Rare Deficiency Types of α_1-Antitrypsin: Electrophoretic Variation and DNA Haplotypes

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Summary

A deficiency of the plasma protease inhibitor α_1-antitrypsin (α1AT), is usually associated with the deficiency allele PI^*Z. However, other alleles can also produce a deficiency. Some of these rare deficiency alleles produce a low concentration (3%–15% of normal) of α1AT and include Mmalton, Mduarte, Mheerlen, and Mprocida. Null, or nonproducing, alleles are associated with trace amounts (<1%) of plasma α1AT. We have identified, using isoelectric focusing, the deficiency alleles in 222 patients (68 children and 154 adults) with α1AT deficiency. In addition to PI^*Z, we found low-producing alleles $PI^*Mmalton$ and $PI^*Mcobalt$ and four null (PI^*QO) alleles. On the basis of a population frequency of .0122 for PI^*Z, frequencies for other deficiency alleles are 1.1×10^{-4} for $PI^*Mmalton$, 2.5×10^{-5} for $PI^*Mcobalt$ (which may be the same as that for $PI^*Mduarte$, and 1.4×10^{-4} for all null alleles combined. Using 12 polymorphic restriction sites with seven different restriction enzymes, we have obtained DNA haplotypes for each of the rare deficiency types. All of the rare deficiency alleles can be distinguished from PI^*Z by their DNA haplotype, and most can be distinguished from each other. DNA haplotypes are useful to indicate the presence of new types of null alleles, to identify genetic compounds for rare deficiency alleles, and to identify the original normal allele from which each deficiency allele is derived.

Introduction

α_1-Antitrypsin (α1AT, also called α1-protease inhibitor), is the major protease inhibitor in human plasma. More than 60 genetic variants have now been identified (Cox et al. 1980; D. W. Cox, unpublished data); almost all are normal electrophoretic variants with no physiological significance. A deficiency of α1AT is often associated with early-onset emphysema (Laurell and Eriksson 1963) and infrequently with childhood liver disease (Sharp et al. 1969); however, an unknown proportion of those with the deficiency escape significant clinical illness. The deficiency alleles are usually considered those which result in the production of <20% of the normal amount of α1AT, since a higher plasma concentration is not usually associated with an increased risk for disease (reviewed in Cox, in press).

The most common deficiency allele is PI^*Z, which has a frequency of .01–.02 in the North American white population (Pierce et al. 1975; Cox et al. 1981) and .018 in Scandinavia, as calculated on the basis of population screening (Laurell and Sveger 1975). Several rare deficiency alleles have been reported in which the amount of α1AT produced is similar to that of the PI^*Z allele or somewhat less, generally in the range of about 3%–15% of normal: Mmalton (Cox 1976), Mduarte (Lieberman et al. 1976), Mheerlen (Kramps et al. 1981), and Mprocida (Takahashi et al., in press). The mutation of Z α1AT results in impaired secretion and liver storage (Sharp 1971), apparently owing to a pronounced tendency to aggregate (Cox et al. 1986). Mduarte and Mmalton are similarly associated with liver inclusions (Lieberman et al. 1976; Roberts et al. 1984). Mmalton α1AT also shows a pronounced tendency to aggregate (Cox et al. 1986). In addition, null (or QO) alleles have been described in which only trace amounts, generally <1% of normal, of α1AT are detected in the plasma (reviewed in Cox and Levison 1988). DNA sequencing studies indicate that null alleles are produced by several different mutations (Brantly et al. 1988).

Elsewhere we have described restriction-site polymorphisms at the PI locus which result in specific DNA haplotypes associated with common M subtypes and have shown that PI^*Z is associated with one unique haplotype (Cox et al. 1987a).

In the present report, we compare the electrophoretic characteristics of several of the rare deficiency alleles for which a plasma product can be identified. By using a number of restriction enzymes, we have obtained, for many of the rare deficiency alleles, DNA haplotypes which contribute to their identification. We have used DNA haplotypes to determine the origin of the deficiency alleles from their normal ancestral alleles.

Material and Methods

Plasma from blood collected in EDTA was obtained from probands and from selected first-degree relatives. Probands had sought medical attention for clinical symptoms, usually relating to the liver or lung. Part of this group of patients was included in a previous preliminary report (Cox et al. 1981). Patients from outside of the usual referral area, referred specifically because they were unusual, e.g., null homozygotes, were excluded from estimates of gene frequencies.

