A de novo Mutation in the Alpha-1-Antitrypsin Gene detected in a Case of disputed Paternity by DNA Sequence Analysis

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INTRODUCTION

Alpha-1-antitrypsin (α1AT) is the major protease inhibitor (PI) in human plasma. It is a 52-kD glycoprotein which consists of a single polypeptide chain of 394 amino acids with three complex asparaginyl-linked carbohydrate chains (Carrell et al. 1982). The α1AT-gene comprises seven exons (three noncoding exons IA-C followed by four coding exons II-V) and six introns over 12.2 kb of chromosome 14q31-32.3 (Long et al. 1984; Brantly et al. 1988). Alpha-1-antitrypsin shows an extensive genetic variation. More than 75 PI variants have been identified by either isoelectric focusing of serum and/or sequence analysis. At the DNA level, mutations are known in all four coding exons.

MATERIALS and METHODS

Phenotyping by Isoelectric Focusing (IEF)

Blood samples from three individuals -the index case, mother and alleged father- were available for analysis. They were collected in plastic tubes containing K EDTA. Plasma was separated after centrifugation of whole blood and used without treatment. Phenotyping of α1AT was performed by IEF on 0.5 mm thin flat-bed polyacrylamide gels containing pharmalytes (pH range of 4.2-4.9) according to the method of Weidinger et al. (1985).

Haplotyping, PCR-Amplification and DNA-Sequencing

Genomic DNA was extracted from peripheral white blood cells by standard procedures. For haplotyping the DNA was digested with the appropriate restriction enzymes (Sst I, Ava II), electrophoresed in 1.0-1.8% agarose gels, and transferred to nitrocellulose membranes by Southern blotting. The α1AT specific genomic DNA probes used for detection of the restriction fragment length polymorphisms (RFLPs) and the hybridization protocol have been described elsewhere (Meisen et al. 1988).

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HLA DQα typing was carried out using an AmpliType kit (Cetus). The D1S58 locus which contains a variable number of tandem repeats (VNTRs) was analyzed according to the method of Kasai et al. (1990).

Polymerase chain reaction (PCR)-amplification of all coding exons of the α1AT-gene was performed as reported previously (Faber et al. 1990). The PCR-products were directly sequenced using an automated fluorescent DNA sequencing method (McBride et al. 1989) and our standard protocol (Faber et al., submitted).

RESULTS and DISCUSSION

Analysis of alpha-1-antitrypsin by IEF revealed the subtype PI M1M2 in the mother and putative father. The one-year old child has clearly shown a variant in the so-called "P" range in addition to PI M1 (Fig. 1A). In comparison with standards the variant phenotype was classified as PI M1Pdonauwörth (abbreviated Pdon according to the PI nomenclature). All three individuals had α1AT-plasma concentrations in the normal range. Testing of the three individuals in 29 other genetic markers (8 blood group-, 12 serum protein-, and 9 enzyme systems) showed that the man could not be excluded from fatherhood. Adding the data for HLA-A, B, and C a strong evidence for paternity has been obtained. The PI data, however, were not compatible with paternity. Biostatistical evaluation of all serological markers (with the exception of PI) gave a paternity probability of W = 99.9972%. These serological data were confirmed by a DNA study.

First, restriction site variation in and around the α1AT-gene was studied by use of two different restriction enzymes and three different genomic DNA probes (1.17 kb and 4.6 kb 5'probes and a 6.5 kb 3'probe). The segregation pattern

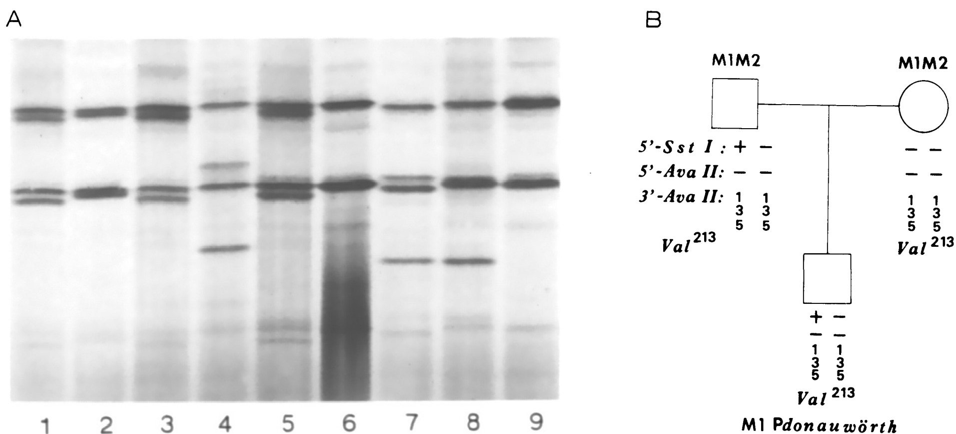

Fig. 1. (A) Demonstration of PI phenotypes as revealed by isoelectric focusing of serum and plasma samples in a polyacrylamide gel, pH range of 4.2-4.9. Lanes: (1) M1M2, (2) M1M3, (3) M1M2, mother, (4) M1Pdon, child, (5) M1M2, alleged father, (6) M1, (7) M3S, (8) M1S, and (9) M1. Anode is at the top. (B) Segregation of the DNA haplotypes in the case of paternity

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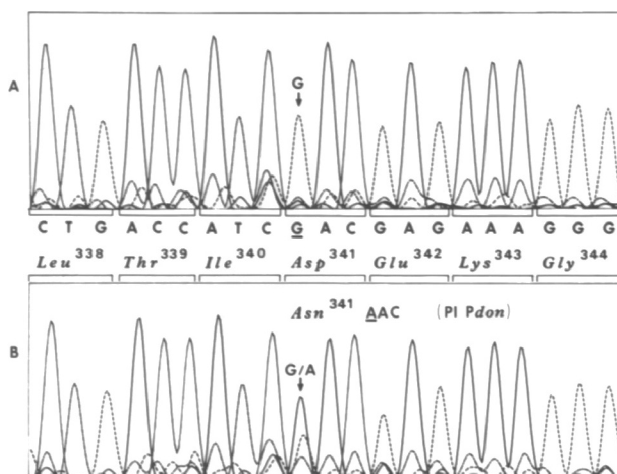

Fig. 2. Sequence analysis of the coding exons of α1AT-genes M1 and Pdon. A guanine (G) to adenine (A) transition causes an amino acid substitution of Asn341(AAC) for Asp341(GAC) in the gene Pdonauwörth

of the DNA haplotypes was compatible with paternity (Fig. 1B). Secondly, analysis of the polymorphic HLA DQα locus was performed. The alleged father and the child were both determined to be DQα type 1.1, 3, and the mother was determined to be type 3, 4 (Fig. not shown). These typing data are also compatible with paternity. Additional evidence in favor of paternity of the alleged father has been obtained by investigation the D1S58 VNTR-locus. To determine the precise nature of the discrepancy within the PI system, we have conducted automated direct sequencing of the childs α1AT-genes. Sequence analysis of all coding exons of the childs Pdon gene has shown a point mutation in exon V which causes an amino acid substitution of Asn341(AAC) for Asp341 (GAC) (Fig. 2). This mutation was not found after testing both parental DNAs, thus confirming a de novo mutation in the childs α1AT-gene.

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