PGM1 SYSTEM: A RARE ALLELE AND AN INTRAGENIC RECOMBINATION IN TWO CASES OF DISPUTED PATERNITY

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INTRODUCTION

The PGM1 system polymorphism was first studied by Spencer et al in 1964. There are at least 30 rare variants already described (Dykes and Polesky 1981; Bertrams et al 1988). In cases of disputed paternity, unexplained maternity and single paternity exclusions have been observed (Martin 1981; Wetterling 1986). Some of these can be explained by the occurrence of a null allele (Brinkmann et al in 1973; Raczek 1990). Carter et al (1979) proposed a phylogeny theory of the PGM1 locus that was developed by Takahashi et al (1982) that explains the remain exclusions. This theory has been verified since 1988, namely by Wetterling. In this report we have found a maternity exclusion corresponding to an intragenic recombination between one of the only genotypes capable of generating recombinant gametes (1B2A) carried by the mother. This study was completed by RFLP analysis. We also have found a rare allele, considered here as W12.

MATERIALS AND METHODS

Isoelectric Focusing (PAGIF)

Blood samples from 378 trios, involved in paternity cases during 1990 and the first 7 months of 1991, were collected in heparinized tubes and stored at -20ºC until tested (a few weeks later). Haemolysates were obtained by freezing and thawing and by adding an equal volume of 0.05 M dithiotreitol (DTT). The PGM1 phenotype determinations were carried out by isoelectric focusing. The PGM1 phenotypes were visualized by an agar overlay method described by Sutton and Burgess (1978). Additionally, blood samples were examined in other conventional markers including HLA.

RFLP Analysis

From the 199 trios studied during 1990, 19 were analysed using RFLP's. Blood samples were collected in EDTA-K3 tubes. DNA was isolated by haemolysis of erythrocytes and digestion in Proteinase K. Extraction was carried out using phenol (Brinkmann 1991). High molecular weight DNA samples were digested with restriction enzyme Pst I. Restriction fragments were separated in a 1.2% agarose gel(Gomes et al 1991). Following electrophoresis DNA was transfered to Hybond-N membranes (Amersham), by southern blotting.

Advances in Forensic Haemogenetics 4
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The probe 3'HVR (Kindly donated by Dr. Carracedo, Faculty of Medicine, University of Santiago de Compostela, Spain), was labelled with 32P using the multiprime method (Multiprime Labelling System, Amersham). Following hybridization, radioactive bands were visualized by auto radiography carried out at -70ºC, in x-ray film, 1-4 days with intensifying screens.

RESULTS AND DISCUSSION

During 1990 and 1991, 378 trios were typed for various systems, including PGM1. In one case we have found a mother-child imcompatibility only by PGM1, according to the first rule of heredity. This result was verified at least three times with identical results by PAGIF "Fig. 1". The phenotypes presented by the putative father, the mother and the child are 1A2B, 1B2A and 2B, respectively. Many maternity exclusions and isolated paternity exclusions have been reported within the PGM1 system in other populations (Martin 1981; Bertrams et al 1988; Wetterling 1988). This is in agreement with an intragenic recombination theory developed by Takahashi et al (1982) and recently verified by Wetterling (1990). In order to complete the study of this family, we have made RFLP analysis. We used the probe 3'HVR labelled with 32P "Fig. 2". This is a preliminary study, but it shows that one of te child's band is inherited from the alleged father and other from the mother.

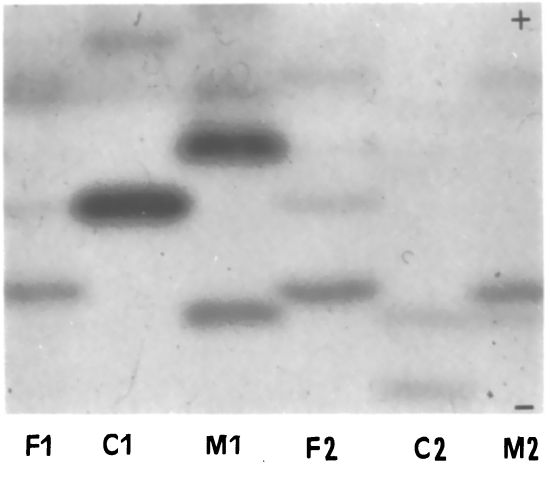

Fig. 1. PGM1 phenotyping of two paternity trios (F-father; C-child; M-mother) on polyacrylamide gel by isoelectric focusing. From left to right: 1A2B (F1); 2B (C1); 1B2A (M1); 1A2B (F2); 1BW12 (C2); 1A1B (M2)

Here we also report a case of a paternity exclusion where there is a rare allele involved, located cathodically to allele 1B "Fig. 1", that we think is allele W12 (personal information given to us by Dr. Weidinger). There have been reported many rare variants in the PGM1 system (Dykes and Polesky 1981; Lin-Chu et al 1991), but it was not possible to compare this rare allele with others in order to confirm if it really corresponds to W12. Further analysis of this allele is needed.

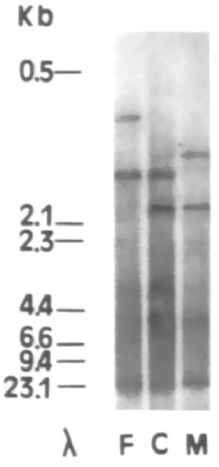

Fig. 2. Auto radiogram of DNA samples from 1 paternity trio, digested to completion with the enzyme Pst I and hibridized with the probe 3'HVR labelled with 32P (λ=molecular weight marker digested with Hind III; F=father; C=child; M=mother)

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