PI typing and $\alpha 1AT$ quantification were carried out on serum from available relatives including children, parents, and sibs of patients. PI typing was carried out by isoelectric focusing in polyacrylamide gels (PIEF) according to a method described elsewhere (Cox 1981), except that the ampholine was Pharmalyte, pH 4.2–4.7 (Pharmacia). Immunofixation was carried out after PIEF (Arnaud et al. 1977; Jeppsson and Franzen 1981), using specific $\alpha 1AT$ antibody (Atlantic Antibodies, Scarborough, ME). Agarose electrophoresis was carried out in barbital buffer, pH 8.6 (Johansson 1972). Sera from the original probands carrying Mduarte and Mheerlen variants were made available, for comparison, by Drs. Lieberman and Klasen. Serum $\alpha 1AT$ concentration was determined by electroimmunoassay, with conditions as outlined elsewhere (Laurell 1966; Cox 1981). $\alpha 1AT$ concentration was expressed as percentage of a normal pool that was based on a standard of 1,000 normal donors obtained at the University of Washington. The 100% value is equivalent to 1.3 mg/ml (Jeppsson et al. 1978).

DNA was extracted from the buffy coats of blood, collected in EDTA (Madisen et al. 1987), from probands and from selected first-degree relatives. In addition, DNA was obtained from additional patients referred to our laboratory specifically because of suspected unusual PI variants. DNA was digested with various restriction enzymes according to conditions recommended by the manufacturer. Fragments were separated in agarose and were transferred to Hybond-N™ (Amersham) or Biodyne™ (Pall) membranes according to the method of Southern (1975). Prehybridization, hybridization with the appropriate $\alpha[32P]dCTP$-labeled probe, washing, and exposure to X-ray film were carried out as described for our laboratory (Cox et al. 1987a). Genomic probes used in the present study were as follows: (1) a 4.6-kb probe including the first exon of $\alpha 1AT$ and (2) a 6.5-probe including most of the coding region of $\alpha 1AT$ (Kidd et al. 1983; Cox et al. 1985); both probes were provided by Dr. S. C. L. Woo.

The restriction enzymes used to detect common polymorphisms were as follows: with probe 4.6 — $SstI$, $MspI$, and $AvaII$ (Cox et al. 1985); with probe 6.5 — $AvaII$ (Cox et al. 1985), $MaeIII$ and $TaqI$ (Cox et al. 1987a), $BstEII$–$BglII$ (Cox et al. 1987b), and $EcoRI$ (Hodgson and Kalsheker 1987). The size of polymorphic fragments and the use of these enzymes to obtain a DNA haplotype have been described elsewhere (Cox et al. 1987a), except for the latter two enzyme-probe combinations. For $EcoRI$, detecting a polymorphism of the downstream PI-like gene, fragments are 7.7 or 5.9 kb. For the $BstEII$-$BglII$ combination, major polymorphic fragments from the $\alpha 1AT$ gene are 5.1 or 4.9 kb, detecting the same amino acid–213 restriction site as does $MaeIII$, and the less intense fragments, from the PI-like gene, are 2.9 or 2.1 kb (Cox et al. 1987b), owing to a polymorphic $BglII$ site (Cox and Coulson 1987).
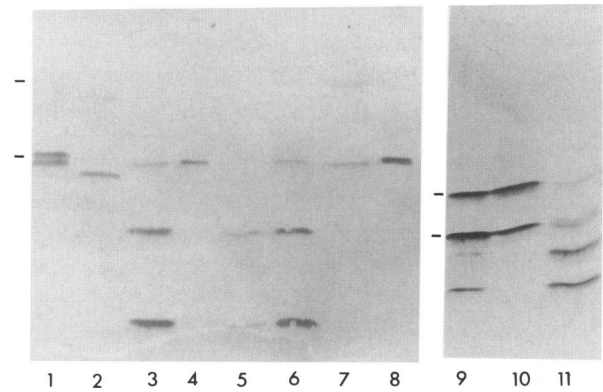
Results

