PI M Subtyping: Indication for an Alpha$_1$-Antitrypsin Null Allele in three Paternity Cases

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INTRODUCTION

Alpha$_1$-antitrypsin (α1AT), or α_1-protease inhibitor (PI), is a 52-kDa glycoprotein in human plasma with an unusual microheterogeneity. Its major physiological function appears to be the inhibition of neutrophil elastase (Travis and Salvesen 1983). Genetic variation in the PI system was first described by Laurell and Eriksson (1963), and Fagerhol and Braend (1965). The technique of isoelectric focusing (IEF) has permitted a division of the most common type PI M into the polymorphic subtypes M1, M2 (Constans and Viau 1975; Frants and Eriksson 1976), M3 (Klasen et al. 1977; Genz et al. 1977), and M4 (Constans et al. 1980; Weidinger et al. 1982). More recently, three further PI M variant types, tentatively named M5, M6 and M7 were revealed by IEF with immobilized pH gradients (Weidinger et al. 1985; Görg et al. 1985; Weidinger and Cleve 1986). Sequence analysis of the protein coding region has permitted the distinction of two M1 haplotypes (M1 Ala^{213} and M1 Val^{213}) in the PI*M1 gene (Nukiwa et al. 1987). Hitherto approximately 75 alleles were identified at the protein and/or gene level (Fagerhol and Cox 1981; Brantly et al. 1988). The PI locus has been assigned to chromosome 14q31-32.3 (Long et al. 1984).

The most common deficient allele in the PI system is PI*Z which has a frequency of 1-2% in most European populations. The molecular basis of the deficient haplotype PI Z has been identified at the protein and gene level (Kidd et al. 1983; Cox et al. 1985). In contrast to PI Z only little is known about the rare deficient PI null state. Existence of PI*Q0 was first described in a proband who had severe emphysema with almost complete absence of α1AT in serum (Talamo et al. 1973). Heterozygous carriers cannot be detected by subtyping if there is not an apparent isolated maternal or paternal exclusion constellation in the PI system which is not confirmed by the other marker systems.

In this paper we report indication for a silent PI allele in three different cases of disputed paternity.

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MATERIALS and METHODS

Serum samples were obtained from apparently healthy individuals for paternity testing. For PI typing the samples were used without previous treatment. Isoelectric focusing (IEF) with pharmalytes pH 4.2-4.9 was carried out in 0.5 mm thin flat bed polyacrylamide gels (dimension 250 x 115 mm) which were fixed on silanized glass plates (Weidinger et al. 1985). In some cases PI M subtypes and PI variants were classified by hybrid-IEF with a very narrow immobilized pH gradient (Weidinger and Cleve 1986). Alpha$_1$-antitrypsin concentration in sera was determined by single radial immunodiffusion using NOR-Partigen plates from Behringwerke, Marburg.

RESULTS and DISCUSSION

Figure 1 presents the band patterns of several PI phenotypes including the PI M subtypes of individuals involved in paternity cases no.2 and 3. In case no. 1 an apparent maternal exclusion and in case no. 2 an apparent paternal exclusion were found in the course of paternity examinations (Fig. 2). The phenotype constellation of case no. 1 was: child PI M1, mother PI M3 and father PI M1. The inverse homozygosity between the mother and her son would mean an exclusion of maternity, but there was no evidence for an exchange of blood samples and any other exceptions in the study of 26 red cell antigens, serum proteins and red cell enzyme systems. In case no. 2

