

PHOSPHOGLUCOMUTASE PROTEINS IN HUMAN MILK

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ABSTRACT

Phosphoglucumutase polymorphism observed at the PGM 4 locus was investigated in 652 colostrum samples (collected 24-48 hs. post partum) in Porto Alegre, Brazil. From 175 of these women, milk samples were also obtained at about 17 days of lactation. In colostrum a great deviation from Hardy-Weinberg expectations was found, with an excess of homozygotes and deficit of heterozygotes. In milk, on the other hand, the phenotype distributions were in accordance with the Hardy-Weinberg law. The differences between the two distributions are due to the computation, in the second analysis, of samples without enzyme activity in colostrum, as well as a possible variability in enzyme activation which may occur in the post partum period. The gene frequencies observed in milk ($n = 175$) were: Whites ($n = 127$) $PGM4^*1 = 0.20$, $PGM4^*2 = 0.41$, $PGM4^*3 = 0.38$ and $PGM4^*4 = 0.01$; Blacks ($N = 48$), $PGM4^*1 = 0.15$, $PGM4^*2 = 0.52$, $PGM4^*3 = 0.32$ and $PGM4^*4 = 0.01$.

INTRODUCTION

A new phosphoglucumutase locus, PGM 4, expressed in human milk has been described by Cantú and Ibarra (1982). They studied a Mexican mixed population and interpreted the results as due to the occurrence of four alleles with polymorphic frequencies.

Phosphoglucumutase acts in the production of glucose-1-phosphate, the first intermediate in the pathway to the synthesis of the galactose moiety of lactose, the main carbohydrate of milk (White *et al.*, 1973). Cantú and Ibarra (1982) suggested then that the need for an efficient enzyme to catalyze this reaction has probably been an evolutionary selective force favoring the appearance of an independent locus, with such a localized effect.

In this paper we describe the phenotype and gene distributions found for PGM 4 in a sample from Porto Alegre, the capital of Brazil's Southernmost State, Rio Grande do Sul. It will be seen that the study of this system poses some problems that do not occur in polymorphisms expressed in blood.

MATERIAL AND METHODS

Porto Alegre has about one million inhabitants, 84% of whom are Whites of mainly Portuguese descent; the remaining are predominantly of African extraction, the presence of Asiatics amounting to only one in one thousand. Information about other genetic markers are further details can be found in Franco *et al.*, (1982).

Samples of colostrum were obtained by pump or manually from 652 women (60% Whites and 40% Blacks), 24 to 48 hours after delivery. From 175 of these women, samples of milk were also obtained at about 17 days of lactation. The samples were put in an ice bath shortly after collection, carried to the laboratory as soon as possible, and kept at 4°C until analysis, which was performed within a maximum of two weeks after collection.

The samples were defatted by adding CCl₄ (1:1), as recommended by Cantú and Ibarra (1982), followed by centrifugation at 3000 r.p.m. for 20 minutes.

The phenotypes were identified by horizontal starch gel electrophoresis according to Spencer *et al.* (1964), using the buffer dilution suggested by Blake and Omoto (1975) and putting on each run, blood samples of known phenotype as cocontrols.

RESULTS AND DISCUSSION

Table I shows the results observed in colostrum samples. As can be seen, 8 phenotypes were found, that can be explained by the segregation of four alleles as suggested by Cantú and Ibarra (1982). Two findings in these data, however, are noteworthy: the first is a large deviation from Hardy-Weinberg expectations in both ethnic groups: Whites - $\chi^2 = 89.9$; 5 df.; $P < 0.001$; Blacks; $\chi^2 = 108.02$; 5 df.; $P < 0.001$. There are in general an excess of homozygotes and deficit of heterozygotes. The second is that 15% of the samples do not show any detected pattern. In these cases sample concentration was made in repeat runs by three methods: a) using rods of dried hydrophilic polyacrylamide gels to remove water (Curtain, 1964); b) evaporating the samples at room temperature with ventilation; and c) using in electrophoresis two sheets of filter paper Whatmann 17 mm. Despite these concentration techniques, it was not possible to detect any phenotype in these samples. No quantitative analysis was made, but it seems that the lack of a detectable pattern in them is due to a very low activity of this locus or to a delay in enzyme activation. Gestational age or the number of previous pregnancies do not seem to have effect on such presumed enzyme activation.