Sera from 222 patients (68 children and 154 adults) with $\alpha 1AT$ deficiency were subjected to isoelectric focusing, both with and without immunofixation. Immunofixation was effective for confirming non-Z-deficiency $\alpha 1AT$ types, which are not clearly visualized consistently by routine PIEF. Five individuals were found to carry non-Z variants whose products could be visualized by PIEF. Three of these were identical, by PIEF and by agarose-gel electrophoresis, to Mmalton, in the proband from the family in which the Mmalton variant was described (Cox 1976). One, which we have named PI Mcobalt according to nomenclature guidelines (Cox et al. 1980), was indistinguishable, by PIEF, from both Mduarte and the normal M3 variant, except for its very low concentration. These variants were compared with variants Mheerlen and Mduarte. The positions of the rare deficiency variants Mcobalt and Mmalton shown by PIEF in figures 1 and 2 are as follows, anodal to
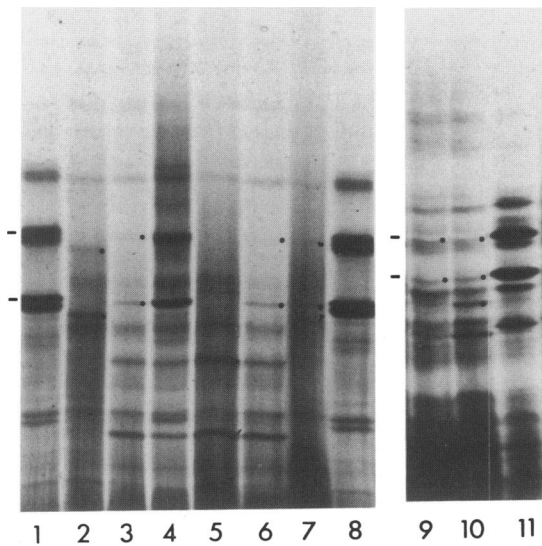
Figure 1   Rare deficiency variants by PIEF. Dashes (–) indicate two major bands of M1; black dots (·) indicate two major bands of each variant. The anode is at the top. Lane 1, M1M2; lane 2, Mmal; lane 3, McobaltZ; lane 4, M3Z; lane 5, ZZ; lane 6, McobaltZ; lane 7, Mheerlen; lane 8, M1M2; lane 9, Mduarte; lane 10, McobaltZ; lane 11, M1.


Figure 2   Rare deficiency variants as shown by print immunofixation after PIEF. Dashes (–) indicate two major bands of M1. The anode is at the top. Lane 1, M1M2; lane 2, Mmal; lane 3, McobaltZ; lane 4, M3; lane 5, ZZ; lane 6, McobaltZ; lane 7, Mheerlen; lane 8, M3; lane 9, M3; lane 10, Mduarte; lane 11, McobaltZ.

cathodal: M1 > M3 = Mcobalt = Mduarte = Mheerlen > M2 > Mmalton. The appearance of the deficiency variants by agarose-gel electrophoresis is shown in figure 3, with relative mobilities as follows: Mmalton > Mcobalt > Mduarte = M3 = M2 = M1. In our preliminary report, we had considered Mcobalt to be identical to Mduarte and had suggested that the slight difference in mobility could be due to molecular interaction (Cox et al. 1981). Because of recent information that

there are many different deficiency alleles, we are considering the two as different variants until the mutant sites are known for each. The deficiency alleles Mmalton and Mcobalt could usually be visualized by routine PIEF, although confirmation by immunofixation was important because of the various minor protein components observed for protein-stained PIEF. Mmalton is more frequently visualized in the heterozygous state because it is clearly separable from the normal M subtypes. However, Mcobalt would not likely be visualized in the presence of M2, and certainly not in the presence of M1 or M3.

The numbers and percentages of the deficiency variants are shown in table 1 for probands only, excluding relatives. Although the numbers are small, it is interest-
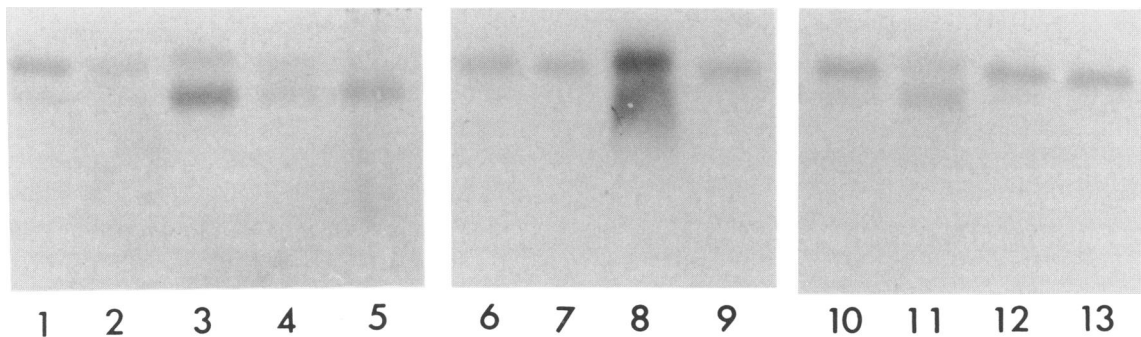
Figure 3   Rare deficiency variants by immunofixation following agarose-gel electrophoresis. The anode is at the top. Lane 1, M3Z; lane 2, Mduarte; lane 3, McobaltZ; lane 4, Mduarte and ZZ mixture; lane 5, ZZ; lane 6, M2; lane 7, M3; lane 8, Mmal; lane 9, M1; lane 10, M2Z; lane 11, MmalZ; lane 12, M2Z; lane 13 M1Z.

