Deletion/frameshift mutation in the α₁-antitrypsin null allele, PI*QObolton

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Summary. The most common deficiency allele of the protease inhibitor (PI) α₁-antitrypsin (α₁AT) is PI*Z. Other rare deficiency alleles of α₁AT are of two types: those producing low but detectable amounts of α₁AT (<20% of normal serum concentrations), and null alleles producing <1% of normal α₁AT and therefore not detectable by routine quantitative methods. We have previously used DNA polymorphisms and family data to determine heterozygosity in an individual producing low levels of serum α₁AT (12% of normal) of PI type Mmalton. By DNA analysis we observed the typical haplotype associated with PI*Mmalton and a unique null haplotype associated with the allele PI*QObolton. The QObolton allele produces no detectable serum α₁AT. We have cloned and sequenced the QObolton allele from a phage genomic library. Deletion of a single cytosine residue near the active site of α₁AT in exon V results in a frameshift causing an in-frame stop codon downstream of the deletion. This stop codon leads to premature termination of protein translation at amino acid 373, resulting in a truncated protein. The truncated protein is predicted to have an altered carboxy terminus (amino acids 363-372) and will lack structurally important amino acids.

Introduction

α₁-Antitrypsin (α₁AT), a 52-kilodalton glycoprotein, is the primary inhibitor of the serine protease, leukocyte elastase (Travis and Salvesen 1983). α₁AT is synthesized primarily in the liver, is secreted into the blood, and diffuses into the interstitial fluids (for recent reviews see Cox 1989; Brantly et al. 1988). The levels of α₁AT in the lower respiratory tract reach approximately 10% of those found in serum (Wewers et al. 1987). Like other secretory proteins, α₁AT contains a signal peptide, which is cleaved off to generate a 394 amino acid mature protein (Carrell et al. 1982; Carlson and Stenflo 1981). α₁AT is a highly polymorphic protein, having more than 60 electrophoretic variants (Fagerhol and Cox 1981; Cox et al. 1981). A deficiency of α₁AT associated with emphysema, liver disease, and hepatocellular carcinoma (Laurell and Eriksson 1963; Sharp et al. 1969; Eriksson and Hagerstrand 1974). The most common deficiency allele of α₁AT is PI*Z, occurring at a frequency of 0.012 in Caucasians (Cox et al. 1981). Z α₁AT is associated with low plasma concentrations (<20% of normal, Laurell and Eriksson 1963) and liver inclusions (Sharp 1971) apparently due to the tendency to aggregate in vitro (Cox et al. 1986).

The null alleles are associated with the most pronounced deficiency states where no serum α₁AT is detected. The null alleles are rare and their combined frequencies are estimated to be 10⁻⁴ among Caucasians (Cox and Billingsley 1989). We have studied a large kindred in which both the QObolton and the Mmalton alleles are segregating. This family was the basis for the original description of PI*Mmalton (Cox 1976). By examining DNA polymorphisms and haplotypes, we have shown that both of the apparent Mmalton homozygotes in this pedigree are actually Mmalton/QObolton genetic compounds. We report here the cloning and sequencing of the QObolton allele from one of these individuals.

Materials and methods

Protease inhibitor (PI) types and plasma concentrations of α₁AT

Blood samples were collected in ethylenediaminetetraacetate (EDTA) tubes from the proband, her father, and from the other 42 members of her family, as described (Cox 1976). PI typing of serum α₁AT was initially done by starch gel electrophoresis (Fagerhol 1968) and by agarose gel electrophoresis followed by immunofixation using α₁AT antibodies as previously described (Cox 1976; Arnaud et al. 1977). PI types of additional family members were determined by polyacrylamide isoelectric focusing gel (PIEF) analysis as described (Cox 1981).

Haplotype analysis

DNA was extracted from leukocytes from EDTA blood (Madisen et al. 1987). A 3-μg aliquot of DNA was digested with each of the restriction enzymes, MspI, SstI, AvaII, MaelIII, BstEII, BglII, EcoRI, and TaqI according to manufacturer’s recommendations. DNA fragments were separated by size in a 0.8% agarose gel and transferred to Hybond™ (Amersham). Hybridization probes were labelled with α³²P-dCTP using an oligonucleotide labelling kit (Pharmacia). Hybridizations, washes, and autoradiography were performed as described (Cox et al. 1987). Two of the probes used in this study, designated as 4.6 and 6.5 (Cox et al. 1985) were kindly provided by S.L.C. Woo (Kidd et al. 1983). The 4.6 probe in-
includes the 5' region of the α1AT gene, exon I, and adjacent regions. The 6.5 probe includes the coding regions, exons II–V, and the 3' untranslated region (Fig. 2). The restriction enzyme polymorphisms observed with these probes and the resulting DNA haplotypes have been described and are listed in Table 1 in an order that represents the location of the polymorphic sites of the α1AT gene and the related PI-like (PIL) gene (D. W. Cox, unpublished data). The last five restriction sites listed, not contained within the 6.5 probe, represent polymorphisms in the PIL gene. The PIL gene is located 8–12 kb downstream of the α1AT gene (Hofker et al. 1988; Bao et al. 1988). The previous identification of the unique PI*Z haplotype in 55 of 58 individuals examined (Cox et al. 1987) allowed assignment of the Mmalton haplotype in the proband, an MmaltonZ heterozygote. Pedigree analysis of PI types in conjunction with DNA haplotype in the proband and close relatives were used to determine the QObolton haplotype of the father of the proband.