Table I - PGM 4 phenotypes and putative gene frequencies in colostrum samples.

Phenotypes	Whites		Blacks		Total
	Number	%	Number	%	
1-1	30	9	23	10	53
2-1	51	15	18	8	69
2-2	74	22	59	27	133
3-1	19	6	15	7	34
3-2	84	25	45	21	129
3-3	66	20	47	21	113
4-1	8	2	11	5	19
4-2	2	1	1	1	3
Number	334		219	15	553
Without activity	59	15	40		99
Total	393		259		652
Putative gene frequencies					
<i>PGM4*1</i>	0.21		0.20		
<i>PGM4*2</i>	0.43		0.42		
<i>PGM4*3</i>	0.35		0.35		
<i>PGM4*4</i>	0.01		0.03		

Cantú and Ibarra (1982) do not refer to Hardy-Weinberg deviations or absence of patterns in their Mexican sample; but they did not study colostrum samples, only milk collected from 3 to 10 days after delivery. We then reinvestigated 175 women at about 17 days of lactation (73% Whites and 27% Blacks). As can be seen in Table II, there is a small difference in gene frequencies between the two racial groups, but the phenotype distributions do not differ statistically. There is also no significant Hardy-Weinberg deviation (Whites - $\chi^2 = 7.5$; 4 df.; $P > 0.10$; Blacks - $\chi^2 = 2.2$; 3 df.; $P > 0.50$) and only one woman shows no activity of PGM 4 in milk at 15 days post partum. The gene frequencies are somewhat dissimilar from those observed in the Mexican population (*PGM4*1*: 0.35; *PGM4*2*: 0.47; *PHM4*3*: 0.11; *PGM4*4*: 0.06; Cantú and Ibarra, 1972), perhaps due to differences in the ethnic composition of the two populations.

The differences between colostrum and milk phenotype distributions are due to two reasons. The first was the computation, in the second analysis, of samples without enzyme activity in colostrum. The second and most interesting is that 36% of the woman who had detectable patterns in colostrum showed different phenotypes

Table II - PGM phenotypes and gene frequencies in milk samples.

Phenotypes	Whites		Blacks		Total
	Number	%	Number	%	
1-1	4	3	1	2	5
2-1	24	19	8	17	32
2-2	14	11	11	23	25
3-1	17	13	3	6	20
3-2	50	40	20	42	70
3-3	14	11	4	8	18
4-1	1	1	1	2	2
4-2	1	1	0	0	1
4-3	1	1	0	0	1
Number	126		48		174
Without activity	1	1	0	0	1
Total	127		48		175
Gene frequencies					
<i>PGM4*1</i>	0.20		0.15		
<i>PGM4*2</i>	0.41		0.52		
<i>PGM4*3</i>	0.38		0.32		
<i>PGM4*4</i>	0.01		0.01		

in milk. When discordant phenotypes between colostrum and milk of the same woman were found the samples were run side by side in the same gel electrophoresis, to check the results. The differences were always confirmed. Figure 1 shows two examples of women who presented different patterns in colostrum and milk. As can be seen in Table III, the phenotype changes always consisted in the alteration of putative homozygotes to heterozygotes, with the maintenance of all bands of the homozygote plus the appearance of new ones.

Three explanations can be given for these findings. The first is technical errors. If these occurred, however, they should not have been important, since care was taken in the preservation and testing of the samples. Storage changes were found if the samples were frozen, but not when they were kept at 4°C. The only storage effect seen on the refrigerated milks was a gradative weakening of the patterns after 15 days from collection. All analyses were made, however, as soon as possible. To be sure that there were no technical interference some samples were tested more than once, and the same result was always found. On the other hand, when a woman showed different phenotypes in the two periods, in most cases it was possible to compare and confirm the results side by side in the same migration.

