

GENETIC ASPECTS OF *Schistosoma mansoni* INFECTION SEVERITY

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ABSTRACT

Genetic variability of 13 proteins have been investigated in a sample of 61 subjects with hepatosplenic (H) and 61 with intestinal (I) schistosomiasis, living in an endemic area (Catolandia, State of Bahia). Only one significant difference was found in the genotypic distributions between H and I patients. *GLO*1/GLO*1* homozygotes have a relative incidence of hepatosplenic schistosomiasis four times and *GLO*1/GLO*2* heterozygotes three times higher than *GLO*2/GLO*2* homozygotes.

INTRODUCTION

Several investigations have suggested that the severity of *Schistosoma mansoni* infection in Brazil may be related to ethnic group, Whites developing the hepatosplenic infection more frequently than Blacks. The prevalence of the disease, however, seems to be the same in both racial groups (review in Tavares-Neto, 1987). Racial classifications are generally based on morphological characteristics and therefore subjected to misclassification, the problem being greater in trihybrid populations. Is this therefore a spurious association related to errors in racial classification or is there a genetic factor associated with the putative Black resistance to the severe

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(hepatosplenic) form of the disease? To test this we investigated 13 genetic systems, as well as the quantitative degree of racial admixture, in samples of hepatosplenic and intestinal schistosomiasis subjects.

MATERIAL AND METHODS

The samples came from Catolandia, BA (12°08' S, 44°50' W), where schistosomiasis is endemic, 70% of the population being infected with *Schistosoma mansoni* eggs (Tavares-Neto and Prata, 1989).

Blood samples for this study were obtained from 61 hepatosplenic (H) and 61 intestinal (I) schistosomiasis patients. The two series were selected using the following criteria: same sex, same age (± 3 years), about the same socioeconomic level and residence in the same neighborhood. Inclusion in the H group was made when the patient's spleen was palpable on the coastal rim or beyond without inspiration. As for the intestinal schistosomiasis patients, they should have lived in Catolandia for at least 10 years and should have arrived there before age 30. Blood was collected in ACD and refrigerated as soon as possible. The plasma and the packed red blood cells (the latter in vials with an equal proportion of a glycerol solution, as described by Conceição *et al.* (1987) were frozen at -20°C and sent to Porto Alegre, RS, where laboratory determinations were made. The techniques used have been described (Franco *et al.*, 1981; Weimer *et al.*, 1981; Kvitko and Weimer, 1988; Rieger *et al.*, 1988).

Allele frequencies were estimated by gene counting, while the quantitative estimates of racial admixture were obtained by the method of Krieger *et al.* (1965). The relationship between genotypes and forms of the disease was examined using the method of Woolf (1955).

RESULTS AND DISCUSSION

No variation was found in either group for albumin, ceruloplasmin, phosphoglucomutase (locus 2) and phosphogluconate dehydrogenase, all showing the usual types. The phenotypic distributions of the other genetic markers are shown in Table I. Only one significant difference was found between H and I patients. For glyoxalase I there was an excess of the 2-2 phenotype in the intestinal schistosomiasis subjects. The relative incidence of hepatosplenic schistosomiasis in the different GLO phenotypes is shown in Table II. As can be seen, the relative incidence of the hepatosplenic form of the disease is about four times higher in $GLO*1/GLO*1$ homozygotes and three times more frequent in $GLO*1/GLO*2$ heterozygotes than in $GLO*2/GLO*2$ homozygotes.

Table I - Phenotype distributions of schistosomiasis patients in Catolandia, BA, Brazil.

Systems	Phenotypes	H ^a		I ^b	
		No.	%	No.	%
Adenylate kinase - AK	1-1	55	93	58	98
	2-1	4	7	1	2
Phosphoglucomutase, locus 1 - PGM1	1-1	26	46	35	58
	2-1	29	51	19	32
	2-2	2	3	6	10
Glucose-6-phosphate dehydrogenase - G6PD ^c	B	40	95	41	93
	AB	1	2	1	2
	A'B	1	2	1	2
	AA ⁻	0	0	1	2
Acid phosphatase - ACP	B	32	54	29	48
	AB	25	42	28	47
	A	1	2	2	3
	BR	1	2	1	2
Esterase D-ESD	1-1	32	57	40	68
	2-1	21	38	15	25
	2-2	3	5	4	7
Glyoxalase I - GLO	1-1	10	18	5	8
	2-1	28	50	19	34
	2-2	18	32	35	58
Haptoglobin - HP	1-1	17	28	14	23
	2-1	31	51	37	61
	2-2	8	13	10	16
	0	5	8	0	0
Hemoglobin - HB	A	59	97	57	93
	AS	2	3	4	7
Transferrin - Tf	C	61	100	59	98
	CD	0	0	1	2

H^a: Hepatosplenic schistosomiasis patients; I^b: Intestinal schistosomiasis patients.

^c: Sex distribution of the individual tested. H: 23 males and 19 females; I: 21 males and 23 females.

Table II - Relative incidence of GLO phenotypes in hepatosplenic versus intestinal schistosomiasis.

