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Research Article

The most common deficiency allele of the plasma protease inhibitor alpha 1-antitrypsin (alpha 1AT) is PI*Z. Some rare deficiency alleles of alpha 1AT produce low but detectable amounts of plasma alpha 1AT (1-20% of normal), which can be differentiated by isoelectric focusing. Others, designated null (QO) alleles, produce no alpha 1AT detectable by routine quantitative methods. We have previously described a method using DNA polymorphisms, haplotypes, and polyacrylamide isoelectric focusing gels, to differentiate various deficiency alleles. Based on haplotypes, we previously identified, in eight patients, five different null alleles, four of which had been previously sequenced. We have now analyzed all 12 null alleles in these eight patients, using allele-specific oligonucleotide probes, and have identified six different null alleles. We have cloned and sequenced one of these, PI*QOludwigshafen, which has a base substitution in exon II, replacing isoleucine 92 in the normal sequence with an asparagine. This substitution of a polar for a nonpolar amino acid occurs in one of the alpha-helices and is predicted to disrupt the tertiary structure. A total of 13 different alpha 1AT deficiency alleles, 6 of them null alleles, have been sequenced to date.

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A Null Deficiency Allele of α_1 -Antitrypsin, QO_{ludwigshafen}, with Altered Tertiary Structure

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Abstract

The most common deficiency allele of the plasma protease inhibitor α_1 -antitrypsin (α_1 AT) is PI^*Z . Some rare deficiency alleles of α_1 AT produce low but detectable amounts of plasma α_1 AT (1–20% of normal), which can be differentiated by isoelectric focusing. Others, designated null (QO) alleles, produce no α_1 AT detectable by routine quantitative methods. We have previously described a method using DNA polymorphisms, haplotypes, and polyacrylamide isoelectric focusing gels, to differentiate various deficiency alleles. Based on haplotypes, we previously identified, in eight patients, five different null alleles, four of which had been previously sequenced. We have now analyzed all 12 null alleles in these eight patients, using allele-specific oligonucleotide probes, and have identified six different null alleles. We have cloned and sequenced one of these, $PI^*QO_{ludwigshafen}$, which has a base substitution in exon II, replacing isoleucine 92 in the normal sequence with an asparagine. This substitution of a polar for a nonpolar amino acid occurs in one of the α -helices and is predicted to disrupt the tertiary structure. A total of 13 different α_1 AT deficiency alleles, 6 of them null alleles, have been sequenced to date. (*J. Clin. Invest.* 1990. 86:1878–1884.) Key words: protease inhibitor • α_1 -antitrypsin deficiency • chromosome 14

Introduction

α_1 -antitrypsin (α_1 AT),¹ a 52-kD plasma glycoprotein, is the primary inhibitor of the serine protease leukocyte elastase (1). α_1 AT is synthesized primarily in the liver, is secreted into the blood, and diffuses into the interstitial fluids (for reviews, see references 2 and 3). α_1 AT is a highly polymorphic protein with more than 60 electrophoretic variants (4, 5). A deficiency of α_1 AT is associated with emphysema and with childhood liver disease (6, 7). The most common deficiency allele of α_1 AT is PI^*Z , occurring at a frequency of 0.012 in caucasians (8). The Z allele is associated with a low plasma concentration, less than 20% of normal (8), and with liver inclusions (9) apparently due to the tendency to aggregate in vitro (10).

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1. Abbreviations used in this paper: α_1 AT, α_1 -antitrypsin; ASO, allele-specific oligonucleotide; PCR, polymerase chain reaction; PIL, PI-like; QO, null alleles; QO_{lud}, QO_{ludwigshafen}.

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Alleles associated with the most pronounced deficiency states, in which no serum α_1 AT is detected by routine methods, are designated as null (QO) alleles. The null alleles are rare, with a combined frequency estimated to be 10^{-4} among caucasians (11). With the goal of delineating the spectrum of mutations that lead to α_1 AT deficiency, we examined DNA from eight individuals with null alleles. By haplotype analysis, following the algorithm of Cox and Billingsley (11), we identified a unique null allele, designated QO_{ludwigshafen} in an individual of German ancestry. The QO_{ludwigshafen} gene was cloned, and sequenced using asymmetric polymerase chain reaction (PCR) amplification. The limitation of the haplotype approach of screening for unique null alleles is that different null mutations may have occurred on the same haplotype background. Therefore, allele-specific oligonucleotide probes were used to further distinguish between different null alleles.

