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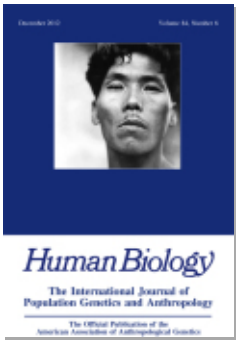

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Brief Communications

Frequencies of ABO, MNSs, and Duffy Phenotypes Among Blood Donors and Malaria Patients from Four Brazilian Amazon Areas

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Abstract We compared the serological phenotypic frequencies of *ABO*, *MNSs*, and Duffy in 417 blood donors and 309 malaria patients from four Brazilian Amazon areas. Our results suggest no correlation between *ABO* phenotype and malaria infection in all areas studied. We observed significant correlation between the S+s+, S+s−, and S−s+ phenotypes and malaria infection in three areas. Some of the Duffy phenotypes showed significant correlation between donors and malaria patients in different areas. These data are an additional contribution to the establishment of differential host susceptibility to malaria.

A large proportion of all malaria cases in South America occurs in the Brazilian Amazon region, where the incidence increased greatly between the 1970s and the early 1990s, with approximately 500,000 cases reported annually. Of the four known human malaria parasites, only *Plasmodium falciparum*, *P. vivax*, and *P. malariae* have been detected in Brazil. Invasion of red blood cells (RBCs) occurs when the extracellular form of the parasite, the merozoite, attaches to the surface of an uninfected RBC. This rapid process includes phases of recognition and attachment followed by reorientation and entry (Bannister and Mitchell 2003). Malaria parasites specifically invade certain species and types of RBCs. The demonstrated specificity of malaria parasites for RBCs of particular species and

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ages appears to depend on a number of ligand-receptor interactions, some of which have been recently defined. RBC receptors related to the invasion process include the *ABO* system, *MNSs* (glycophorins A and B), and the Duffy blood group antigen (Daniels 1997). The present study describes the *ABO*, *MNSs*, and Duffy phenotype frequencies among blood donors and malaria patients from four malaria-endemic regions of the Brazilian Amazon.

Materials and Methods

To be included in the study, the malaria patients ($n = 409$) had to meet the following criteria: They were seeking medical assistance because of clinical malaria symptoms, were older than 18 years old, and had thick blood exam positive results. The control group consisted of blood donors ($n = 417$), and, according to the Brazilian blood bank policy, they filled the following criteria: They were older than 18 years old, of either sex, belonged to any blood group, were asymptomatic for malaria clinical signs, had thick blood film exam negative results, had a place of birth in the studied area, and showed no signs of malaria during early interview. The control subjects were matched to the patients with respect to age (± 5 years), sex, and ethnicity. All the control subjects were genetically independent.

The study subjects came from the following four areas of the Brazilian Amazon: Macapá, Amapá State; Belém, Pará State; Porto Velho, Rondônia State; and Rio Branco, Acre State.

A blood sample was collected from both the patients and the control subjects after informed consent. The *ABO* phenotypes were classified using a hemaglutination standard test. *MNSs* and Duffy (Fy^a and Fy^b antigens) were phenotyped using a microtyping kit (DiaMed-ID Microtyping System, DiaMed AG, Morat, Switzerland). To assess the significance of the variables and to obtain independence among the proportions, we used Fisher's exact test.

The mean ages of patients and control subjects were 29 years ($\pm$ 14 SD) and 28 years ($\pm$ 8 SD), respectively. All the studied groups showed no statistically significant difference in mean ages or ethnicity, indicating a well-matched population. The same results were obtained when we compared both groups in each area.

Results and Discussion

Our results showed similar frequencies of *ABO* phenotypes between blood donors and malaria patients. Regarding the *MNSs* system, we observed higher frequencies of the S+s+ phenotype among *P. falciparum* malaria patients from Belém and Rio Branco, higher frequencies of the S−s+ phenotype in blood donors from Belém, Porto Velho, and Rio Branco, and higher frequencies of the S+s− phenotype in blood donors from Belém ($p < 0.05$). However, there was

no significant association when both S phenotypes were taken into account in Macapá.

The Duffy phenotype analysis showed a higher frequency of the FY A−,B+ phenotype in donors from Macapá, whereas the FY A+,B+ phenotype was more frequent in *P. vivax* malaria patients from the same region and from Belém and Macapá. These findings were significant between both groups ($p < 0.05$). On the other hand, we observed a significant frequency of the FY A−,B− phenotype in blood donors from Belém ($p < 0.05$) (Table 1).

The results presented in this study on *ABO* phenotypes are in accordance with previous reports, because they also showed no association between this blood group system and malaria infection in the Brazilian population from another Amazonian area (Beiguelman et al. 2003) and also in the Amazonian Colombian population (Montoya et al. 1994). Although the A antigen has been implicated as a coreceptor for *P. falciparum*, the effect of *ABO* phenotype on the susceptibility to malaria infection does not seem to be important (Barragan et al. 2000).

