

## PLACENTAL POLYMORPHISMS IN SOUTHERN BRAZIL AND THEIR RELATIONSHIP TO RACE AND MOTHER-CHILD INTERACTIONS

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### ABSTRACT

A total of 440 placentas were studied for alkaline phosphatase (PL), soluble (ACONS) and mitochondrial (ACONM) aconitase and acid alpha-glucosidase (alpha-GLUA) systems. In addition, the mothers' plasmas were examined for placental alkaline phosphatase levels. Nineteen (two apparently undescribed) phenotypes were observed in the PL system, 94% corresponding to combinations between the three common *PL\*1*, *PL\*2* and *PL\*3* alleles. ACONM showed no variability, while in the ACONS and alpha-GLUA systems 96% of the individuals were respectively *ACONS\*1/ACONS\*1* and alpha-GLUA\*1/alpha-GLUA\*1. PL and ACONS distributions vary with race, *PL\*1* and *ACONS\*4* being good markers of African ancestry. On the other hand, variability in PL, ACONS and alpha-GLUA showed no association with birth and placental weight, or Apgar indices. PL phenotype prevalences also did not differ in ABO or Rh compatible and incompatible matings. Equally negative results were obtained for the relationships between maternal placental alkaline phosphatase levels and PL phenotypes, and between the former and the perinatal factors indicated above.

### INTRODUCTION

Due to the importance of the placenta in human reproduction, it is to a certain extent surprising that the information available concerning its genetic variability is much less than that for blood. This is certainly due to the fact that it is

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easier to obtain blood than placenta samples. But the latter are not so difficult to secure, and the understanding of the role of polymorphisms in our and other species must consider variation among tissues and at different (DNA, protein) levels. In Brazil, previous genetic studies in placentas involved four systems (aconitase, hexokinase, phosphoglucomutase 3, alkaline phosphatase) and three populations (Salvador, Aracaju, Porto Alegre) only (Azevêdo *et al.*, 1979, 1983; Aguiar *et al.*, 1980; Silva *et al.*, 1985; Sousa *et al.*, 1986; Kvitko and Weimer, 1988). The present report extends the data available for alkaline phosphatase, the mitochondrial and soluble forms of aconitase, and furnishes the first results concerning acid alpha-glucosidase in Brazilian groups. The distribution of these alleles will be considered in relation to race differences and to a series of parameters which reflect perinatal health.

### MATERIAL AND METHODS

Placentas and chord bloods were obtained at the moment of parturition at the Maternidade Mario Totta of Porto Alegre's Santa Casa de Misericórdia. Immediately afterwards a sample of blood was collected from the mother and both the placenta and bloods placed in an ice box and transported to the laboratory.

The following day an interview was performed with the mother and attending physician, in which information concerning pregnancy, childbirth and the baby's condition were registered. A racial classification of the mother and her child was then made, considering skin color, nose shape, hair type, and lip thickness. In the children genitalia color would also be verified, since it is not easy to establish unequivocally newborns' skin color. We therefore adopted a four-scale classification for mothers (White, Light Mulatto, Dark Mulatto and Black), but a three-scale only for babies (Light, Medium, Dark).

Blood samples were centrifuged at 2,000 rpm for five minutes at 4°C, and the plasmas stored at -20°C until testing. The level of placental alkaline phosphatase was determined in the maternal plasmas according to the technique described by Fishman *et al.* (1972). Red cells were washed in isotonic saline and tested for the ABO and Rh blood groups using standard procedures, commercial antisera, and an extract from *Ulex europaeus* prepared in our laboratory as described by Boyd and Shapleigh (1954). Finally, the placental samples were handled and tested according to the techniques presented in Harris and Hopkinson (1978).

### RESULTS

Table I presents the phenotype and Table II the allele frequencies observed in the alkaline phosphatase system. Some of the patterns found are reproduced in

Figure 1, the respective diagram being shown in the upper part of Figure 2. In the lower part of this figure a general outline of the pattern expected in homozygotes for 19 alleles described by Donald and Robson (1974) and in the present communication is shown.

Table I - Phenotype frequencies in the placental alkaline phosphatase system considering the racial classification of the newborn.