Methods

Protein Studies. Serum α_1 AT was quantified using electroimmunoassay (5, 12). All sera were examined by isoelectric focusing using narrow range ampholine, Pharmalyne pH 4.2–4.9 (LKB, Sweden), followed by immunoprinting (5, 13). By these assays, in combination with family studies or haplotype analysis, eight individuals were identified previously as having a null allele (11).

Haplotype analysis. These individuals were classified using the algorithm for limited haplotype analysis as described (11). The restriction enzymes used to determine the polymorphic sites forming the haplotypes are listed in Table I in an order that represents the location of the polymorphic sites of the α_1 AT gene and the related PI-like (PIL) gene. Probes used, designated 6.5 (pAT 6.5) and 4.6 (pAT 4.6) (Fig. 1), were provided by S. L. C. Woo. The last five restriction sites listed, not contained within the 6.5 probe, represent polymorphisms in the PIL gene (unpublished data), located 8 to 12 kb downstream of the α_1 AT gene (16, 17). The previous identification of the unique PI^*Z haplotype in 55 of 58 individuals examined (14) allowed the assignment of the complete QO_{ludwigshafen} (QO_{lud}) haplotype in the proband, a QO_{lud}Z compound heterozygote. The QO_{ludwigshafen} allele was initially identified as a unique null allele based on its haplotype.

Gene cloning. High molecular weight DNA suitable for cosmid cloning was extracted from leukocytes from the proband (18). DNA was partially digested with Mbo I, fractionated by sucrose density gradient centrifugation (19) and fractions containing 23 to 50 kb DNA fragments were ligated to the double cos site vector, C2RB (20). Clones were screened (21) with an exon I-specific probe (Fig. 1). Positive clones were digested with Eco RI to determine the integrity of the full-length genes. QO_{ludwigshafen} clones were distinguished from Z clones by two restriction site differences using either Msp I with probe 4.6 or a double digest using Bst EII and Pst I with probe 6.5. Locations of the two polymorphic sites are indicated (Fig. 1).

Sequencing. Cosmid DNA containing the full-length gene was prepared from one QO_{ludwigshafen} clone (22). Single-stranded DNA was prepared for each of the coding exons (II through V) by asymmetric PCR amplification as used on genomic DNA (23), except that 15–150 pg of cosmid DNA was amplified. 11 synthetic primers were used and 7 regions were amplified (Fig. 1). Amplification primers were as fol-

Table I. DNA Haplotypes of a QOudwigshafen Z Heterozygote as Defined by 12 Polymorphic Restriction Sites*

Allele	Probe 4.6 [§]						Probe 6.5 [§]					
	S	Msp	A	A 3/2	M/B	A 5/7	T	Bgl	A 1/4	M	RI	T
Z	—	+	+	+	—	+	+	—	—	—	—	+
QOud [‡]	—	—	—	+	+	—	+	+	—	—	—	—

* Polymorphic restriction enzyme sites are as follows: S, Sst I; Msp, Msp I; A, Ava II; M, Mae III; B, Bst E II; T, Taq I; Bgl, Bgl II; RI, Eco RI; +, presence of site; —, absence of site. [§] Alleles are as described (11, 14, 15). [‡] The QOudwigshafen (QOud) haplotype was initially deduced from the assignment of the proband's Z haplotype, based upon the previous identification of a unique Z haplotype in 55 of 58 individuals examined (53).

