DNA Polymorphism of Alkaline Phosphatase Isozyme Genes: Linkage Disequilibria between Placental and Germ-Cell Alkaline Phosphatase Alleles

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Summary

The use of human placental alkaline phosphatase (PLAP) cDNA as a probe allows the detection and identification of restriction DNA fragments derived from three homologous genes, i.e., intestinal alkaline phosphatase (AP), germ-cell AP (GCAP), and PLAP. In previous RFLP studies we have reported linkage disequilibria between an RsaI and two PstI (a and b) polymorphic restriction sites and electrophoretic types of PLAP. In this report we present evidence that, in spite of the strong correlation with PLAP types, PstI(b) is an RFLP of GCAP. The data indicate close linkage between the PLAP and GCAP loci.

Introduction

Human alkaline phosphatases (APs; orthophosphoric monoester phosphohydrolase [alkaline optimum]; E.C.3.1.3.1) occur in multiple molecular forms (isozymes). Cloning and sequencing of the genes for placental AP (PLAP), germ-cell AP (GCAP), and intestinal AP (IAP) have shown that there is a high degree of homology between these three genes, in particular, a 98% amino acid sequence identity between PLAP and GCAP (Kam et al. 1985; Millán 1986; Henthorn et al. 1988; Knoll et al. 1988; Millán and Manes 1988).

In PLAP, three common alleles and a large number of rare alleles have been discovered by means of starch gel electrophoresis of placental extracts and histochemical detection of activity (Robson and Harris 1965; Beckman et al. 1966; Donald and Robson 1974). Additional (genetic) variations of PLAP have been demonstrated by using immunochemical methods (Slaughter et al. 1981; Millán et al. 1982). Two RFLPs of PLAP have been found after cleavage with RsaI (Martin et al. 1987) and PstI (Tsavaler et al.

1987, 1988). They have been found to be correlated with electrophoretic types of PLAP (Beckman et al. 1989; 1991).

In a previous study of a Finnish population sample, we described a PstI RFLP strongly correlated with electrophoretic PLAP types and believed to be caused by a variable restriction site at position 367 in exon II of PLAP (Beckman et al. 1991). In the current study we present evidence that, in spite of the correlation with PLAP types, PstI(b) is an RFLP of GCAP.

Material and Methods

High-molecular-weight DNA was isolated from 201 Swedish placentas and was digested and electrophoresed according to standard procedures (Maniatis et al. 1982). A 2.0-kb EcoRI-KpnI fragment of a 2.8-kb PLAP cDNA (Millán 1986) was used as a probe for hybridization according to a method described elsewhere (Beckman et al. 1991).

Extracts of placental tissue were prepared and typed by starch gel electrophoresis according to a method described elsewhere (Beckman et al. 1967). Statistical analysis of associations between RFLPs and PLAP phenotypes was performed by means of the χ^2 test. Linkage disequilibria between RFLPs were estimated, omitting double heterozygotes.

Table I

Positions of *PstI* Restriction Sites and Fragment Size, in GCAP, PLAP, and IAP

AP Isozyme	Site (bp)	Fragment Size (bp)
GCAP	1108	1,410
	2518	378
	2896	227
	3123	184
	3307	
PLAP	291	76
	367	1,723
	2090	226
	2316	185
	2501	770
	3271	777
	4048	
IAP	246	268
	514	1,375
	1889	422
	2311	1,209
	3520	

NOTE.—The nucleotide position of the sites is an arbitrary number based on the currently available sequence information of the respective genes (Millán and Manes 1988; Knoll et al. 1988 for PLAP; Henthorn et al. 1988, and GenBank). Variable sites are underlined.

Results and Discussion

Table 1 details the positions of the *PstI* restriction sites and the sizes of the fragments in GCAP, PLAP, and IAP. Figure 1 shows schematically the electrophoretic pattern of DNA fragments in an individual heterozygous for the *PstI* RFLPs, as well as the probable assignment of these fragments to GCAP, PLAP, and IAP. The assignment of the constant 2.1-kb fragment is uncertain. The 1,375-bp and 1,209-bp fragments are constant in this population and are derived from IAP. The polymorphism described elsewhere (Tsavaler et al. 1987, 1988), here called "*PstI*(a)," shows variable fragments at 1.0 and 0.8 kb (more precisely 955 bp and 770 bp, respectively).