Phenotype	Racial classification							
	Light		Medium		Dark		Total	
	N	%	N	%	N	%	N	%
1	100	42	92	59	33	69	225	51
2-1	75	32	32	21	9	19	116	26
2	18	8	10	6	1	2	29	7
3-1	14	6	10	6	2	4	26	6
3-2	8	3	3	2	1	2	12	3
3	3	1	1	< 1	0	-	4	1
4-1	0	-	1	< 1	0	-	1	< 1
4-2	2	1	1	< 1	0	-	3	< 1
5-2	6	3	1	< 1	0	-	7	2
8-1	1	< 1	0	-	0	-	1	< 1
9-1	1	< 1	1	< 1	0	-	2	< 1
9-3	1	< 1	0	-	0	-	1	< 1
10-2	1	< 1	0	-	0	-	1	< 1
PA1-10	1	< 1	2	1	1	2	4	1
12-3*	1	< 1	0	-	0	-	1	< 1
12-4*	3	1	0	-	1	2	4	< 1
16-2	1	< 1	0	-	0	-	1	< 1
18-3	0	-	1	< 1	0	-	1	< 1
PA1-PA2	1	< 1	0	-	0	-	1	< 1
Total	237	-	155	-	48	-	440	-

\* Since we could not test the neuraminidase effect in these samples, it is possible that in some the responsible allele is the less frequent *PL\*13* and not *PL\*12*.

Table II - Allele frequencies in the placental alkaline phosphatase system considering the racial classification of the newborn.

Allele	Racial classification			Total
	Light	Medium	Dark	
<i>PL*1</i>	0.614	0.736	0.802	0.677
<i>PL*2</i>	0.272	0.184	0.126	0.225
<i>PL*3</i>	0.063	0.052	0.032	0.056
<i>PL*4</i>	0.011	0.007	0.010	0.009
<i>PL*5</i>	0.013	0.003	-	0.008
<i>PL*8</i>	0.002	-	-	0.001
<i>PL*9</i>	0.004	0.003	-	0.003
<i>PL*10</i>	0.004	0.006	0.010	0.006
<i>PL*12*</i>	0.009	-	0.010	0.006
<i>PL*16</i>	0.002	-	-	0.001
<i>PL*18</i>	-	0.003	-	0.001
<i>PL*PA1</i>	0.004	0.006	0.010	0.006
<i>PL*PA2</i>	0.002	-	-	0.001

\* See the footnote of Table I.

Of the 19 phenotypes observed, 94% correspond to combinations between the common alleles *PL\*1*, *PL\*2* and *PL\*3*. The remaining 6% are composed of heterozygotes between one common and one rare, or two rare alleles. Patterns PA1-10 and PA1-PA2 were interpreted as arising due to the presence of *PL\*10* and two new rare alleles, designated *PL\*PA1* and *PL\*PA2* (PA being an abbreviation of Porto Alegre; we avoided giving numbers in sequence to 18 due to the absence of a reference center in which the variant samples could be compared and their distinctness established without doubt).

Phenotype distribution is in accordance with that expected under Hardy-Weinberg equilibrium. Combining all the other alleles, besides *PL\*1* and *PL\*2*, and comparing the three sets of figures in the three different racial groupings yields a clear difference ( $\chi^2$ : 20.1; 4 d.f.;  $P < 0.001$ ). There is an increase in frequency of *PL\*1* and a decrease of *PL\*2* as African ancestry increases.

Table III shows the results for the soluble aconitase system. Five phenotypes in Hardy-Weinberg equilibrium have been observed, explicable by the presence of four alleles (*ACONS\*1* to *ACONS\*4*). Most individuals (96%) are