lows: exon II, 5' GGTGTCAATCCCTGATCACT, 3' TTGCAAGG-AGAGTTCAAGA; exon III, 5' TTCTGTACACTCTTCCAAA, 3' AGTCCCAACATGGCTAAGAG; exon IV, 5' GTCCAGAAGAA-CAAGAGGA, 3' CACATTCTTCCCTACAGATA; exon V, 5' TCA-GCCTTACAACGTGTCTC, 3' CAGAGAAAACATCCCAGGGA. Exon IV sequence was confirmed by priming from a site within exon V resulting in a 1.3-kb single-stranded template (Fig. 1). DNA was concentrated and unincorporated nucleotides were removed with a Centricon-30 microconcentrator (Amicon Corp., Danvers, MA). The 0.5-kb Pst I fragment containing exon I was subcloned in both orientations into pBluescript (pBS; Stratagene Inc., La Jolla, CA). The hepatocyte promoter region and exon I were sequenced using the T7 primer binding site in pBS. Six primers used to sequence the remaining exons were: exon II, GGGTGTCAATCCCTGATCACT, AACCAGCCAGAC-AGCCAGCT, TTGCAAGGAGAGTTCAAGA; exon III, CTG-CTACACTCTTCCAAACC; exon IV, GAAGAATGTGTGGGG-GCTGC; exon V, TCACATGCAGGCAGGGACCA. The regions sequenced are shown in Fig. 1 as solid bars. All sequencing was done with Sequenase (US Biochemical Corp. Cleveland, OH) using the dideoxy chain termination method. The sequence of the QOudwigshafen mutation was verified by amplifying and sequencing the opposite strand of exon II.

Verification of the mutation

(a) *Hybridization of the QOudwigshafen allele-specific oligonucleotide to polymerase chain reaction amplified DNA.* Genomic DNA from the proband, and 10–100 pg of each control DNA, was amplified using Taq polymerase (24) as described (25). QOudwigshafen control DNA was the cosmid clone used for sequencing. Normal control DNA was a clone containing exon II on a 6.5-kb Bam HI fragment, determined by sequencing to be normal in the region of the mutation (25). Oligonucleotides specific for the QOudwigshafen mutation and its corresponding normal sequence (Fig. 3) were 5' end-labeled following manufacturer's recommendations (Boehringer-Mannheim Diagnostics

Inc., Houston, TX). The analysis of PCR-amplified DNA was as described (25) except that for the QOudwigshafen probe, the hybridization temperature was 45°C and the final wash temperature in 5× SSPE was 50°C.

DNA from 10 normal individuals was selected for testing based on previous haplotyping results (14).

(b) *Xho II digestion of PCR amplified exon II DNA.* Genomic DNA from the proband, his daughter, a normal individual with the identical M2 haplotype, and individuals having α 1AT deficiency variants Mmalton and Mduarte, were amplified by PCR and purified using a Centricon-30 (Amicon Corp.) microconcentrator. DNA was digested with Xho II according to manufacturer's specifications and the samples were visualized on a 2% agarose gel with ethidium bromide staining.

Hybridization of other null alleles with allele-specific oligonucleotides

In order to fully characterize the 12 null alleles in our eight patients with rare deficiency alleles, particularly those that had identical haplotypes, leukocyte DNA was amplified from individuals with null alleles and haplotypes consistent with the QObellingham mutation, a previously sequenced QO allele (26). The QObellingham control was a cosmid clone that had been demonstrated to contain the QObellingham mutation by allele-specific oligonucleotide (ASO) hybridization (Hofker, M. H., unpublished observation). The normal control was the cosmid clone used for sequencing the QOudwigshafen allele, determined to be normal in the region of the QObellingham mutation (this study). The hybridization temperature for the QObellingham probe was 55°C and the final temperature of the washes was 62°C in 5× SSPE.

Results

The QOudwigshafen mutation

Protein studies. The proband of PI type QOudZ had a serum α 1AT concentration of 13% of normal, or 0.17 mg/ml (normal concentration 1.32 mg/ml) (27). When the serum of the proband, observed to be of PI type Z on isoelectric focusing, was examined by isoelectric focusing with immunoprinting, only Z α 1AT could be visualized. The presence of a null allele had originally been recognized through testing of his daughter, who was of PI type M1, with an α 1AT concentration of 48% of normal (0.63 mg/ml). Several years later, when taking oral contraceptives, her serum concentration of α 1AT was 118% of normal (1.56 mg/ml).

