models: 6 vs. 4; $\chi^2_1 = 0.48$, $P = 0.488$). The transmission probabilities were tested for an additive major gene. The Mendelian hypothesis was not rejected (models: 6 vs. 8; $\chi^2_3 = 2.46$, $P = 0.483$), while the non-transmission hypothesis was rejected (models: 9 vs. 8; $\chi^2_2 = 27.77$, $P < 0.001$). Furthermore, the additive hypothesis is the most parsimonious model determined by AIC criterion. The additive major gene accounted for 60% of the residual phenotypic variance. Approximately 2.3% (q^2) of the sample are homozygous for the high genotype. Therefore, there is evidence of an additive major gene responsible for the number of malaria episodes manifested by an individual.

As seen above, a possible new genetic mechanism seems to be present in this riverine hypo-endemic population. Differently from the unconfirmed results of Abel et al. (1998), the present study used an uncomplicated trait, which solely measured the amount of malarial episodes, while in their study, although also based on segregation analyses, they found evidences of a major gene acting on a distinct trait, parasitemia. It is known that parasitemia is a trait difficult to be handled, since the number of infested red blood cells in a particular moment of the epidemioIogical process depends upon such a variety of factors as to make difficult, if not impossible, to single out a particular causal factor.

The various simple mechanisms known to affect the process of infection were mainly observed in Africa, since there are very few genetic epidemiological surveys in other geographic areas, mainly in hypo-endemic regions, like the Amazon region. Aside its characteristic epidemiological pattern, this region is also typified by the presence of both types of malarial parasites (*vivax* and *falciparum*). Therefore, it is possible that these findings could be restricted to the type of population living under this particular epidemiological pattern.

Finally, it should be emphasized that both the estimated gene frequency of this major genetic mechanism is much higher than the pooled frequencies in Rondônia of the recognized genetic loci involved with malaria (Hb, Fy or G-6-PD) and also that the expression of the detected genetic mechanism is distinct (additive).

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