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ACP1 Polymorphism: Five New Variants Detected by Multiple Electrophoretic Methods

S. A. Miller, D. D. Dykes, and H. F. Polesky

Memorial Blood Center of Minneapolis, 2304 Park Avenue South
Minneapolis, Minnesota 55404, U.S.A.

INTRODUCTION

Human red cell acid phosphatase (ACP1, EC 3.1.3.2) is known to be genetically polymorphic. Three codominant autosomal alleles ACP1*A,B and C were first described by Hopkinson (1963) using differences in electrophoretic mobilities. Other variants published to date include ACP1*D, E, F, G. H, I, K, M, R, GUA-1 and TIC-1. This report describes five new variants identified by both isoelectric focusing and conventional electrophoresis. The new alleles show genetic transmission from family data and are named ACP1*N, P, S, T, and U. Further evidence of an allele with reversed ACP1 A band intensity and a variant with reduced ACP1 C activity are discussed.

METHODS

Variant samples were obtained from paternity cases tested in our laboratory. Blood was collected in ACD anticoagulated tubes. The cells were washed three times with saline and hemolysed with an equal volume of deionized water and stored at -20°C until tested.

The ACP1 phenotypes were determined by agarose isoelectric focusing (IEF) on 0.5 mm thick gels containing 0.225 g agarose IEF (Pharmacia) 2.7 g sucrose, 20 ml deionized water and 1.4 ml ampholyte pH 4-6.5 (LKB). Anode and cathode strips contained 1M H_3PO_4 and 0.2 M NaOH respectively. The hemolysate samples were treated with 0.1% 2-ME for 30 minutes and applied 1 cm from the cathode using 3 x 5 mm filter paper wicks. Focusing was performed at 2000 V, 20 mA and 5W for 10 minutes. Sample wicks were removed and the power increased to 10W for an additional 35 minutes. Variant samples were also identified by conventional starch gel electrophoresis (SGE) at both pH 5.7 and pH 5.9 according to Nelson (1984, 1985). On all three techniques band patterns were developed by placing over the entire gel a piece of filter paper soaked in 4-methylumbelliferyl phosphate in a sodium citrate buffer.

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Edited by W. R. Mayr
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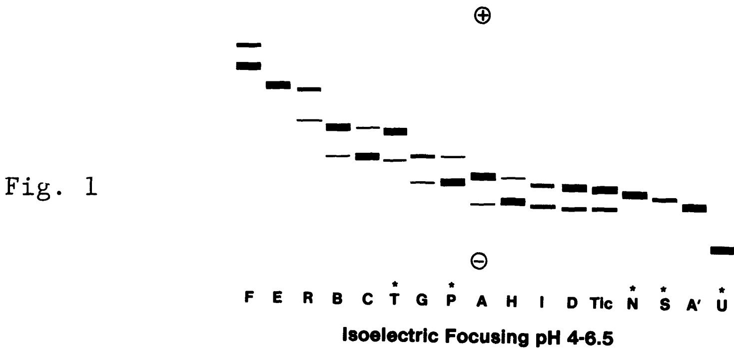
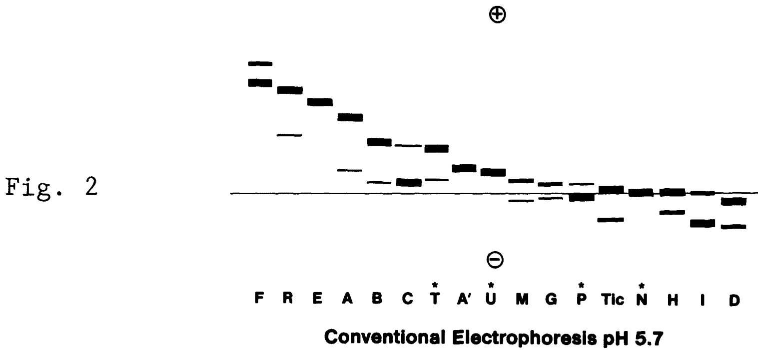
Fig. 1



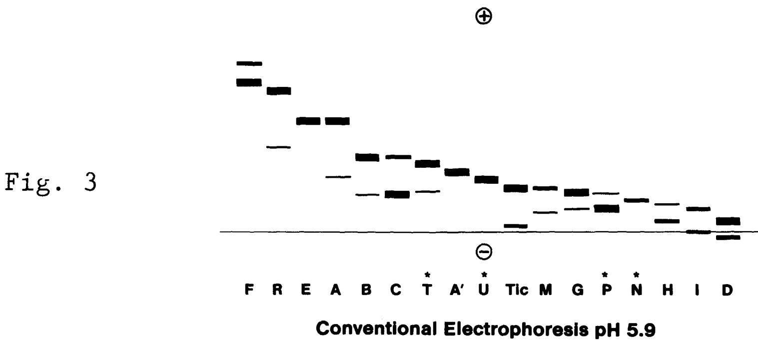
Isoelectric Focusing pH 4-6.5

Fig. 2



Conventional Electrophoresis pH 5.7

Fig. 3



Conventional Electrophoresis pH 5.9

RESULTS AND DISCUSSION

Figure 1-3 show diagrams of the positions of the five new alleles (indicated by *) in comparison to the previously published variants on the three methods of separation. Some of the bands demonstrate shifts in position on IEF. ACP1 M is not distinguishable on IEF from ACP1 A. The new ACP1*S which was found in a white family cannot be distinguished from the ACP1 C band on either conventional method. On IEF, it is seen as a single band migrating just cathodal to the primary ACP1 A band.