Table 1

Frequency of PI Type in Probands

PI TYPE	No. (%) OF Children	No. (%) OF Adults
ZZ	67 (98.5)	146 (94.8)
MmaltonZ	1 (1.5)	3 (1.9)
McobaltZ[a]	0	1 (.6)
ZQO	0	3 (1.9)
QOQO	0	1 (.6)
Total	68	154

[a] Similar to Mduarte but may be a different variant.

Table 2

Frequencies of PI Deficiency Alleles in Adults and Children (444 alleles)

Allele	Frequency in Series	Population Frequency[a]
Z	.978	1.22×10^{-2}
Mmalton	.009	1.1×10^{-4}
Mcobalt	.002	2.5×10^{-5}
QO	.011	1.4×10^{-4}

[a] Non-Z frequencies are calcualted from Z frequency (see text).

ing to note that the rare variants have been almost entirely found in adult probands, most of whom have lung disease, and not in children with liver disease.

The frequency of the PI deficiency alleles is shown in table 2. The PI^*Z frequency was calculated from population data (Cox et al. 1981). The rare deficiency-allele frequencies were obtained from probands ascertained through illness but provide appropriate population frequencies if all rare deficiency alleles have similar risks for clinical disease. Because of the relatively small numbers, they should be considered as only approximate.

Sera of probands and their relatives were used for the calculation of serum concentration of α1AT associated with various PI types. For PI type ZZ, the mean ± SD serum concentration was 17.8% ± 5.2% of normal for 193 adults and 23.9% ± 8.0% of normal for 61 children. The concentration of α1AT in the probands was rarely >25% of normal in adults (6%) and was more frequently so in children (36.1%). Seven (11.5%) children had more than 30% of normal α1AT in serum. The concentration appears to be elevated particularly in association with liver disease in children. The concentration of α1AT in various deficiency types is shown in table 3 for all adults (>18 years old) tested,

including relatives. From these values, it can be calculated that, on average, the Z allele contributes approximately 18% of normal, Mmalton 13%, Mcobalt (and Mduarte) 7%, and null <1%. The concentration of α1AT in heterozygotes for rare deficiency alleles is shown in table 4, in comparison with that of MZ heterozygotes. Normal M individuals tested in our laboratory had a mean ± SD serum α1AT concentration of 100% ± 23.5% of normal (Cox et al. 1976).

DNA Haplotypes Associated with Deficiency Alleles

DNA from all individuals with rare deficiency alleles and from one or more first-degree relatives was digested with various restriction enzymes and was hybridized to specific probes as indicated in Material and Methods. Haplotypes were derived from segregation in families, where the patient was not homozygous, or, in the case of heterozygotes with Z, the usual haplotype associated with the Z allele was assumed. Numbers of samples studied were as follows: Mmalton apparent homozygote, 1; MmaltonZ 2; McobaltZ, 1; QO homozygotes, 3; ZQO heterozygote, 1. In addition to these samples from our own series, DNA was generously made available, for QOhongkong (Sifers et al. 1988), by Drs.

Table 3

Serum Concentration (expressed as % of normal levels) of α1AT in Adults with Deficiency

	PI TYPE ZZ	QOQO	MmalQO	MmalZ	McobZ	ZQO
Mean	17.8	<1	13.4	14.8	12.5	11.3
SD	5.2		3.0	4.2	2.1	3.8
No. tested	193	5	6	9	2	3

Table 4

Serum Concentration (expressed as % of normal levels) of α1AT in Adults Heterozygous for Deficiency[a]

	PI Type			
	MZ[b]	MMmal	MMdua[c]	MQO
Mean	64	64	48	56
SD	15.2	17.4	12.3	8.7
No. tested	77	29	4	27

[a] Heterozygotes with one normal and one deficiency allele.
[b] Source: Cox et al. (1976).
[c] Includes three MMduarte and one MMcobalt (see text).

So and Muensch and, for Mheerlen (Hofker et al., in press), by Drs. Hofker and Frants.

Results of the restriction digestions are indicated in table 5. Typical Southern blots are shown for selected enzymes useful for identifying the rare variants: BstEII-BglII and TaqI with probe 6.5; MspI and AvaII with probe 4.6 (fig. 4). The restriction sites have been arranged in order from 5' to 3', on the basis of cosmid mapping (D. W. Cox, G. D. Billingsley, and M. H. Hofker, unpublished data). We have elsewhere shown that the PI*Z allele occurs with the specific haplotype indi-

cated in table 5 (Cox et al. 1985, 1987a, 1987b), to which we have added additional enzymes (D. W. Cox, unpublished data). The results indicate that PI*Mmalton and PI*Mcobalt have DNA haplotypes that differ from each other and from that associated with PI*Z. PI*Mheerlen, which is associated with an α1AT concentration of <2% of normal (Kramps et al. 1981) and has a mobility similar to that of Mcobalt and the normal M3 allele, has an entirely different haplotype, being derived from the M1 form with an alanine substitution at amino acid 213, designated as M1ala 213, or M1A (Cox et al. 1987a).

