PI*Mpalermo: A NEW ALPHA-1-ANTITRYPSIN DEFICIENCY ALLELE DETECTED BY DNA SEQUENCE ANALYSIS IN TWO FAMILIES

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INTRODUCTION

Alpha-1-antitrypsin (α1AT) is a member of the serine proteinase inhibitor (serpin) superfamily. The 52-kD glycoprotein is mainly synthesized in the liver and functions as the major plasma inhibitor of neutrophil elastase. α1AT deficiency is an inherited autosomal disorder associated with chronic obstructive lung disease and childhood liver disease (Crystal 1990). The highly polymorphic single-copy gene has been mapped to chromosome 14q32.1. Approximately 80 different allelic variations are known (Cox 1990). These alleles can be categorized into four groups: (1) normal, (2) deficient, (3) null, and (4) dysfunctional. The major clinical importance relates to the deficiency alleles (associated with reduced serum α1AT levels) and null alleles (no detectable α1AT in serum). According to their allelic frequencies PI*Z (0.01-0.02) and PI*S (0.02-0.04) are the most common deficient variants in European populations whereas all other abnormal PI variants are rare.

In the present study we characterize the gene and protein of the highly deficient α1AT allele, PI*Mpalermo.

MATERIALS and METHODS

1. Study of families

The allele PI*Mpalermo was evaluated in two unrelated families from the Mediterranian area (Fig. 1). Family A. (southern Italy) contained the index case, a one-year-old male heterozygote who was clinically treated for asthma. Quantitative determination of the patient's and her mother's α1AT serum levels by radial immunodiffusion have shown a reduction to nearly 50 %. According to the mother's birth place the new deficiency allele was named PI*Mpalermo. In an unrelated Turkish family two boys (three and four years of age) were found to be homozygous for Mpalermo. The only physical abnormality detected in these two children was a hyperreactive bronchial system. In none of the affected family members a liver disease is known.

2. Phenotyping and haplotyping

PI phenotypes in serum were classified by isoelectric focusing (IEF) on polyacrylamide gels using two different techniques (Weidinger 1992). Genomic DNA of the eight individuals examined was isolated from peripheral leukocytes of EDTA blood using standard procedures. DNA haplotyping was performed according to Meisen et al. 1988.

3. Amplification and direct sequencing

All coding exons (II-V) of the α1AT gene were amplified by polymerase chain reaction (PCR) using specific primers and genomic DNA as template (Faber et al. 1990). Sequencing was performed by a radioactive and an automated non-radioactive method (Faber et al. 1993). For automated DNA sequencing

fluorescent oligonucleotide primers and T7 DNA polymerase (Sequenase 2.0 USB Co.) were used. Fluorescent labeled reaction products were analyzed with an Applied Biosystems 373 automated DNA sequencer.

RESULTS and DISCUSSION

The PI phenotypes were identified by IEF and immunoprinting (Fig. 2). Mother and child of family A. as well as both parents of family S. have shown slightly reduced α1AT band patterns. They were assumed to be heterozygous carriers of a deficient PI M variant which is located only a trace cathodal to the normal M2 subtype. Two individuals were found to be homozygous for this variant (lanes 7 and 8) which has been tentatively designated Mpalermo. Sequence analysis of this mutant demonstrated a 3bp deletion in exon II (TTC → Phe) which also occurs in the deficiency allele Mmalton (Fig. 3).

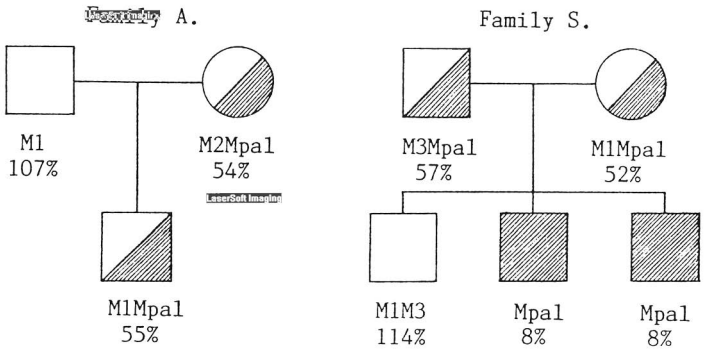

Fig. 1. Pedigree of two unrelated families with the deficiency allele PI*Mpalermo (Mpal). The phenotype is listed below each family member together with the serum α1AT level (% of normal value). Hatched symbols represent the rare Mpal variant.