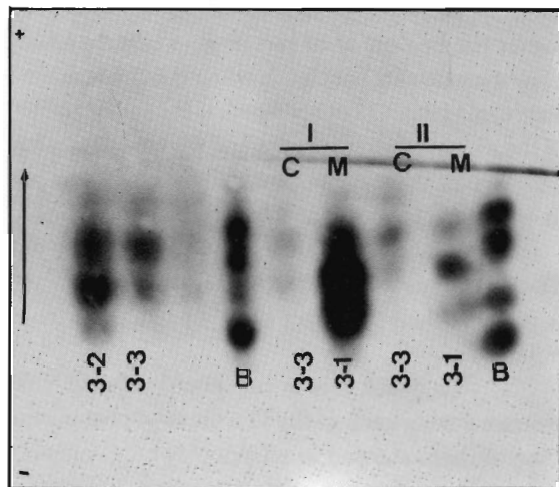


Figure 1 - Phenotypic changes between the colostrum (C) and milk (M) of two women (I and II). B-blood.

Table III - Types and number of changes observed in subjects with activity in colostrum, and retested in milk.

Phenotype changes	Number	%
1-1 → 2-1	5	3
1-1 → 3-1	1	1
2-2 → 2-1	11	8
2-2 → 2-3	14	10
2-2 → 2-4	1	1
3-3 → 3-1	10	7
3-3 → 3-2	9	6
3-3 → 3-4	1	1
Number	52	36
Without phenotype change	91	64
Total	143	

The second is post-translational changes. As was indicated above, *in vitro* changes were found only when the samples were frozen. Since all of them were kept at 4°C, no storage effects would be expected. It is possible, however, the occurrence of *in vivo* post-translational changes with lactation time. Ibarra (personal communication) suggests possible *in vivo* changes with lactation, and *in vitro* alterations by freezing the samples.

The third is differential activation of PGM 4 alleles. If only one allele is active at the beginning of lactation, a heterozygote woman would be identified as an homozygote, and only later, when the other allele is activated, she would be typed as a heterozygote. On other hand, if the initial activity is low, it is possible to miss some of the electrophoretic zones and make a wrong interpretation of the phenotypes. We believe that the most important cause of these pattern variations may be differences in enzyme activities at the beginning of lactation.

In Mexico, Cantú and Ibarra (1982) did not refer to phenotype changes. Their samples were collected between the 3rd and 10th day after the child's birth. Is is possible then that after the 3rd day all women would have this locus fully active in milk.

To check this in our population we collected, in a pilot test, milk of four women during each of the first six days post partum, as well as at 15 days of lactation. One of them showed in all samples a 2-3 phenotype. The second did not show any pattern in the first two samples and a 2-3 phenotype from the 3rd day. The two others showed homozygote phenotypes (2-2 and 3-3) in the first six days and heterozygote ones (2-3 and 3-1) on the 15th day. The study of a greater number of women for a period longer than six days, as well as enzyme activity dosages must be made before a definitive interpretation of these data can be made. There is, however a suggestion of variability in regulation of this locus, perhaps by the segregation of two regulatory alleles: one for precocious and the other for late activation. The homozygote for the late activation gene would have no detectable pattern in colostrum, the homozygote for the precocious allele would have no pattern changes during lactation, and the heterozygote would have pattern changes.

These data indicate that population genetic studies of milk's polymorphism must not be made on colostrum, since differences in enzyme activation may lead to errors in gene frequencies estimates.

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RESUMO

O polimorfismo do loco 4 da fosfoglicomutase foi investigado em uma amostra de colostro obtida de 652 mulheres (60% brancas e 40% negras), coletada 24 a 48 hs. após o parto, em Porto Alegre, Brasil. Uma nova amostra de leite foi obtida de 175 dessas mulheres com cerca de 17 dias de lactação. No colostro observou-se um acentuado desvio no equilíbrio de Hardy-Weinberg, havendo em geral um ex-

cesso de homozigotos e deficiência de heterozigotos. No leite, no entanto, esse desequilíbrio não ocorreu. As diferenças entre as duas distribuições são devidas à detecção de padrões nas amostras que não apresentavam atividade no primeiro período, assim como a variabilidade na ativação enzimática, que pode ocorrer no início da lactação. As frequências gênicas no leite ($n = 175$) foram: brancos ($n = 127$) $PGM4^*1 = 0,20$, $PGM4^*2 = 0,41$, $PGM4^*3 = 0,38$, $PGM4^*4 = 0,01$; negróides ($n = 48$) $PGM4^*1 = 0,15$, $PGM4^*2 = 0,52$, $PGM4^*3 = 0,32$ e $PGM4^*4 = 0,01$.

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