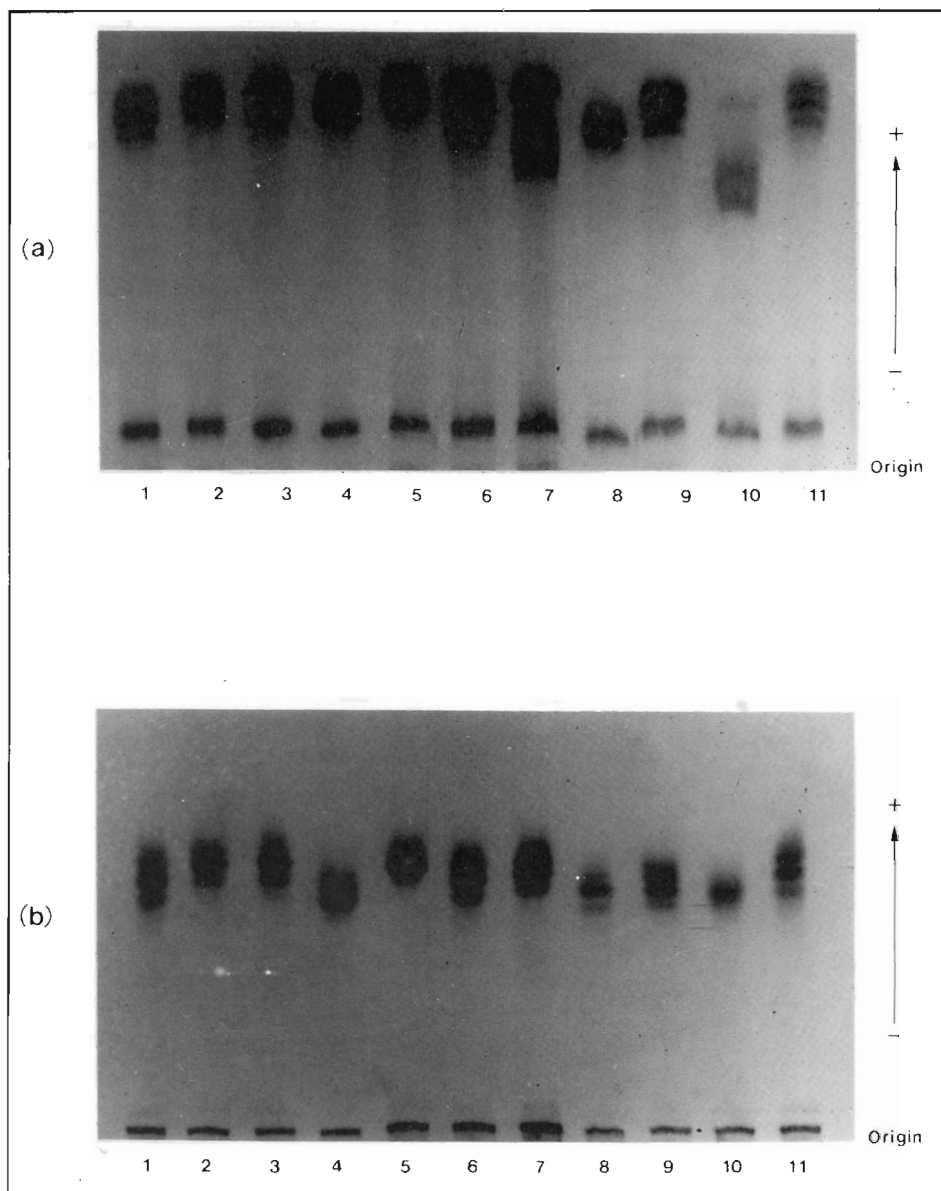


Figure 1 - Starch gel electrophoretic patterns from heterozygotes for some of the rare PL alleles found in the present study. (a) In pH 8.6; (b) in pH 6.0. Samples 1, 6 and 11:2-1 controls; 2 and 3: PA1-10; 4:12-3; 5:10-2; 7:16-2; 8:12-4; 9:PA1-PA2; 10:18-3. In relation to the samples 12-3 and 12-4, see the footnote of Table 1.

