# COMPARATIVE SUBTYPING OF ACP-1, PGM-1 AND ESD IN HUMAN PLACENTA AND CORD BLOOD BY ISOELECTRIC FOCUSING: PRACTICAL CONSIDERATIONS OF FORENSIC SIGNIFICANCE

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## INTRODUCTION

Human red cell enzymes with genetic polymorphism (ACP-1, PGM-1, ESD...) were previously analyzed in placenta and other tissues by conventional starch electrophoresis (Blake et al. 1973; Harris and Hopkinson 1976). However, to our knowledge, analysis of these enzymes from human placenta by isoelectric focusing (IEF) have not been performed hitherto.

In this study, the genetic polymorphism of ACP-1, PGM-1 and ESD was analyzed in placental extracts and red cell lysates from the corresponding cord and maternal bloods by IEF using miniaturized polyacrilamide gels.

## MATERIALS AND METHODS

98 samples of human placenta (maternal and fetus sides) and the corresponding cord and maternal bloods were collected after delivery. Small pieces of placenta were homogenized with a glass/glass homogenizer in 100 µl of distilled water, centrifuged at 3000g for 5 min. and stored at -40ºC until use. The red cells were washed three times with saline and stored at -40ºC until use. Placental extracts were diluted with 0.05 M DTT (1:1 for ACP-1 and ESD typing and 1:60 for PGM-1 typing). Red cell hemolysates were diluted 1:4 with 0.05M DTT. ACP-1 typing was performed by IEF in miniaturized polyacrylamide gels as previously described (Alonso and Gascó 1987) with the following modifications: 12% Glycerol was used as a density agent instead of sucrose and the pH gradient was created with a mixture of Ampholine pH 5-7 and Pharmalyte pH 6-8 (3:1 v/v). The ACP-1 patterns were detected using 4-methylumbelliferyl phosphate (MUP) as substrate, while the specific detection of the ACP-2 and ACP-3 bands was done by enzyme blotting using α-Naphthyl phosphate (α-NP) as substrate (Harris and Hopkinson 1976). PGM-1 typing was also carried out by IEF in miniaturized polyacrylamide gels using the Ampholine pH 5-7. The PGM-1 phenotypes were revealed by the overlay technique previously described (Sutton and Burgess 1978). ESD typing was done by IEF under reducing (0.05M DTT) and mild denaturing (1.5M Urea) conditions using a narrow pH gradient (Pharmalite pH 4.5-5.4) in combination with two separators (HEPES and ACES) according to Alonso et al. (1991).

## RESULTS AND DISCUSSION

Figures 1A and 1B show the ACP band patterns from cord bloods and the corresponding placental extracts after IEF followed by MUP staining, respectively. As can be seen, the most striking difference between these two tissues was the presence of three monomorphic bands in

Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider

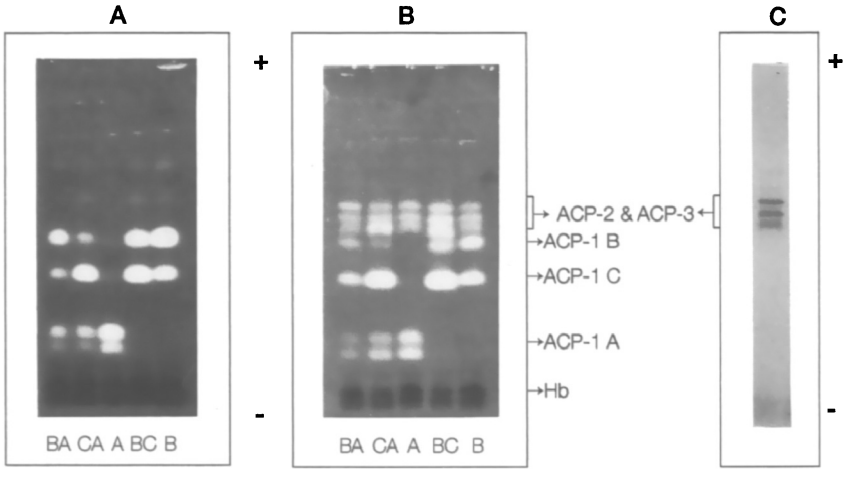


Fig. 1. Analysis of ACP by IEF: A) ACP-1 phenotypes from cord bloods, B) the corresponding placental extracts and C) ACP-2 and ACP-3 from placenta, stained with α-NP