Patients	Phenotype comparisons		Relative incidence	χ^2	P
	1-1 vs 2-2				
Hepatosplenic	10	18	3.89	4.81	< 0.05
Intestinal	5	35			
	1-1 vs 2-1				
Hepatosplenic	10	28	1.36	0.24	> 0.50
Intestinal	5	19			
	2-1 vs 2-2				
Hepatosplenic	28	18	2.86	6.43	< 0.05
Intestinal	19	35			

Glyoxalase is a ligase that catalyses the conversion of glutathione and methylglyoxal into S-lactoylglutathione. Since methylglyoxal can inhibit cell division, Beretta *et al.* (1983) suggested that GLO plays a role in this process. GLO activity in *GLO*1/GLO*1* homozygotes is only 91%-92% of that of *GLO*2/GLO*2* homozygotes (Parr *et al.*, 1977; Sparkes *et al.*, 1983). The circulating levels of methylglyoxal and glutathione could therefore be higher in *GLO*1/GLO*1* subjects and those of S-lactoylglutathione greater in *GLO*2/GLO*2* subjects. Could these metabolites interfere in the egg production of the parasites or in the resistance to reinfection? Schistosomiasis originated from Africa and there the disease now runs a benign course (see review in Prata and Schroeder, 1967). In Blacks *GLO*1* has a frequency of 28%, while in European Whites it is 44%. The lower frequency of this allele in Blacks could perhaps be related to selection against it in hyperendemic schistosomiasis areas at the time when the hepatosplenic form was common. Of course, there is no guarantee that the activities obtained *in vitro* are reproduced *in vivo*; and since the differences in activity between the two homozygotes are small, they may account for only a part of the gene frequency differences. But the hypothesis is reasonable and can be tested in other populations or models.

The GLO locus has been mapped on chromosome 6, exactly at 6p21.3 - 6p21.2, at about 3 cM from the HLA region. An association between the HLA A1/B5 haplotype and the severe form of schistosomiasis has been reported (Salam *et al.*, 1979; Kamel *et al.*, 1984). Therefore, another possibility is that the association between

GLO and hepatosplenic schistosomiasis is a reflection of a differential susceptibility linked to HLA differences.

Due to the previously reported association between the prevalence of the forms of schistosomiasis and race, a quantitative estimate of the relative contributions of Africans, Caucasians and Indians to the two groups of patients was performed, considering eight systems. For these estimates we used as parental frequencies data from Africa's West Coast, Portugal and Spain, and Tupi and Ge Indians respectively. They were compiled from Franco *et al.* (1982), Conceição *et al.* (1987), Salzano and Callegari-Jacques (1987) and Roychoudhury and Nei (1988), and are presented in Table III. The errors attached to each estimate are large, especially among the hepatosplenic patients. But the difference between the two series (H vs I) in relation to Black ancestry is much larger if the GLO system is included (16 vs 42) than if it is excluded (17 vs 31). Moreover, the GLO results do not show a good fit with those of the other systems, in the I series, the respective chi-square (9.2, 2 degrees of freedom) being significant at the 1% level. We conclude that the proposed resistance of Blacks to the hepatosplenic form of *Schistosoma mansoni* infection may be related to an association with the glyoxalase system only, or to another genetic marker (HLA?) not investigated in this paper.

Table III - Allele frequencies postulated to be present in the ancestors of the Catolandia population.

System	Parental frequencies			Catolandia frequencies	
	Black	Indian	White	H ^a	I ^b
<i>Ak*1</i>	.997	1.000	.963	.97	.99
<i>Ak*2</i>	.003	0.000	.037	.03	.01
<i>PGM1*1</i>	.825	.842	.740	.71	.74
<i>PGM1*2</i>	.175	.158	.260	.29	.26
<i>GLO*1</i>	.277	.419	.439	.43	.25
<i>GLO*2</i>	.723	.581	.561	.57	.75
<i>ACP*A</i>	.156	.137	.373	.23	.27
<i>ACP*B</i>	.821	.863	.627	.76	.72
<i>ACP*R</i>	.023	.000	.000	.01	.01

Continued

Table III - Continued.

System	Parental frequencies			Catolandia frequencies	
	Black	Indian	White	H ^a	I ^b
<i>ESD*1</i>	.900	.620	.853	.76	.80
<i>ESD*2</i>	.100	.380	.147	.24	.20
<i>Hp*1</i>	.654	.603	.403	.58	.53
<i>Hp*2</i>	.346	.397	.597	.42	.47
<i>Hb*A</i>	.915	1.000	1.000	.98	.97
<i>Hb*S</i>	.085	.000	.000	.02	.03
<i>Tf*C</i>	.965	1.000	1.000	1.00	.99
<i>Tf*D</i>	.035	.000	.000	.00	.01

	Admixture estimates (%)					
	Including the GLO system			Excluding the GLO system		
	Black	Indian	White	Black	Indian	White
^a H	16 ± 25	38 ± 34	46 ± 33	17 ± 29	38 ± 37	45 ± 35
^b I	42 ± 13	17 ± 13	41 ± 14	31 ± 7	22 ± 7	47 ± 8

^aH: Hepatosplenic schistosomiasis patients.

^bI: Intestinal schistosomiasis patients.

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RESUMO

A variabilidade genética em 13 sistemas proteicos foi investigada em uma amostra de 61 pacientes com hepatosplenomegalia (H) esquistossomótica e 61 com a forma intestinal (I) da doença.

provenientes de uma região endêmica (Catolandia, Estado da Bahia). Foi verificada apenas uma diferença significativa nas distribuições genotípicas de pacientes H e I. Os homozigotos *GLO*1/GLO*1* têm uma incidência relativa da forma hepatosplênica 4 vezes maior e os heterozigotos *GLO*1/GLO*2* 3 vezes maior do que os homozigotos *GLO*2/GLO*2*.

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