DNA haplotype analysis of the QOudwigshafen allele. The two different haplotypes found in the proband are shown in Table I. These haplotypes are associated with two alleles: Z, and a unique null allele, which we named QO*udwigshafen according to nomenclature guidelines (28). The QOudwigshafen mutation must have occurred in an M2 allele, as the

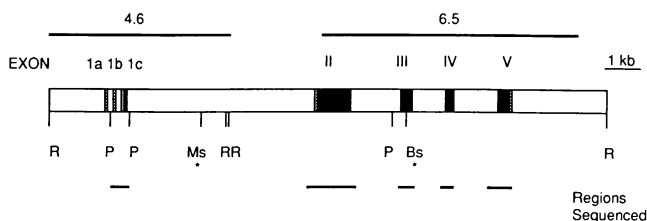


Figure 1. Map of α 1AT gene (exons I–V). Coding exons are filled boxes and untranslated regions are hatched boxes. Exons Ia, Ib, and part of Ic are found only in monocyte, not in hepatocyte transcripts. Probes used: pAT 4.6 and pAT 6.5. Two polymorphic restriction sites, Msp I (*Ms**) and Bst EII (*Bs**), were used to identify the alleles in isolated clones, and Eco RI (*R*) sites were used to determine gene integrity of isolated clones. Pst I sites (*P*) were used for subcloning exon I. Solid heavy bars indicate regions sequenced.

associated haplotype is identical to the most common M2 haplotype for all enzymes tested. The same M2 haplotype is also associated with the Mmalton deficiency allele (25).

Cloning and sequencing. Six cosmid clones were purified; full-length genes were obtained in five as determined by digestion with Eco RI and hybridization with the probes 4.6 and 6.5. The absence of the polymorphic Msp I site in the intron 3' of exon I and the presence of the polymorphic Bst EII site in exon III (Fig. 1 A) differentiated the QOludwigshafen clone from the Z clone.

Sequence was obtained for all five exons, all intron/exon junctions and the hepatocyte transcriptional control region (29) and compared with the genomic sequence of S (30) and cDNA of M2 (31). A single T to A base transversion in exon II was the only mutation found (Fig. 2), except for the mutations characteristic of S and M2. The substitution results in an altered amino acid 92. The other differences observed were the absence of the S mutation (valine was found at amino acid 264) and the presence of the asparagine at 376 and histidine at 101 as in M2 (31).

Hybridization of the QOludwigshafen ASO to polymerase chain reaction amplified DNA. The QOludwigshafen mutation was confirmed by oligonucleotide hybridization after PCR amplification of both the cloned allele and genomic DNA from the proband (Fig. 3). Since the QOludwigshafen haplotype is identical to both the haplotype associated with the normal variant M2 and the Mmalton deficiency allele (25), it was important to determine if the mutation was unique to the QOludwigshafen allele. Genomic DNA from six independent M2 haplotypes, other deficiency alleles having the same M2 haplotype, the proband, and his daughter were amplified along with the QOludwigshafen cosmid control and a subclone containing a normal exon II. The QOludwigshafen ASO hybridized to amplified genomic DNA from the proband and his daughter, confirming the inheritance of this null allele. Conversely, the QOludwigshafen ASO did not hybridize to the normal control, normal M2, Mmalton deficiency, or QOmat-tawa alleles.

NORMAL			LUDWIGSHAFEN		
DNA Sequence	aa	Codon No.	DNA Sequence	aa	Codon No.
G A G G C T C A G A T C C A T G A A	glu	89	G A G G C T C A G A T C C A T G A A	glu	89
	ala	90		ala	90
	gln	91		gln	91
	iso	92		asn	92
	his	93		his	93
	glu	94		glu	94

Figure 2. Sequence of the region around the QOludwigshafen mutation in exon II, obtained by the dideoxynucleotide chain termination method. Codon numbers and corresponding amino acids in the mature protein are indicated. The sequence of the QOludwigshafen allele is identical to that for M2 except for the T → A alteration (depicted in bold), resulting in the substitution of asn for ile 92.