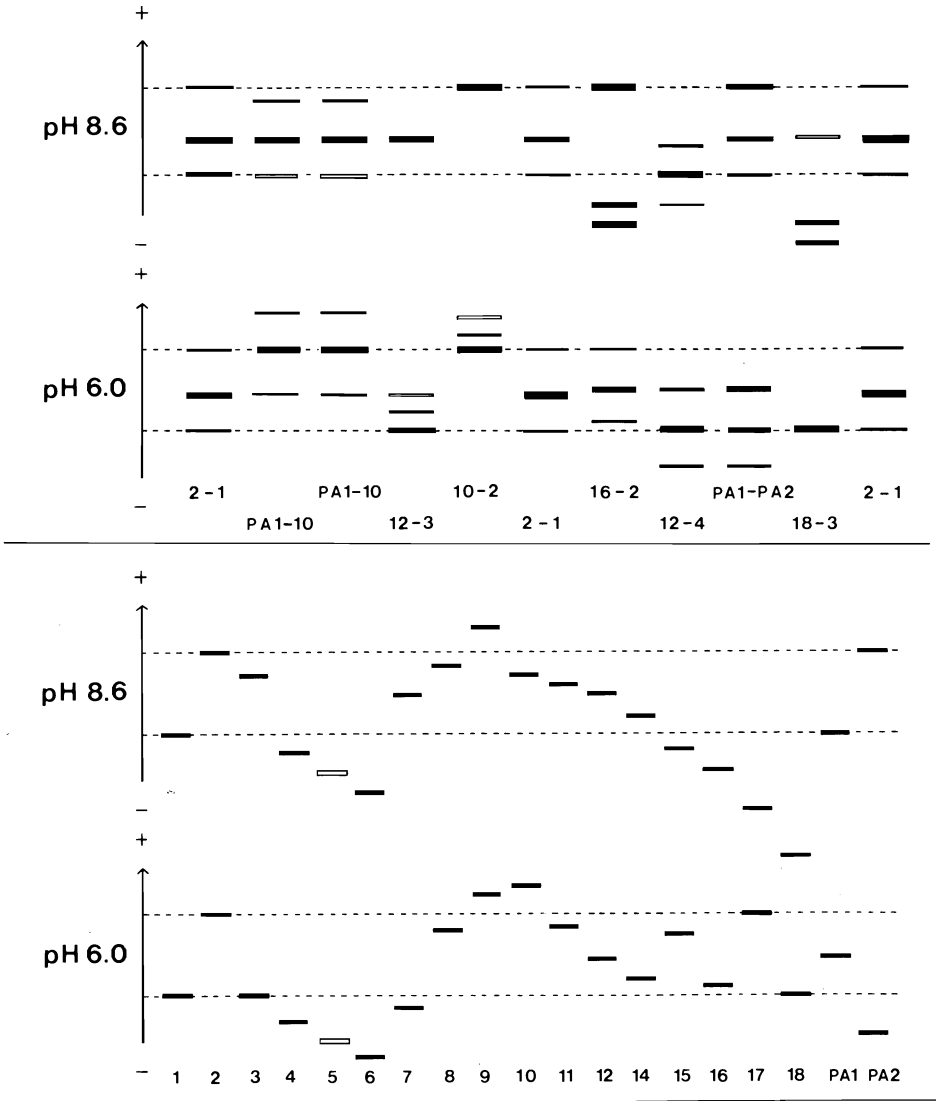


Figure 2 - Upper part: diagram of the electrophoretic patterns shown in the photographs of Figure 1. Lower part: diagram of the electrophoretic patterns expected in homozygotes for the alleles present in the different heterozygotes described by Donald and Robson (1974) and in the present work. The dotted lines indicate the relative positions of the bands synthesized by *PL\*1* and *PL\*2*. Bands 12 and 13 have the same electrophoretic mobility in the indicated buffers, being differentiated by the action of neuraminidase, which modifies the migration of band 13 but not of 12.

*ACONS\*1/ACONS\*1* homozygotes, *ACONS\*4* being the next most frequent allele. Again, there is a clear difference in the distribution of *ACONS\*4* and the other alleles according to race ( $\chi^2$ : 13.6; 2 d.f.;  $P < 0.01$ ), *ACONS\*1* decreasing and *ACONS\*4* increasing with rising African influence. Five of the placentas did not show detectable soluble aconitase activity, despite the fact that mitochondrial aconitase level was normal in each. The mitochondrial aconitase locus showed no variation in the 440 individuals tested.

Table III - Phenotype and allele frequencies in the soluble aconitase system considering the racial classification of the newborn.

Phenotype		Racial classification			Total
		Light	Medium	Dark	
1	N	228	147	42	417
	%	97	96	88	96
2-1	N	0	1	0	1
	%	-	< 1	-	< 1
3-1	N	1	0	0	1
	%	< 1	-	-	< 1
4-1	N	5	5	5	15
	%	2	3	10	3
4	N	0	0	1	1
	%	-	-	< 1	< 1
Total		234	153	48	435*
Allele					
<i>ACONS*1</i>		0.987	0.981	0.927	0.978
<i>ACONS*2</i>		-	0.003	-	0.001
<i>ACONS*3</i>		0.002	-	-	0.001
<i>ACONS*4</i>		0.011	0.016	0.073	0.020

\* In five placentas no detectable soluble aconitase activity was found.

The data on the acid alpha-glucosidase system are given in Table IV. Three phenotypes, conditioned by two alleles, were observed. The distributions are in Hardy-Weinberg equilibrium, without significant interracial differences.

Table IV - Phenotype and allele frequencies in the acid alpha-glucosidase system considering the racial classification of the newborn.

Phenotype		Racial classification			Total
		Light	Medium	Dark	
1	N	228	151	43	422
	%	96	97	90	96
2-1	N	8	4	5	17
	%	3	3	10	3
2	N	1	1	0	1
	%	< 1	-	-	< 1
Total		237	155	48	440
Allele					
<i>alfa-GLUA*1</i>		0.979	0.987	0.948	0.978

A search for an association between allele distribution in these three systems and the variables birth and placental weight, as well as Apgar index (an indication of the child's health at birth), was made and yielded negative results. Also, PL phenotype prevalences did not differ in ABO or Rh compatible and incompatible matings.