Our DNA haplotypes indicate that there are several varieties of the null (QO) allele. One null allele, which we have named PI*QOludwigshafen, according to nomenclature guidelines (Cox et al. 1980), has the same haplotype as Mmalton. We have considered these as separate variants because no product is visible for QO*ludwigshafen, even when immunofixation is carried out on undiluted serum. We have reported three sisters homozygous for a QO allele who are in fact genetic compounds, with one allele that we have named PI*QOmattawa and with another allele with a different haplotype, found by sequencing (R. G. Crystal, personal communication) to be PI*QObellingham (Satoh et al. 1988). This second haplotype was found in one

Table 5

Rare Deficiency Haplotypes

| Allele (N)	Ancestral Allele[b]	4.6 Probe[a]			6.5 Probe[a]								
		SstI	MspI	AvaII	AvaII 3/2	MaeIII BstEII[c]	AvaII 5/7	TaqI 2.0/1.4	BglII*	AvaII* 1/4	MaeIII* 2.3/2.5	EcoRI*	TaqI* 6.7/4.8
Z		−	+	+	+	−	+	+	−	−	−	−	+
Mmalton (4)	M2	−	−	−	+	+	−	+	+	−	−	−	−
Mcobalt (1)	Rc(M2,M3)[d]	−	−	−	+	+	−	+	−	−	−	−	−
Mheerlen (2)	M1ala213	+	+	+	+	−	+	+	+	+	+	+	0
Null types:													
QOmattawa (1 or 3)	M3	+	+	+	+	+	−	+	−	−	−	−	+
QOludwigshafen (1)	M2	−	−	−	+	+	−	+	+	−	−	−	−
QOhongkong[e] (1)		+	−	±	+	+	−	+	−	−	−	−	+
QObellingham[e] (5)	S,M1val213, M3	+	−	+	+	+	−	+	−	−	−	−	+

[a] Numbers for AvaII refer to band numbers published previously (Cox et al. 1985) as follows: band 2 (1.2 kb), band 3 (1.0 kb), band 5 (0.68 kb), band 7 (0.48 kb), band 1 (2.7 kb), and band 4 (0.72 kb). For MaeIII and TaqI two polymorphisms each are revealed: one in the α1AT gene and 3' flanking region, respectively, given on the left; another in the PI-like gene. All RFLPs in the PI-like gene are indicated by an asterisk (*).

[b] Based on haplotypes with these common variants (Cox et al., 1987a).

[c] This restriction site is detected with either BstEII or MaeIII.

[d] Rc = recombinant, from ancestral haplotypes associated with stated alleles.