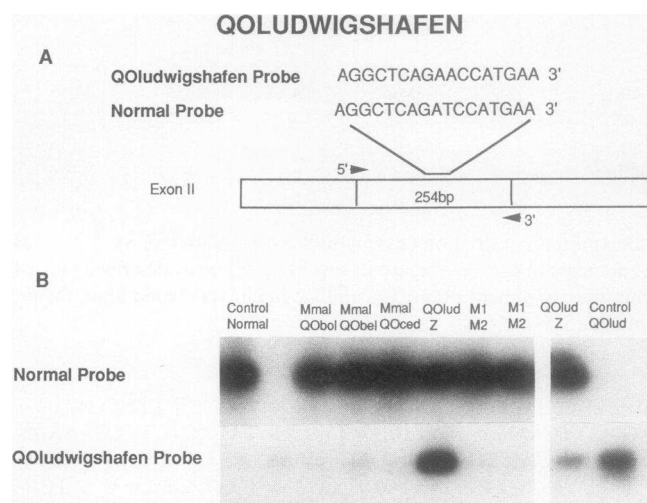


Figure 3. QOludwigshafen allele ASO hybridization to amplified genomic DNA. (A) Arrows indicate the location of the 5' and 3' PCR amplification primers which flank the substitution. Sequences of the normal and QOludwigshafen ASO probes are listed (B) The QOludwigshafen ASO or its normal counterpart were hybridized to DNA amplified by PCR. Genotypes tested are indicated above each lane: M1, M2, M3, Z, QObellingham (QObel), QOcedarisland (QOced), QObolton (QObol), QOludwigshafen (QOlud), and Mmalton (Mmal). Plasmid clones containing exon II from either the QOludwigshafen or the normal allele were amplified for homozygous controls.

Identification of QOludwigshafen with the restriction enzyme Xho II. The T → A transversion of QOludwigshafen results in the loss of an Xho II site. The PCR amplification product of exon II was 254 bp in length. DNA without the QOludwigshafen allele was predicted to produce products of 216 and 38 bp, whereas DNA with the QOludwigshafen allele would remain intact at 254 bp. The controls used were an Mmalton cosmid clone determined to be normal in this region by sequencing (25) and the QOludwigshafen cosmid clone known to contain the mutation by sequence analysis. Amplified DNA from the proband and his daughter both showed, on agarose electrophoresis, fragments of 254 and 216 bp. This is consistent with their heterozygous state at the α 1AT locus, QOludZ, and QOludM1, respectively (Fig. 4).

Examination of other null alleles. In our eight patients with QO alleles we had identified five different haplotypes associated with a QO allele. Of the 12 QO alleles, seven had the M1val213 haplotype associated with the QObellingham mutation (11), so we determined if all seven carried the QObellingham allele (data summarized in Table II). This was done by hybridization with the QObellingham ASO after PCR amplification of genomic DNA (Fig. 5). Six of the seven alleles were QObellingham DNA samples from five normal individuals having haplotypes identical to that of QObellingham, and were amplified along with control clones containing exon III from either the QObellingham or the QOludwigshafen allele, the latter being normal in this region. The QObellingham ASO did not hybridize to any of the normal alleles, including those with an M1ala213 haplotype or with M1val213 (the ancestral allele for M2), or to any of the deficiency alleles with haplotypes different from M1val213. Based on haplotype analysis, we had previously suggested that the second QO allele found in