In our previous study of a population sample of 83 Finnish placentas, all individuals were found to have the 1.72-kb band (1,723 bp; see table 1). In 40 of the 83 individuals, a 1.80-kb band was found in addition to the 1.72-kb band. Since the presence of this 1.80-kb band showed a very strong correlation with electrophoretic PLAP phenotypes and with the *PstI*(a) RFLP
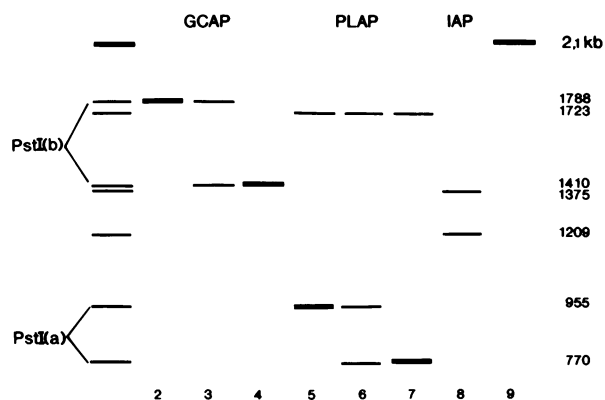

Figure I Schematic representation of DNA fragments obtained in Southern blot after digestion of genomic DNA with *PstI*, electrophoresis, and hybridization with a PLAP-cDNA probe (a 2.0-kb *EcoRI-KpnI* fragment). The cathode is at the top. Lane 1, DNA fragments found in an individual heterozygous for the *PstI*(a) and *PstI*(b) RFLPs. Lanes 2–4, Three *PstI*(b) types of GCAP—2-2, 2-1, and 1-1, respectively. Lanes 5–7, *PstI*(a) types of PLAP—2-2, 2-1, and 1-1, respectively, and the constant 1,723-bp fragment. Lane 8, IAP fragments. Lane 9, 2.1-kb fragment of uncertain origin.

of PLAP, the 1.80-kb band was interpreted as the result of a loss of the *PstI* site 367 in the PLAP gene (see table 1), giving rise to a 1,723 bp + 76 bp = 1,799-kb fragment. If the 1,799-bp and 1,723-bp fragments are allelic, one would have expected about 5 of the 83 individuals to have only the 1,799-bp fragment. We observed none and discussed possible reasons for this deviation from the Hardy-Weinberg equilibrium, e.g., negative selection against this phenotype. The *PstI*(b)2 allele showed a strong association with the electrophoretic 2(F) PLAP variant, which has been found to be associated with an increased risk for spontaneous abortion (Beckman et al. 1972).

Southern blot analyses of the 1,799-bp and 1,723-bp fragments, however, indicate that these fragments are unlikely to be allelic, for the following reasons.

1. In this series of 201 individuals, all showed the 1,723-bp fragment. If the 1,799-bp and 1723-bp fragments were allelic, we should have expected, according to the Hardy-Weinberg equilibrium, about 22 individuals to be lacking the 1,723-bp fragment. We found none, and this deviation from expectation ($P = 3 \times 10^{-6}$) excludes the hypothesis that the 1,799-bp and 1,723-bp fragments are allelic. Thus, the 1,723-bp band is a constant fragment of PLAP.
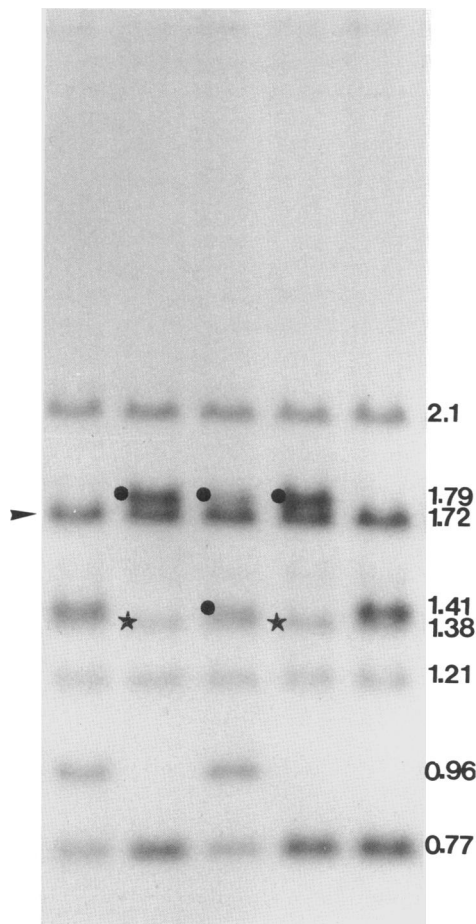
Figure 2   Southern blot analysis of *Pst*I digests of DNA from five individuals, showing constant bands at 2.1, 1.72 (arrow), 1.38, and 1.21 kb. In GCAP, polymorphic bands (black dots) are seen at 1.79 and 1.41 (*Pst*I[b]), and, in PLAP there are polymorphic bands at 0.96 and 0.77 kb. The constant IAP band is indicated by a star.

