PiM4: An Additional PiM Subtype

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Summary. The authors studied Pi polymorphism using the Separator isofocusing method with slight modification. A new Pi allele was observed. Family pedigrees confirmed co-dominant inheritance with other Pi alleles. According to the electrophoretic mobility of its isoprotein bands, and to its frequency (0.04) this new allele is considered as a fourth PiM subtype: PiM4.

Introduction

Since the α1-antitrypsin polymorphism or Pi system polymorphism was first described by Fagerhol and Braend (1965) and Laurell and Persson (1973), the techniques used for its determination have benefited from several improvements (for reviews see Kueppers (1976) and Massi et al. (1979). Isoelectric focusing (IEF) procedures in particular have contributed to show that this polymorphism was even larger than first expected. According to starch gel or agarose electrophoresis the principal allele of the Pi system was PiM (Milford Ward et al., 1977). After IEF became available two subtypes were observed, PiM1 and PiM2, also described by Constans and Viau as PiN (1975). Some time later, the existence of an additional subtype, PiM4, was clearly revealed by Frants and Eriksson (1978), who used the Separator-IEF method.

Based on a minor modification of this technique, the procedure we used enabled us to present evidence for a fourth subtype, PiM4.

Materials and Method

A sample of 163 sera from healthy individuals living in the Toulouse region was examined for Pi polymorphism by the Separator-IEF procedure: a 0.5-mm-thick polyacrylamide gel (4.85% acrylamide-0.15% bisacrylamide) was prepared from a mixture of ampholyte solutions in pH ranges 4–5 and 4–6.5 (40/60 vol./vol.) after the addition of ACES (N₂-acetamido) 2-aminethane sulfonic acid). The electrophoresis conditions were 1400 volts, 10 mA, 7.5 w and migration time 4.5 h at 10°C.

After electrophoresis the gel was stained with Coomassie Brilliant Blue (R 250) in ethanol solution. The identity of the electrophoretic patterns with the Pi protein was monitored after immunofixation and development on cellulose acetate strip (Constans and Cleve, 1979). PiM1M2, M1M3, and M1S, M2S, M3S sera samples were kindly examined by D.W. Cox for comparison with standard sera. The nomenclature used in this paper corresponds to the one recommended after the Pi workshop (Cox, 1978).

Results and Discussion

After IEF, the pattern obtained by using the ampholyte mixture mainly corresponded to the Pi protein bands, as confirmed after immunofixation. Different patterns were observed, which are presented in the left-hand part of Fig. 1. These patterns, owing to the genetic heterogeneity of the molecule, cannot be properly interpreted if we assume the existence of only three PiM subtypes. An additional allele has to be postulated. In the right-hand part of the figure, the diagram shows the mobilities of the bands obtained in the presence of different phenotypes. As observed in any Pi allele protein, eight isoprotein bands are associated with the new allele products: two major (nos. 4 and 6) and six minor bands (nos. 1, 2, 3, 5, 7, and 8). Among the two main bands, the anodic one (no. 4) presents an electrophoretic location between the homologous bands of PiM3 and PiM2 gene products and the second band (no. 6), with a cathodal mobility, is located between the homologous bands of PiM3 and PiM2 isoprotein bands. This is shown in the picture and in the diagram.
Fig. 1. Left. The picture shows the IEF patterns obtained with the following sera: samples 1, 4, and 9, Pi M1M2 phenotype; sample 2, Pi M1M3 phenotype; sample 3, Pi M1M4 phenotype; sample 5, Pi M1S phenotype; sample 6, Pi M3S phenotype; sample 7, Pi M4S phenotype; sample 8, Pi M2S phenotype. In the heterozygous phenotypes Pi M1M4, Pi M1M3, and Pi M1M2 the no. 4 isoprotein bands are not clearly separated, whereas for the isoprotein bands (nos. 6 and 8) very sharp separations are obtained. With PiS heterozygous phenotypes, isoprotein band PiS no. 4 is well separated from PiM isoprotein band no. 6. These differences may correspond to a nonlinear pH gradient in this zone caused by a particular drift of the ampholytes in the presence of ACES. The isoprotein bands only are well separated in the cathodic zone. Examination of some sera classified as PiM1M1 shows a blurred pattern of their bands nos. 6 and 8. These pictures do not correspond to the Pi M3 subtype. Further genetic heterogeneity of the Pi M1 subtype might be revealed when supplementary technical improvements are brought to the IEF procedures.

Right. The diagram represents the relative mobility of the two major bands (nos. 4 and 6) observed with any Pi gene products. The mobility of the Pi M1 bands is used as reference to compare the electrophoretic location of other PiM subtype isoproteins. In this analytical scheme the Pi M4 bands (nos. 4 and 6) are located between the homologous bands of Pi M3 and Pi M2. Owing to the drift of the ampholytes from gel to gel it is sometimes difficult to separate Pi M4 bands from Pi M2 bands satisfactorily. Technical improvements are still needed.

Fig. 2. Family pedigrees showing the segregation of the different PiM subtypes and the new PiM4 allele

Family investigations were carried out. In four pedigrees we were able to follow the transmission of the new allele as a co-dominant gene with the Pi M1 bands or the Pi S allele (Fig. 2). According to the IEF patterns obtained, the new allele was in previous works misclassified with other PiM bands. It cannot be mistaken for known Pi variants; it corresponds to a supplementary PiM subtype. For these reasons we called it Pi M4. A sample of 163 sera taken from healthy individuals was examined. The Pi phenotype distribution obtained and the gene frequencies calculated are presented in Table 1. No significant deviation was observed between the numbers observed and those expected in the different classes of phenotypes. If related individuals are ex-