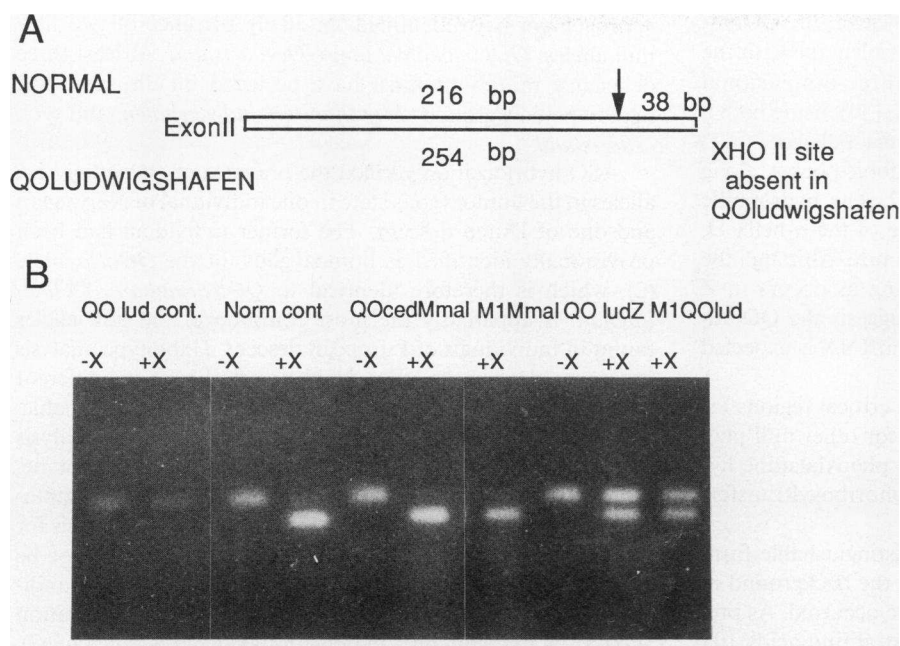


Figure 4. Identification of *QO ludwigshafen* by digestion with Xho II. Genomic DNA was amplified by PCR, producing a 254-bp fragment. Amplification products were run in the agarose gels as controls (–X) or after digestion with Xho II (+X). The T to A mutation in *QO ludwigshafen* destroys an Xho II site in exon II. Genotypes tested are indicated, abbreviations are as in Figs. 3 and 4. Plasmid clones containing exon II from *QO lud* or a normal allele were digested and used as homozygous controls.

the proband in association with *QO hongkong* (32) might be a *QO bellingham* allele (11), but this is not the case, as demonstrated by ASO hybridization. We have tentatively designated the different allele as *QO hongkong2*. Another potential *QO bellingham* allele, found in combination with the deficiency allele *Mmalton*, was demonstrated to be a different null allele, which we designated *QO cedarisland* according to nomenclature guidelines (28). ASO hybridization confirmed the presence of the *QO bellingham* allele in four individuals, two of whom were homozygous for the *QO bellingham* allele (data not shown) and two heterozygous for other deficiency alleles.

Discussion

The null alleles of α_1 AT described to date, which have less than 1% of the normal concentration of α_1 AT, have base substitutions, deletions, or insertions that result in the generation of a premature translational stop codon. However, other possible mechanisms for generating null alleles are promoter/en-

hancer defects, splice site mutations, or critical amino acid substitutions. The *QO ludwigshafen* mutation is a T to A transversion in exon II, resulting in replacement of isoleucine 92 by asparagine. A comparison of amino acids found at the equivalent of position 92 in human and baboon α_1 AT, human α_1 -antichymotrypsin, human antithrombin III, chick ovalbumin, and rat angiotensin reveals that uncharged nonpolar resi-

Table II. Null Alleles Analyzed by Hybridization with Probes

Allele	Number observed*	Haplotype derivation	ASO verification
<i>QO ludwigshafen</i>	1	M2	+ASO <i>QO lud</i>
<i>QO bolton</i>	1	M1 val213 = Taq	+ASO <i>QO bolton</i>
<i>QO bellingham</i>	6	M1 val213	+ASO <i>QO bel</i>
<i>QO mattawa</i>	1	M3	–ASO <i>QO bel</i>
<i>QO hongkong1</i>	1	M2†	–ASO <i>QO bel</i>
<i>QO hongkong2</i>	1	M1 val213§	–ASO <i>QO bel</i>
<i>QO cedarisland</i>	1	M2	–ASO <i>QO lud</i>

* Independent allele observations in unrelated individuals.

† Rare M₂ haplotype for Europeans, frequency in Asian population unknown.

§ Derivation based on European haplotypes may not apply to Asian population.

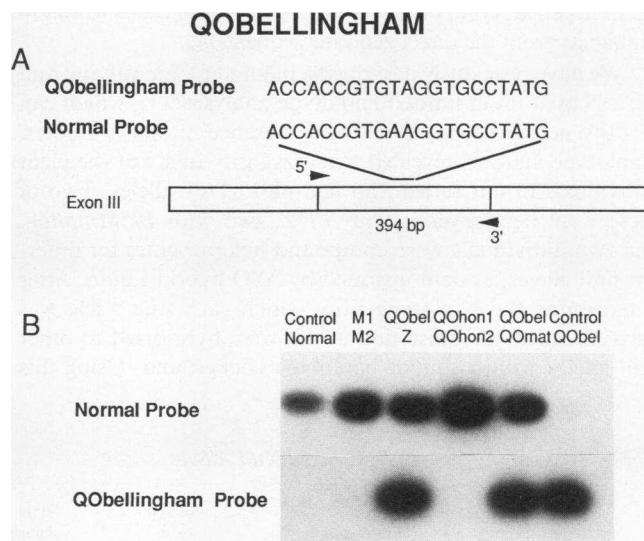


Figure 5. QObellingham ASO hybridization to amplified genomic DNA. (A) Arrows indicate the location of the ASO probes and the 5' and 3' PCR amplification primers which are separated by 394 bases and flank the substitution. Sequences of the normal and QObellingham ASO probes are listed. (B) The QObellingham ASO or its normal counterpart was hybridized to DNA samples amplified by PCR. Haplotypes tested are indicated above each lane: M1, M2, Z, QO mattawa (QO mat), QO hongkong (QO hon), and QObellingham (QO bel). Plasmid clones containing exon III from either the QObellingham or the normal allele were amplified for homozygous controls.