[e] Genetic compound for two rare deficiency variants. If AvaII (4.6 probe) is (−) with QOhongkong, then the other haplotype would be that of QObellingham.
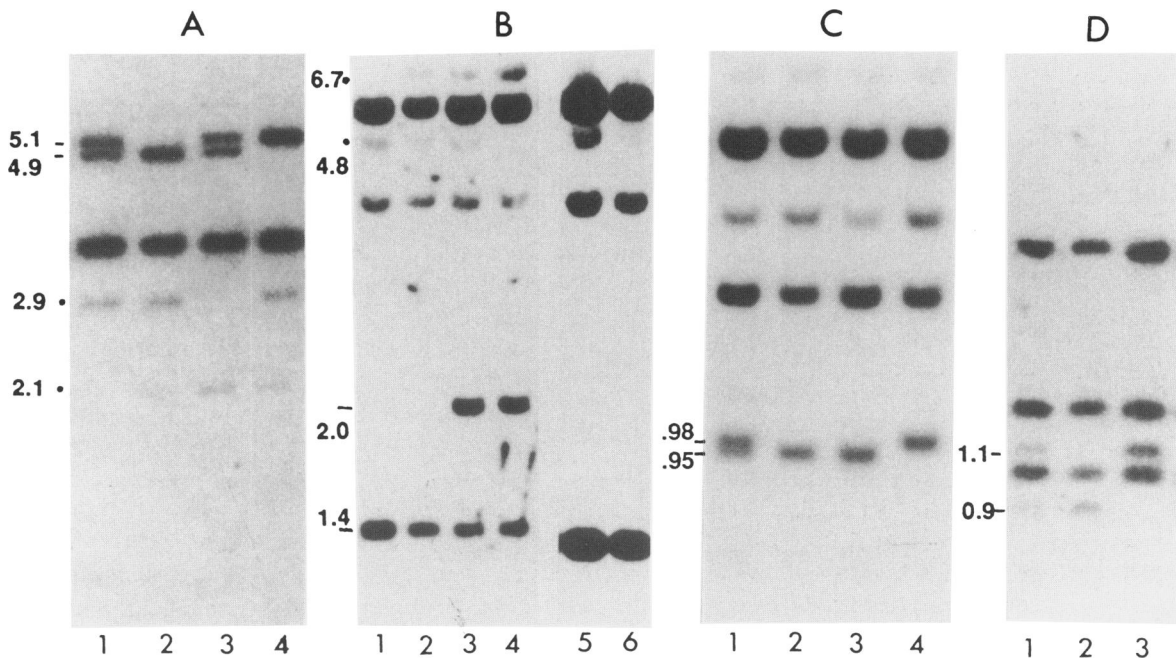
Figure 4   Southern blot hybridization following digestion with restriction enzymes useful in distinguishing rare variants. *A, BstEII-BglII* (probe 6.5), α1AT gene, followed by downstream Pi-like gene polymorphism. Lane 1, +−,−−; lane 2, ++,+−; lane 3, +−,+−; lane 4, −−,+−. *B, TaqI* (probe 6.5) α1AT flanking polymorphism followed by PI-like polymorphism. Lane 1, ++,+0; lane 2, ++,+−; lane 3, +−,+−; lane 4, +−,−−; lane 5, ++,+0; lane 6, ++,00. *C, MspI* (probe 4.6). Lane 1, +−; lanes 2 and 3, ++; lane 4, −−. *D, AvaII* (probe 4.6). Lane 1, +−; lane 2, ++; lane 3, −−. Dashes (−) indicate α1AT polymorphic fragments; black dots (·) indicate the PI-like polymorphic fragments. Fragment sizes are given.

other phenotypic null homozygote and in the patient with the null hongkong variant. This latter patient was discovered to be a genetic compound when we determined the haplotype. Although these all have the same DNA haplotype as does *PI*QObellingham,* sequencing or testing with synthetic oligonucleotide probes specific for the mutant site will be required to determine whether they all carry the *QObellingham* mutation.

On the basis of the DNA haplotypes given, we can derive the most likely normal allele from which these mutant alleles originated, as shown in table 5.

Identification of Specific Rare Deficiency Alleles

Specific haplotypes provide a means to provisionally identify many, if not all, of the individual rare deficiency alleles. PIEF, with and without immunofixation, should identify those alleles which produce a readily detectable protein product generally >3%–4% of normal and usually on the order of about 15% of normal (table 6). All of these variants and at least four null alleles can be specifically recognized by DNA haplotype. A scheme for preliminary identification of all available

rare variants is shown in figure 5. Definitive identification usually requires specific oligonucleotide probes for each different mutation. Null alleles identified as different from each other by haplotype or sequence are shown in table 7. Other null alleles described in homozygotes have not been characterized at the molecular level: QOboston (Talamo et al. 1973; Arnaud et al. 1975); QOrouen or Mrouen (Feldman et al. 1975; Martin et al. 1975).

In addition to using specific haplotypes, there are two situations in which a direct identification can be made using restriction enzymes. *PI*Mprocida,* a rare deficiency allele reported to produce 3%–4% of the normal amount of α1AT (Takahashi et al. 1988), is identified by the restriction enzyme *PvuII,* which produces a 2.9-kb fragment for Mprocida instead of the normal 2.7- and 0.2-kb fragments. Because the DNA haplotype associated with Mprocida is unknown, we carried out a *PvuII* digest on all nonsequenced null alleles for which we have a DNA haplotype; we have not identified any as Mprocida. QOgranitefalls has been sequenced (Nukiwa et al. 1987) and, like Mheerlen, has an alanine at amino acid 213 (as in M1ala213), which indicates

Table 6

Rare Deficiency Alleles of α1AT

Allele	Position by PIEF[a]	Serum Concentration (% of normal)	Liver Inclusions	Mutation	Reference
Mmalton	< M2	13	+	(aggregation)[b]	Cox 1976; present study
Mcobalt	= M3	7	?	?	Present study
Mduarte	= M3	6	+	?	Lieberman et al. 1976
Mheerlen	= M3	2	−	aa 369: pro→leu	Kramps et al. 1981 Hofker et al., in press
Mprocida	> M3	6	−	aa 41: leu→pro	Takahashi et al. 1988
Zaugsburg ...	< Z	<10	?	?	Weidinger et al. 1985
M-like	= M	4	?	?	Kueppers et al. 1977
"Unusual" ...	< Z[c]	2	?	?	Langley et al. 1979

[a] > is anodal; < is cathodal.