Montoya et al. (1994) observed in Colombia that the *MNSs* system seems to confer resistance to malaria infection. Interestingly, we also observed that the S−s+ phenotype in Rio Branco and Porto Velho had a higher frequency among blood donors, and it is possible that this phenotype can contribute to resistance to *P. falciparum* malaria. Beiguelman et al. (2003) suggested no association between MN and Ss phenotypes and malaria infection in the rural area of Rondônia State. This may imply that this population is distinct from other populations investigated in this study.

The higher frequency of the FY A+,B+ phenotype among blood donors also suggests protection against *P. vivax* malaria infection, whereas individuals with the *FY* alleles in heterozygosis (FY A+,B+) can be more susceptible. We also observed that FY A−,B− individuals were less infected by *P. vivax*, according to a previous study of malaria patients in the Western Brazilian Amazon (Rondônia State) (Cavasini et al. 2001).

To our knowledge, this study is the first evaluation of the frequencies of three important blood group phenotypes in malaria patients from four different locations in the Brazilian Amazon region. This study represents an additional contribution to the establishment of differential host susceptibility to malaria, an important public health issue. However, a limitation we acknowledge is related to the fact that, by using blood group phenotyping, we were not able to assess molecular changes, because alterations such as gene promoter disruption or deletion can abolish or reduce antigen expression (Tournamille et al. 1995; Michon et al. 2001; Storry et al. 2001). Therefore we believe that by conducting a genotyping study, we will be able to explore other aspects, such as disruption of a GATA motif in the Duffy gene promoter that can be related to the significant associations between blood group variants and susceptibility or resistance to malaria. Molecular investigations are being conducted in our laboratory to clarify the role of such variants in this group of patients.

Table 1. Frequencies of *ABO*, *MNSs*, and Duffy Phenotypes Among Blood Donors and Patients Infected with *Plasmodium vivax* and *P. falciparum* in the Brazilian Amazon (Samples Collected in 2003–2005)

	Belém			*Macapá*			*Porto Velho*			*Rio Branco*		
		Patients (100)			*Patients (108)*			*Patients (117)*			*Patients (84)*	
Blood Group System	*Donors (100)*	*Pv (85)*	*Pf (15)*	*Donors (117)*	*Pv (64)*	*Pf (44)*	*Donors (100)*	*Pv (83)*	*Pf (34)*	*Donors (100)*	*Pv (56)*	*Pf (28)*
ABO												
A	19.0	25.0	2.0	27.0	17.0	10.0	19.0	22.0	9.0	33.0	15.0	4.0
B	14.0	11.0	4.0	11.0	9.0	6.0	14.0	7.0	3.0	12.0	4.0	3.0
AB	3.0	4.0	–	7.0	–	–	3.0	5.0	2.0	1.0	4.0	–
O	64.0	45.0	9.0	72.0	38.0	28.0	64.0	49.0	20.0	54.0	33.0	21.0
MNSs												
M+N+S+s+	18.0	22.0	7.0[b]	25.0	18.0	12.0	23.0	15.0	7.0	23.0	11.0	4.0
M+N+S+s−	1.0	2.0	–	5.0	1.0	1.0	2.0	6.0	1.0	2.0	–	–
M+N+S−s+	24.0	17.0	3.0	25.0	11.0	11.0	31.0[a]	13.0	7.0	31.0	10.0	7.0
M+N−S+s+	12.0	16.0	2.0	24.0	16.0	6.0	9.0	16.0	6.0	9.0	15.0	9.0[b]
M+N−S+s−	7.0*	1.0	–	4.0	–	1.0	1.0	4.0	3.0	1.0	2.0	1.0
M+N−S−s+	10.0	11.0	–	8.0	6.0	2.0	13.0	17.0	5.0	13.0	11.0	3.0
M−N+S+s+	12.0	10.0	1.0	11.0	5.0	4.0	3.0	4.0	4.0	3.0	3.0	2.0
M−N+S+s−	–	1.0	–	–	–	2.0	–	–	–	–	–	–
M−N+S−s+	16.0[a]	5.0	2.0	15.0	5.0	9.0	18.0[a]	8.0	1.0	18.0[a]	4.0	2.0
Duffy												
FY A+,B−	32.0	25.0	5.0	37.0	18.0	13.0	26.0	25.0	10.0	26.0	13.0	4.0
FY A−,B+	33.0	25.0	9.0	44.0[a]	15.0	7.0	34.0	25.0	12.0	34.0	17.0	7.0
FY A+,B+	27.0	35.0[b]	1.0	28.0	30.0[b]	23.0	37.0	32.0	11.0	37.0	26.0	17.0
FY A−,B−	8.0[a]	–	–	8.0	1.0	1.0	3.0	1.0	1.0	3.0	–	–

To obtain the independence among the proportions, we applied Fisher's exact test ($p < 0.05$).

Pv = *Plasmodium vivax.*

Pf = *Plasmodium falciparum.*

a. $p < 0.05$ in donors.

b. $p < 0.05$ in patients

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