dues always occur at this location (33). However, the QOludwigshafen mutation replaces the nonpolar isoleucine with the polar asparagine. Examination of the three-dimensional model, based on studies of Loeberman et al. (34), using both a computer image and a physical model of crystallized α 1AT, reveals that amino acid 92 lies in a hydrophobic pocket, along with methionine 63 and phenylalanine 82. The hydrophilic asparagine would result in a sharp twisting of the α -helix D, with resulting distortion of the tertiary structure. Although the disruption could result in impaired folding as occurs in Z α 1AT, the complete absence of protein suggests the QOludwigshafen protein is destabilized. Normal mRNA is expected in this type of mutation.

This type of mutation, which identifies critical regions for protein conformation, has been described for other null phenotypes such as found for Factor IX (35), phenylalanine hydroxylase (36), and hypoxanthine phosphoribosyltransferase (37).

The QOludwigshafen haplotype is indistinguishable from that of one of the M2 haplotypes (11, 15), the background of the allele on which the mutation must have occurred. As predicted from haplotype data, the changes at amino acids 101 and 376 associated with M2 (31) are found in the QOludwigshafen sequence. To verify that the substitution in exon II of the QOludwigshafen allele is not characteristic of M2, which constitutes 10% of the normal M alleles, we hybridized the QOludwigshafen mutant oligonucleotide to PCR amplified DNA of the identical M2 haplotype. The QOludwigshafen allele was clearly distinguished from the M2 alleles and from the normal M1 or M3 alleles. Although direct sequencing of amplified exons can be carried out from genomic DNA, the prior cloning allows unambiguous assignment of the common mutations that occur in several exons. Since rare deficiency states will frequently occur in genetic compounds, correct matching of exons will allow ambiguity from the direct genomic sequencing.

We have previously described a method for identifying null alleles based upon limited haplotype analysis (11), which can identify heterozygosity even in the absence of family studies. Haplotype analysis revealed heterozygosity in six of the eight individuals in our series who had null α 1AT alleles. Two of these were heterozygous with PI*Z, two with PI*Mmalton, and two individuals were compound heterozygotes for different null alleles, as demonstrated by ASO hybridization. After determining the haplotype from which each null allele was derived, ASOs for those null alleles were hybridized to other null alleles with a similar haplotype background. Using this

approach we have identified the likely presence of two new null alleles: QOhongkong2 and QOcedarisland. At least three deficiency mutations must have occurred on the same M2 haplotype background: Mmalton, QOludwigshafen, and QOcedarisland.

ASO hybridization verified the presence of QObellingham alleles in the homozygous state in one individual of Norwegian and one of Dutch descent. The former individual had been provisionally identified as homozygous for the QOoslo allele (2), which is therefore identical to QObellingham. QObellingham is apparently the most common of the null alleles found in individuals of European descent. Haplotype analysis is hampered when the individual being studied is of a different ethnic origin than that for which haplotype data are available, as for QOhongkong (i.e., hongkong1). By sequence analysis (32), this allele must have been derived from an M2 haplotype, but the haplotype does not resemble the common M2 haplotype of European populations (15). Our haplotype analysis for QOhongkong1 was incomplete because phase could not be assigned to the one RFLP showing heterozygosity in this individual (4.6 probe and Ava II digestion). ASO hybridization allowed us to distinguish the new null allele (QOhongkong2), from the QObellingham allele in the compound heterozygote, despite haplotypes that differ at no more than one polymorphic restriction site. We have not used ASO hybridization for the QOgranite falls allele (38) because we have found this mutation results in the loss of a Mae II site in exon II (11) and none of the alleles we have examined have lost that site. Furthermore, the haplotype on which the QOgranite falls mutation occurred is the M1ala213 haplotype, which is not present with any of the null alleles in our series.