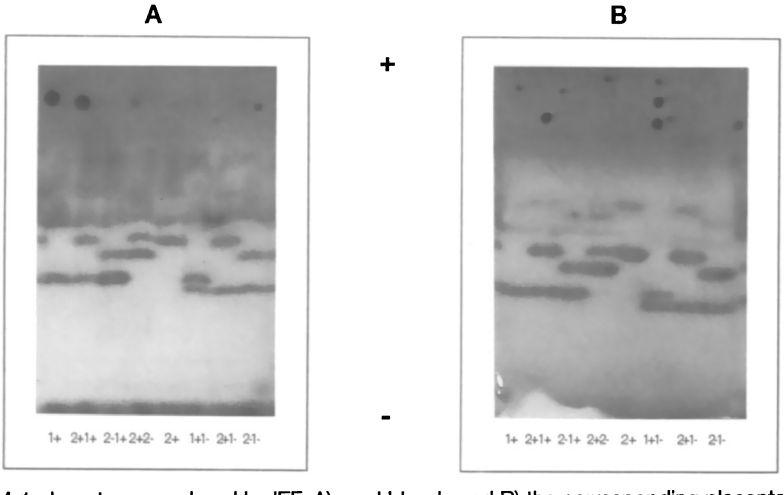


Fig. 2. PGM-1 phenotypes analyzed by IEF: A) cord bloods and B) the corresponding placental extracts

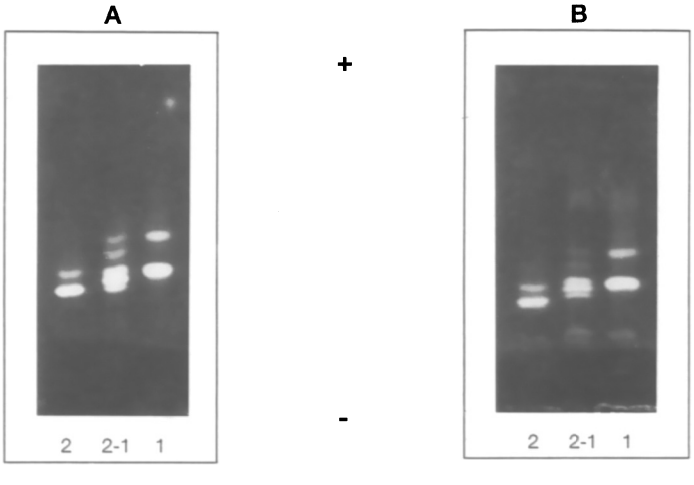


Fig. 3. ESD phenotypes analyzed by IEF: A) cord bloods and B) the corresponding placental extracts

placenta that were absent in cord blood. These monomorphic extrabands were demonstrated to be the genetic products of the ACP-2 and ACP-3 loci, since they were detected with α-NP that specificaly stains this tissue isoenzymes (Blake et al. 1973) (Fig. 1C). On the other hand, the analysis of the ACP-1 allele products from these tissues (Figs. 1A and 1B) revealed that the ACP-1 phenotypes from placenta could be assigned to the corresponding phenotypes from cord blood, making possible a genetic interpretation of the polymorphism displayed by the placental ACP-1 enzyme. However, a more detailed comparison between these two tissues showed a high staining intensity of the placental ACP-1 C band, specially in the B, BC and BA phenotypes. In spite of this difference, that must be taken into account for a correct interpretation of the genetic polymorphism of this enzyme, each phenotype can be differenciated from each other by a particular staining pattern as is shown in Fig. 1B.

The PGM-1 patterns from placenta and the corresponding cord blood as analyzed by IEF are shown in Fig. 2B and Fig. 2A, respectively. As can be seen, the genetic variability of this enzyme from placental extracts diluted 1:6O corresponded to the allelic variation that this enzyme displayed in cord blood. The placental extracts were diluted 1:60 with DTT since less diluted samples exhibited overstained tissue bands with impaired resolution as well as the presence of maternal PGM-1 bands due to blood contamination, making difficult the interpretation of the new born phenotypes in placenta.

Finally, it has been shown (Figs. 3A and 3B) that the three ESD common phenotypes analyzed in this study displayed the same band patterns and staining intensity in placenta and in the corresponding cord blood.

The results presented here clearly show that ACP-1, PGM-1 and ESD fetal phenotypes can be studied from placenta samples, using a fast and reliable IEF methodology. Therefore, we suggest the applicability of these conventional markers in the preliminary genetic analysis of forensic cases (illegal abortions and infanticides) in which fetal fragments are mixed with placental tissue.

## ACKNOWLEDGMENTS

We wish to express our gratitude to Dr. José María Guerra Flecha from the Instituto de Obstetricia y Ginecología, Hospital Gregorio Marañón, Madrid for kindly providing bloods and placental tissue samples. Thanks are also due to Mrs. A. Zamora for the photographic work.

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