No association of maternal placental alkaline phosphatase levels with PL phenotypes and birth and placental weights was observed. These levels were also not significantly different in ABO or Rh compatible or incompatible marriages.

## DISCUSSION

Previous studies in Brazilian groups were performed in relation to the PL and ACONS systems only. The former was investigated in Salvador, Bahia (Silva *et al.*, 1985). Differences in distribution between Porto Alegre and Salvador occur mainly in the Caucasoid fraction of the two populations, and can be explained by a higher degree of Black admixture in Salvador. But since people from that city may also have a certain fraction of Ameridian ancestry the influence of this part of their gene pool cannot be completely dismissed. At present there are no studies of the PL locus in Brazilian Indians. It is important to emphasize, also, that seven "new" alleles observed in Salvador were not found in Porto Alegre, while *PL\*PA1* and *PL\*PA2*

were not encountered in Salvador. The high variability of the placental alkaline phosphatase locus may condition patterns that are almost unique to each population sampled. But this conclusion should await more detailed comparative analyses at both the protein and DNA levels for eventual confirmation or refutation.

As for the soluble aconitase system, the frequencies observed in Salvador and Aracaju (Azevêdo *et al.*, 1979) do not differ in a significant way from those found in Porto Alegre, although there is more similarity of the Porto Alegre prevalences with Aracaju, as would be expected from the racial composition of these two populations.

The clear association of PL and ACONS alleles with racial differences indicates that they can be instrumental in studies aiming to quantify gene exchange between these segments of the Brazilian population, an approach that can also be useful from a historical point of view (Salzano, 1987; Conceição *et al.*, 1987; Schneider *et al.*, 1987; Santos *et al.*, 1987).

Investigations of populational distributions of acid alpha-glucosidase alleles are still relatively scarce. The surveys conducted by Swallow *et al.* (1975) in Europe and USA, Teng and Tan (1979) in Asia, Blake (1984) in Asia and Oceania, and Nickel and McAlpine (1982) in Canada furnished results similar to those obtained here, *alpha-GLUA\*1* uniformly displaying (with one exception) frequencies above 97%.

The observation of five placentas in 440 (1%) without detectable soluble aconitase activity is curious. Azevêdo *et al.* (1979) also could not detect the activity of this enzyme in 6%-7% of their placenta specimens. Sample deterioration is the first obvious explanation for these results, but homozygosis for a "silent" allele can also be considered. In this connection, we verified that such an allele with a frequency of homozygosis of 1% is not incompatible with the phenotype distribution found in Porto Alegre.

Beckman *et al.* (1969); Beck and Ananthakrishnan (1974); Das *et al.* (1975); and Chakraborty *et al.* (1975) were unable, as ourselves, to detect any relationship between PL phenotypes and birth and placental weights. On the other hand, Bottini *et al.* (1972) reported an association between these phenotypes and ABO incompatibility, that we could not confirm. Since sample sizes were not markedly different in the two investigations, this factor cannot be considered to explain the difference. Only additional studies can settle this issue.

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## RESUMO

Um total de 440 placentas foram estudadas para os sistemas da fosfatase alcalina (PL), da aconitase solúvel (ACONS) e mitocondrial (ACONM), e da alfa-glicosidase acida (alfa-GLUA). Além disto, os plasmas dessas mulheres foram examinados quanto aos níveis de fosfatase alcalina placentária. Dezenove fenótipos (dois aparentemente ainda não descritos) foram observados no sistema PL, 94% correspondendo a combinações entre os três alelos comuns *PL\*1*, *PL\*2* e *PL\*3*. ACONM não mostrou variabilidade, enquanto nos sistemas ACONS e alfa-GLUA 96% dos indivíduos eram respectivamente *ACONS\*1/ACONS\*1* e *alfa-GLUA\*1/alfa-GLUA\*1*. As distribuições de PL e ACONS variam com a raça, *PL\*1* e *ACONS\*4* sendo bons marcadores de ancestralidade africana. Por outro lado, a variabilidade em PL, ACONS e alfa-GLUA não mostrou associação com o peso ao nascer ou placentário, nem com os índices Apgar. As prevalências nos fenótipos PL também não diferiram em casamentos compatíveis ou incompatíveis para ABO ou Rh. Resultados igualmente negativos foram obtidos para as relações entre os níveis de fosfatase alcalina placentária e os fenótipos PL, ou entre os primeiros e os fatores perinatais acima indicados.

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