Since the combined frequency for all the null alleles is estimated, based on unbiased ascertainment, to be 1.4×10^{-4} (8), the individual null alleles are even more rare. We have observed the QOludwigshafen allele in only one pedigree to date. This has been the case for all other null alleles described except for the QObellingham allele (26) which has been observed in several different pedigrees of European origin. Of four alleles ascertained without bias in Canadian caucasians, two were QObellingham, which is therefore estimated to have an allele frequency of 7×10^{-5} .

Six QO alleles have now been sequenced (Table III). QOcardiff (41) is not included, as the reported change of asparagine 256 to valine is the same as the mutation described for the PI*P allele (42), coding for a somewhat unstable protein associated with $\sim 30\%$ of the normal serum concentration of

Table III. Summary of the Sequenced Null Alleles

Allele	Ethnic origin	Altered exon	Base change	Amino acid change	Premature stop	mRNA present	Reference
QObellingham	Dutch, other	III	A \rightarrow T	217 Lys \rightarrow stop	217	no	26
QObolton	British	V	delC	362/3glu \rightarrow arg	373	—	39
QOgranitefalls	American, white	II	delC	160 tyr \rightarrow stop	160	no	38
QOhongkong1*	Oriental	IV	delTC	319 ser \rightarrow arg	334	yes	32
QOmattawa	French	V	insert T	353 leu \rightarrow phe	376	yes	40
QOludwigshafen	German	II	T \rightarrow A	92 ile \rightarrow asn	none	—	this study

* Previously called QOhongkong, number has been added because a second null allele (QOhongkong2) has been identified in the same individual, according to haplotype analysis.

α 1AT. The presence of other differences from P has not been excluded. The five previously sequenced null alleles all have premature termination codons. Only two of these null alleles retain the active site of α 1AT at amino acid 358. The *QObel-lingham* alleles (26) and *QOgranitefalls* (43) are apparently associated with unstable transcripts, as no mRNA could be isolated from alveolar macrophages obtained from lung lavage, a normal source of α 1AT mRNA. In contrast, the *QO-mattawa* allele is associated with stable mRNA but no protein is secreted in in vitro expression assays (40). The *QOhongkong* allele codes for a protein that is not transported from the rough endoplasmic reticulum to the Golgi apparatus but is apparently too unstable to form inclusions (32).

The null alleles lie at the extreme of a spectrum of alleles that result in various degrees of deficiency. Seven distinct deficiency alleles, which produce detectable plasma protein product, (\sim 3–18% of normal) have also been sequenced and do not have premature termination codons as are found in the null alleles. Three of the four deficiency alleles associated with inclusion bodies have been sequenced. The PI Z mutation (glutamine to lysine at amino acid 342) (44, 45) and the Mmalton (25) and Mnichinin (46) mutations (deletion of phenylalanine at amino acid 51/52) result in proteins that readily aggregate in vitro and form stable inclusion bodies in the liver. The phenylalanine deletion of the latter two PI types occurred on different backgrounds, M2 and M1val213, respectively. Similarly, the PI Z mutation has occurred on an M2 background in *Zunbridgewells* (47), which appears to be identical to *PI*Zaugsberg* (48). Two of the deficiency alleles, *Mheerlen* (49) and *Mprocida* (50), have amino acid substitutions involving a proline and therefore likely form unstable proteins. The Mheerlen substitution would change the bend of the β -sheet B4; the Mprocida substitution would disrupt α -helix A. Neither are associated with liver inclusion bodies (50, 51). Mmineralsprings, which shows a modest reduction in concentration, is also functionally deficient (14% of normal) (52). *PI*Mduarte* and *PI*cobalt* could be the same from protein studies but are not sequenced (11).

Clinical implications for lung destruction may be similar for all of the deficiency variants, except that there is evidence that even the low amount of α 1AT produced in PI ZZ homozygotes is protective in comparison with PI QO homozygotes (53). Liver disease has only been described in association with *PI*Z* (7) and *PI*Mmalton* (54). Further studies of patients with rare deficiency alleles may eventually help clarify whether the plasma deficiency or liver accumulation of α 1AT is responsible for liver disease.

Haplotype and sequencing studies have now demonstrated a minimum of 15 different deficiency alleles, with different types of molecular defects. Characterization of the spectrum of mutations leading to α 1AT deficiency allows delineation of amino acid residues critical for stability, for normal secretion, and for normal function.

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