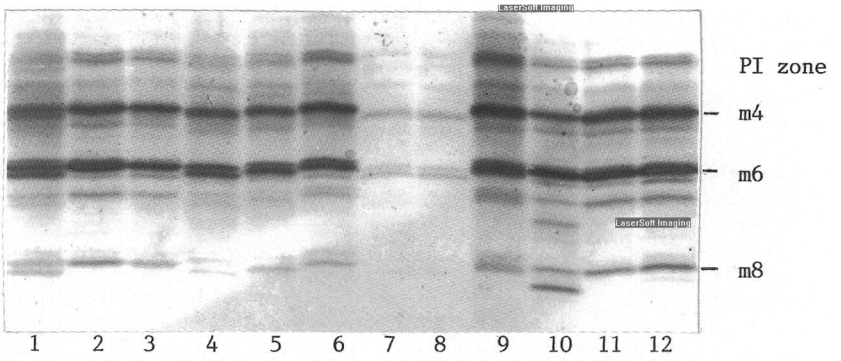

Fig. 2. Presentation of normal and deficient PI phenotypes as obtained by IEF on polyacrylamide gel (pH range of 4.2-4.9) followed by immunoprinting with a monospecific α1AT antiserum. Lane (1) M1M2; family A.: (2) father= M1, (3) child= M1Mpal, and (4) mother= M2Mpal; family S.: (5) father= M3Mpal, (6) mother= M1Mpal, (7) child 1= Mpal, (8) child 2= Mpal, and (9) child 3= M1M3; (10) M1Z; (11) M1, and (12) M1. Anode is at the top.

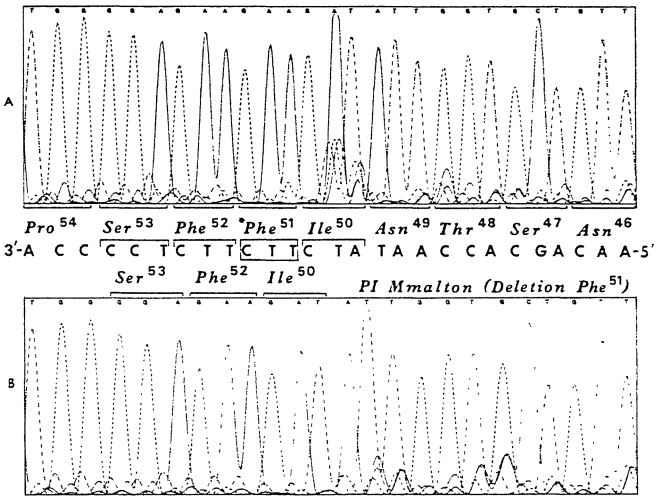

Fig. 3. Identification of the PI Mpalermo mutation in exon II by non-radioactive automated direct sequencing. (A) Normal sequence for the M1(Val213) allele concerning the amino acid 46-54 region. (B) Sequences of Mmalton and Mpalermo are identical in this region. A triple nucleotide deletion at the codon position for amino acid Phe51 characterizes the mutation.

In contrast to Mpalermo with a M1(Val213) background, the Mmalton allele derives from a M2 base allele (Curiel et al. 1989; Fraizer et al. 1989). To clarify the origin of both alleles we employed DNA haplotyping. While the Mmalton allele is associated with the haplotype 5'-SstI(-), 5'-AvaII(-), and 3'-AvaII(135), the Mpalermo allele was associated with the haplotype ++(135) in both families, indicating that the two alleles were generated in two independent mutational events. PI*Mnichinan, another deficiency allele of the M-family is identical to Mpalermo with the exception of a G → A transition at the codon 148 causing Gly → Arg substitution (Matsunaga et al. 1990).

We conclude that sequencing of the PCR amplified DNA is the most efficient technique for differentiation of very low producing alleles in the PI system.

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