**Gene cloning**

High molecular weight DNA was partially digested with SauIII and fractionated as described (Fraizer et al. 1989). Fractions containing DNA fragments ranging in size from 10–28 kb were ligated to the phage vector, λDash (Stratagene). About 1.1 × 10^6 recombinant clones were plated and screened. Fractions containing DNA fragments ranging in size from 10–28 kb were ligated to the phage vector, λDash (Stratagene). About 1.1 × 10^6 recombinant clones were plated and screened (Maniatis et al. 1982), first with an exon-I-specific probe and then with an exon V probe (Fig. 2). The cloned DNA was digested with EcoRI, PstI, and BamHI to determine the integrity of the genes. The QObolton clones were distinguished from the Mmalton clones by one of two restriction site differences using either SsrI or TaqI, with hybridization to the probes 4.6 or 6.5, respectively. Locations of the polymorphic sites are shown in Fig. 2. Five phage clones containing the QObolton allele were obtained.

**Sequencing**

Two overlapping phage clones were used to sequence the full-length gene. Both of the phage clones containing the QObolton allele were digested with PstI and all five exons were subcloned in both orientations into pBluescript (pBS, Stratagene). The PstI sites around the α1AT gene are depicted in Fig. 2, with exons named according to Perlino et al. (1987). Each exon was represented by PstI fragments of the following sizes: 0.5 kb (exon I), 1.6 kb (exon II), 2.4 kb (exons III and IV), and 1.1 kb (exon V). The exon II clone was linearized with SacI, digested with mung bean exonuclease and S1 nuclease following the manufacturer's recommendations (Pharmacia), and religated to form smaller subclones, which could then be sequenced using the T7 primer binding site in the pBS vector. All sequencing was done with sequencing (United States Biochemical Corp.) using the didexo chain termination method. The regions sequenced are shown in Fig. 2 as solid bars. The hepatocyte promoter region, exon I, and exon II were sequenced using the T7 primer binding site in pBS. Five synthetic primers were used to sequence the remaining exons. The sequence of the QObolton mutation was verified by sequencing the opposite strand of the 1.1-kb PstI fragment.

**Oligonucleotide hybridization to polymerase chain reaction (PCR) amplified genomic DNA**

The polymerase chain reaction (PCR) was used to amplify 1 μg of genomic DNA from several individuals of known PI types and haplotypes (Saiki et al. 1988), and 10 pg of control DNA was amplified. The QObolton control DNA was the 1.1-kb PstI clone used for sequencing, and the normal control was a subclone containing a normal exon V on a 6.5-kb BamHI fragment. The normal exon V subclone was obtained from the other allele present in the heterozygote described here and was determined to be normal in this region by sequencing (Fraizer et al. 1989). Oligonucleotides specific for the QObolton mutation and the corresponding normal sequence (Fig. 4) were 5' end-labelled following manufacturer’s recommendation (Boehringer-Mannheim). The mutant probe lacks one G at the site of the deletion but has an additional C at the 3' end so that the melting temperatures of the two probes should be similar. The analysis of PCR-amplified DNA was as described (Fraizer et al. 1989) except that the final temperature of hybridization and washes was increased to 63°C.

**Results**

**PI typing of α1AT**

Polyacrylamide isoelectric focusing gel analysis of plasma collected from this 44-member pedigree demonstrated the segregation of the PI deficiency types Mmalton and Z (Fig. 1). Low level deficiency types were confirmed with immunofixation using α1AT antibodies as previously described (Cox 1976; Ar-

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Fig. 1. Pedigree of part of family with QObolton and Mmalton alleles. The original proband (Cox 1976) is indicated with an arrow; the father is the individual whose QObolton allele was cloned and sequenced. Shaded symbols indicate QObolton alleles (QO); black symbols indicate Mmalton alleles (Mm). Serum α1AT concentrations are shown as the percentage of a normal pool.