2. Southern blot analysis revealed that when the 1.8-kb band was strong, the 1.4-kb band was usually weak and of somewhat different mobility (see fig. 2), because the 1,410-bp GCAP fragment and the

1,375-bp IAP fragment will hardly separate clearly on electrophoresis. The allelic fragments of the *Pst*I(b) RFLP should be derived from GCAP, where *Pst*I(b)1 is the 1,410-bp fragment and *Pst*I(b)2 is the 1,410 bp + 378 bp = 1,788-bp fragment when site 2518 is missing.

3. Furthermore, with the *Pst*I(a) polymorphic bands of PLAP and with 1,723 bp being a constant PLAP band, there is simply no space left for the *Pst*I(b) polymorphism on the PLAP gene (table 1).

In spite of the overlap of the 1,410 and 1,375-bp fragments, a classification into three *Pst*I(b) types is feasible. In type 1-1, the 1,788-bp fragment is missing and the 1,410-bp band is strong; in type 2-1, the 1,788-bp and the 1,410-bp bands are of equal strength; and, in type 2-2, the 1,788-bp band is strong and the 1,410-bp band is missing. Instead we observe a weak 1,375-bp band somewhat shifted toward the anode (see fig 2, lane 2).

Table 2 shows the relationship between *Pst*I(b) genotypes and electrophoretic PLAP types. The frequency of the *Pst*I(b)2 allele was .435, and the observed distribution of the three *Pst*I(b) genotypes was in good agreement with the expected distribution under the assumption of a Hardy-Weinberg equilibrium ($\chi_1^2 = 0.69, P = .41$). Also, the electrophoretic PLAP types showed a close fit to the Hardy-Weinberg distribution ($\chi_3^2 = 1.83, P = .18$). From table 2 it can be seen that there is a strong correlation between the *Pst*I(b) types and the electrophoretic PLAP types. There was a highly significant correlation between the *Pst*I(b) types and PLAP allele frequencies obtained by simple gene counting ($\chi_4^2 = 97.9, P = 3 \times 10^{-20}$). The *Pst*I(b)2 allele was associated with the electrophoretic types 2 and 3 PLAP alleles (or, in the older nomenclature, F and I).

Pairwise haplotype frequencies and linkage disequilibrium coefficients were calculated between the *Pst*I(a),

Table 2

Relationship between *Pst*I(b) Genotypes and Electrophoretic PLAP Phenotypes

*Pst*I(b) Type	No. of Individuals with PLAP Type						Total No. of Individuals
	1	2-1	2	3-1	3-2	3	
1-1	59	7	1	0	0	0	67
2-1	26	42	6	16	3	0	93
2-2	3	15	7	5	8	3	41
Total	88	64	14	21	11	3	201

Table 3

Estimates of Pairwise Haplotype Frequencies and Linkage-Disequilibrium Coefficients (D) between the PstI(a) and RsaI RFLPs of PLAP and the PstI(b) RFLP of GCAP

No. of Chromosomes	ESTIMATED HAPLOTYPE FREQUENCY				D	D_{max} or D_{min}	D (%)	P VALUE
	PstI(a)1-PstI(b)1	PstI(a)1-PstI(b)2	PstI(a)2-PstI(b)1	PstI(a)2-PstI(b)2				
344	.453	.41	.122	.015	−.043	.058	74	2×10^{-6}
	RsaI(1)-PstI(a)1	RsaI(1)-PstI(a)2	RsaI(2)-PstI(a)1	RsaI(2)-PstI(a)2				
326	.432	.003	.451	.114	.048	.051	94	6×10^{-8}
	RsaI(1)-PstI(b)1	RsaI(1)-PstI(b)2	RsaI(2)-PstI(b)1	RsaI(1)-PstI(b)2				
304	.25	.178	.335	.237	−.001	.177	1	.99

PstI(b), and RsaI RFLPs (table 3). A strong linkage disequilibrium was found between the RsaI and PstI(a) alleles ($P = 6 \times 10^{-8}$). A significant disequilibrium was observed also between the PstI(a) and PstI(b) alleles ($P = 2 \times 10^{6}$), but there was no association between the PstI(b) and RsaI alleles. Essentially the same linkage disequilibria were observed in our previous study of a Finnish population sample (Beckman et al. 1991).

The present study illustrates the difficulties that may be encountered in the analysis of Southern blots detecting DNA fragments from three closely related (isozyme) genes. It also shows the usefulness of population-genetic data, e.g., fit to Hardy-Weinberg equilibrium, in the interpretation of RFLP data.

There seems to be little doubt at this point that PstI(b) is an RFLP of GCAP, in spite of the very strong association with electrophoretic types of PLAP and with the PstI(a) RFLP of PLAP. It is somewhat puzzling that PstI(b) shows no association at all with the RsaI RFLP of PLAP. The evidence for these unusual haplotype associations is strong, since they have been found, in two different populations (Finnish and Swedish), with high levels of statistical significance. The linkage disequilibria between GCAP and PLAP indicate close linkage between these loci.

Acknowledgments

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