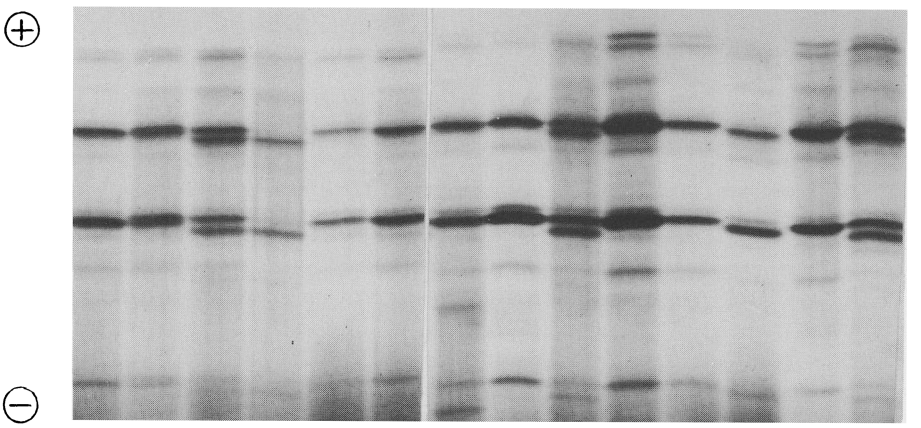

Fig. 1. Band patterns of different PI types obtained by isoelectric focusing in polyacrylamide gel, pH range of 4.2-4.9. The phenotypes were: (1) M3, (2) M1M3, (3) M1M2, (4) M2-Q0, (5) M1-Q0, (6) M1, (7) M1 Z, (8) M1, (9) M1M2, (10) M1, (11) M1-Q0, (12) M3-Q0, (13) M1, and (14) M1M2. Lanes 3,4,5, and 6 are samples from the mother, child, grandfather and grandmother of case no. 3. Lanes 10, 11, and 12 are samples from the mother, child and alleged father of case no. 2

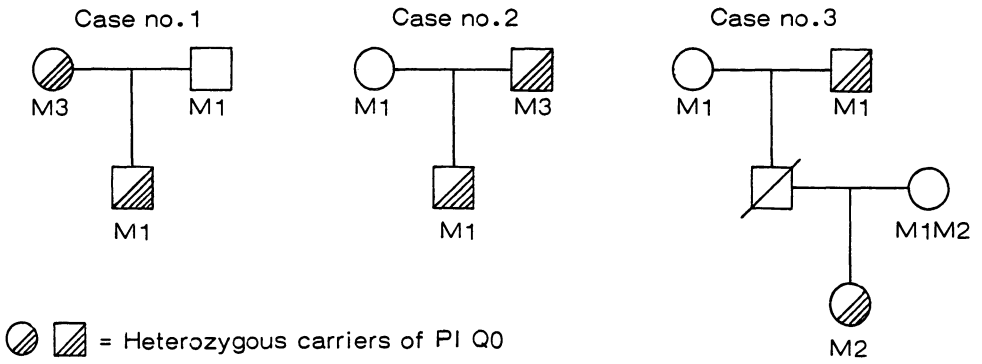

Fig. 2. Transmission of the PI*Q0 allele in three cases of disputed paternity

an incompatibility was observed between the child (PI M1) and the alleged father (PI M3). The man could not be excluded as the father of this child in 26 other genetic systems. Biostatistical evaluation of the combined data yielded a paternity probability of W>99.99%. In case no. 3 the alleged father was deceased. The grandparents were classified as PI M1, the mother PI M1M2, and the child PI M2. Family study has shown no inconcistencies in other systems. In all three cases the existence of a PI null allele was assumed. The band patterns of heterozygous PI Q0 carriers were clearly reduced and the α1AT-concentrations in sera were only approximately 50% of the normal range.

At present the three PI Q0 cases will be investigated at the DNA level. Former molecular studies have shown that α1AT genes were present in PI Q0 carriers but without or with faulty production of mRNA transcripts (Garver et al. 1986; Nukiwa et al. 1987). A possible explanation for faulty α1AT gene transcription could be the presence of a single mutation, deletion, addition, or rearrangement of DNA in the exons as well as introns of the gene.

We indicate that a null allele should be considered in cases of paternity with high probabilities if an exclusion is found only in the PI system. The haplotypic frequency of PI*Q0 in European populations is estimated to 0.1-0.2%, which is approximately one-tenth of the deficient PI*Z allele frequency. The PI Q0 heterozygotes of our paternity cases are apparently healthy, but individuals with certain deficient haplotypes such as PI Z or PI Q0 have a high risk for development of emphysema (Talamo et al. 1973; Muensch et al. 1986).

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