[b] Mutation has now been identified; see "Note added in proof."

[c] By starch- and agarose-gel electrophoresis; not tested by PIEF.
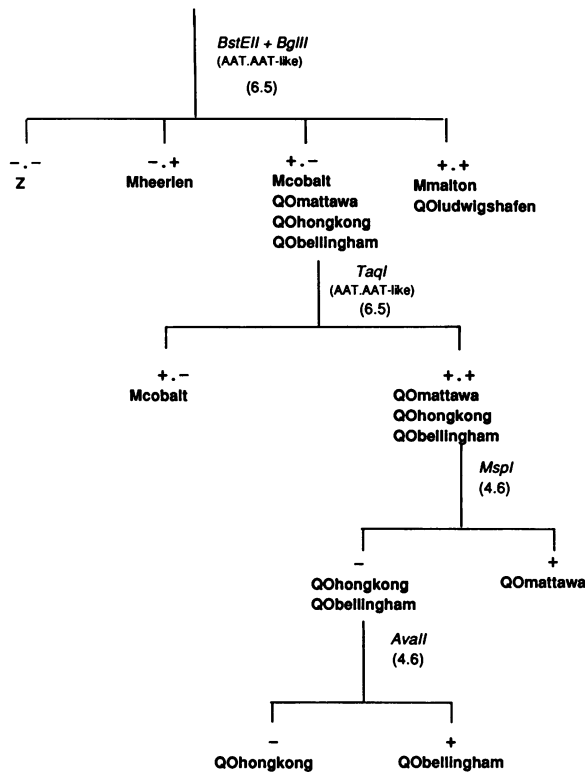
Figure 5   Strategy for tentative identification of rare deficiency alleles by using DNA polymorphisms as indicated by the given restriction enzymes. When two polymorphisms are revealed with one enzyme, these are given for the α1AT gene and downstream gene, respectively, e.g., +.−, −.−, etc.

that a BstEII digest would be negative for the restriction site at this position. This null variant is therefore different from all of the null variants we have tested. It is the same at amino acid 213 as is Mheerlen, which has been sequenced and has a different mutant site (table 6). Furthermore, we have found that the restriction enzyme MaeII does not cleave the QOgranitefalls mutant site in amino acid 160, producing a 2.2-kb fragment for QOgranitefalls and 1.6- and 0.6-kb fragments when the mutant site is not present. DNA from all of our nonsequenced null alleles was digested with MaeII, and none showed a 2.2-kb fragment with probe 6.5. This is in agreement with the BstEII results that none of our reported variants are likely to be identical to QOgranitefalls.

Discussion

The deficiency variants Mmalton and Mcobalt are readily differentiated from Z and from each other by PIEF. Mduarte and Mcobalt, indistinguishable by PIEF, show a slight difference by agarose-gel electrophoresis. Elsewhere we have compared the relative mobilities by starch-gel electrophoresis with immunofixation (Cox et al. 1981). Those results indicate similar mobilities for M1, M3, Mcobalt, and Mduarte; M2 and Mmalton run slightly cathodal by PIEF. The Mduarte proband is now deceased, and we were unable to retest sera with the narrower range of pH gradient that we

Table 7

Null (QO) Alleles of α1AT

Allele	Ethnic Origin	Mutation	Reference
QObolton	British	0	Present study
QOludwigshafen	German	0	Present study
QObellingham	Dutch	aa 217:lys—stop	Garver et al. 1986
			Satoh et al. 1988
QOgranitefalls	American white	aa 160-161: tyr.val—stop	Nukiwa et al. 1987
QOhongKong	Oriental	aa 317-318:leu-ser—leu-arg	Muensch et al. 1986
			Sifers et al. 1988
QOmattawa	French	0	Cox and Levison 1988

ᵃ Includes only those known to be different from each other by haplotype or DNA sequence.

now use for PIEF. Mduarte and Mcobalt are therefore provisionally considered to be different, pending sequencing. Other rare deficiency M-like variants with mobilities similar to that of M3, Mcobalt, and Mduarte are Mheerlen and Mprocida. Both of these can be identified either by a characteristic DNA haplotype (for Mheerlen) or by restriction digestion (PvuII for Mprocida).