ACP1*N, found in a black family migrates just cathodal to the ACP1 C band on SGE but much more cathodal on IEF.

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ACP1*P was discovered in a white family and one other individual. The bands of this allele appear to have activity that is the reverse of the ACP1 G bands reported by Radam (1982). We also found a family with the ACP1*G allele which confirms that ACP1*P and G are two different variants.

ACP1*T migrates very close to the ACP1 B band on all three methods. This allele was found in two different white families.

ACP1*U, the most cathodal variant on IEF, was discovered in a white family. However, on SGE it migrates between the ACP1 B and C bands.

The allele designated A' was observed in one family and five other individuals, one of whom was black. It migrates in the position of the secondary ACP1 A band on all three techniques. Reversed 'A' activity has been described in the literature by Smerling (1973) and named ACP*K by Turowska (1984). Figures 4-7 are photos of the rare variants on the three methods.

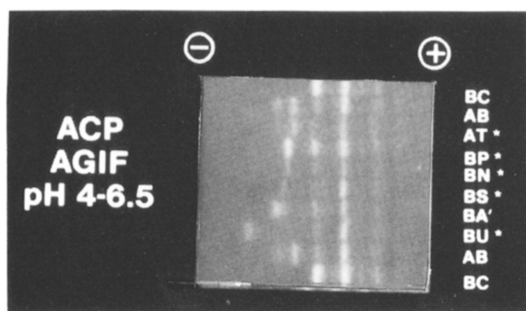
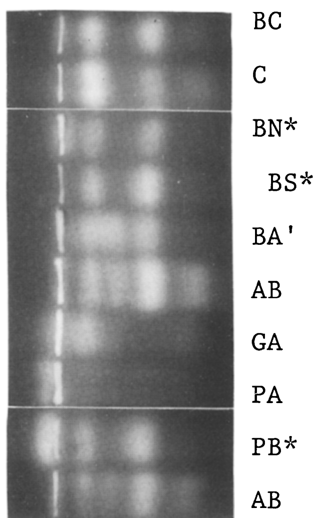
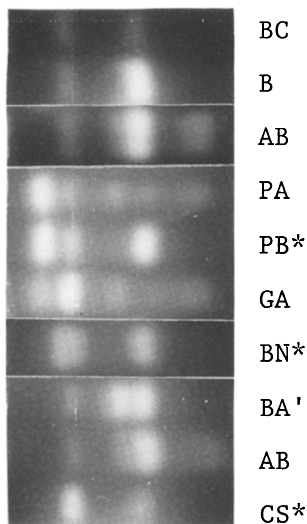


Fig. 4

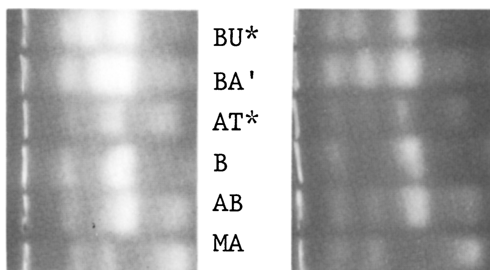


SGE pH 5.7

Fig. 5



SGE pH 5.9



pH 5.7

pH 5.9

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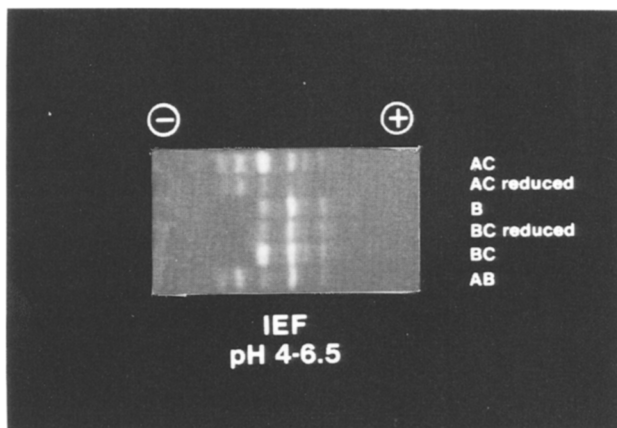
In three families a variant that exhibited a marked decrease in the activity of the ACP1*C gene product was observed on both IEF and conventional electrophoresis. Figure 8 shows a photo of one of the families. The alleged father phenotyped as an ACP1 AC with reduced C activity, the mother as a normal ACP1 B, and the child as ACP1 BC with reduced C activity. Mohrenweiser (1982) describes ACP1*GUA-1, found exclusively in Guaymi Indians, as a variant with low activity migrating in the ACP1 C position on PAGE. He suggests an association of this low activity with an increase in glutathione reductase activity. However, his Indian samples were not run on IEF or compared with our samples from white families. We suspect that they are different quantitative variants.

Fig. 8



CONCLUDING REMARKS

IEF is a fast, easy method for separating ACP1 phenotypes resulting in highly resolved bands. However, many of the rare variants tend to pile up at the cathodal end near the ACP1 A bands. Conventional methods of separation are also needed to confirm the identity of suspected variants.

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