The frequencies of non-Z rare deficiency alleles have been obtained from our series of patients with α1AT deficiency. These other alleles produce at least as pronounced a deficiency of α1AT as does PI*Z. PI*Mcobalt and PI*Mduarte alleles appear to lead to a more pronounced deficiency of α1AT than does PI*Z, although the numbers of individuals tested are presently small. As shown in table 6, only Mduarte and Mmalton are known to have a defect in secretion, which results in accumulation of α1AT and inclusions in the liver. We have shown for Z and Mmalton that this lack of secretion can be explained by a pronounced tendency of these abnormal molecules to aggregate (Cox et al. 1986), but other rare deficiency types have not been tested.

Haplotype studies indicate there is a greater variety of deficiency alleles than previously had been identified. PI*QO is not a single allele but consists of at least six different mutant alleles. We have described four different null haplotypes. For those with similar haplotypes, further studies would be required to identify that the mutation is identical. The combined frequency of all null alleles is considerably less than that of PI*Z. However, the frequency of all null alleles as estimated here (i.e., 1.4 × 10⁻⁴) is a minimum estimate. Both parents were tested for all children in our study, and no null alleles were found. Null alleles could be undetected in PI Z adults, as first-degree relatives were not avail-

able for all and usually included their children, rather than their parents. However, of 26 PI ZZ adults (52 alleles) tested, all were consistent by DNA haplotype with homozygosity for PI*Z (D. W. Cox, unpublished data). Of the eight known null variants, the haplotypes and mutant sites are unknown for QOrouen (Mrouen) and QOboston. QObellingham and QOgranitefalls are nonproductive because of stop codons at amino acids 217 and 160, respectively. QOhongkong, found in an Oriental patient, has a mutation at amino acids 317 and 318, where a TC deletion changes Leu-Ser to Leu-Arg, resulting in a stop codon at amino acid 344.

Haplotype studies are useful, particularly prior to cloning of the mutant gene, for detecting the presence of two different rare deficiency variants. Heterozygosity for two variants appear to be more likely than homozygosity. The QO homozygote in our series is actually a genetic compound, heterozygous for two different haplotypes and two mutant alleles, QOmattawa and QObellingham. This finding has been confirmed by sequencing studies (R. G. Crystal, personal communication). Our haplotype studies indicate that the patient reported to be homozygous for the QOhongkong variant is also a compound, possibly having a PI*QObellingham allele found in Caucasian patients. Because of the different racial origin, however, mutant sites could be different despite occurring on the same haplotype. Alternatively, this null variant may be widespread in various racial and ethnic groups. For both of these genetic compounds, parents were unrelated. The QObellingham proband was shown by family studies to be homozygous for the mutant allele, and, in this case, parents were first cousins.

We have presented a scheme whereby, with or without specific identification by PIEF and without knowl-

edge of the mutant sequence, tentative identification of individual rare deficiency alleles can be made. Identification by haplotype is particularly useful in cases where the mutant alleles have not been sequenced. Specific oligonucleotide probes can be used to identify mutant sites in deficiency variants, as for QObellingham and QOgranitefalls (Nukiwa et al. 1987; Satoh et al. 1988. DNA amplification by the polymerase chain reaction (PCR) (Saiki et al. 1988) can be used in combination with specific oligonucleotide probes to identify specific mutant sites, as for Z (Bruun Petersen et al. 1988). When sequence data become available for more deficiency variants, a tentative identification can indicate which portion of the gene should be amplified and which specific oligonucleotide probes should be selected for definitive identification.

The haplotypes associated with the deficiency alleles are the same as those associated with various normal variants, which allows us to infer the most likely evolutionary pathway from a normal precursor allele. Rare deficiency mutations have arisen on haplotypes found with both types of M1 (M1B or M1val 213, M1A or M1ala 213), M3, and M2. Knowledge of the haplotype of origin is useful in interpreting other mutations observed in addition to the mutant site causing the deficiency. Sequencing of QOhongkong revealed the substitutions, at amino acids 101 and 376, characteristic of M2 (Sifers et al. 1988); but we do not have haplotypes for Orientals and cannot trace QOhongkong's origin. QObellingham has valine at amino acid residue 213, as found in M1 and other variants (Satoh et al. 1988). The associated haplotype is commonly found with PI*S and infrequently with PI*M3 and PI*M1, but sequencing indicates derivation from PI*M1. Mheerlen, in agreement with haplotype data, has an alanine at amino acid position 213 and a PI-like gene as found with M1ala213 (Hofker et al., in press). QOgranitefalls also has an alanine at amino acid 213, compatible with its origin from M1ala213.

Delineation of the specific mutant sites of this protease inhibitor will allow us to learn more about both the control of glycoprotein secretion from the liver and the mechanisms for impaired transcription and translation. Such studies may also add to our knowledge of the basis of the liver and lung destruction associated with the deficiency state.

Note added in proof.—Mutation of Mmalton has now been identified by Frazier et al. (1989).

Acknowledgments

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