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Research Article

α1-Antitrypsin Deficiency, Emphysema, and Liver Disease

Genetic Basis and Strategies for Therapy

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α1-Antitrypsin (α1AT)[1] deficiency, one of the most common lethal hereditary disorders of Caucasians of European descent, is an autosomal recessive disorder characterized by reduced serum levels of α1AT, a 52-kD glycoprotein that functions as an antiprotease (1, 2). The deficiency state is caused by mutations in the α1AT gene, a pleomorphic 12.2-kb, 7-exon gene on chromosome 14 at q31-31.2 (3). Normal α1AT serum levels are 20–53 μM (4, 5). Various homozygous and heterozygous combinations of at least 17 different mutations of the α1AT gene are associated with α1AT levels < 11 μM and a high risk for developing emphysema (5–7). Some individuals with α1AT deficiency develop reactive airways disease. A subset of mutations is associated with hepatitis and cirrhosis (8, 9). Rarely, α1AT deficiency has been linked with hepatoma or relapsing panniculitis (7, 9). One rare mutation is associated with a fatal hemorrhagic disorder (10).

In the context that the major clinical importance of α1AT deficiency relates to its association with emphysema and liver disease (1, 7, 9), this review will focus on the recent advances in defining the genetic basis of these manifestations of α1AT deficiency and therapeutic strategies that have been developed to prevent them. For more details, the reader should consult prior reviews (1–12).

The major clinical manifestations of α1AT deficiency relate to the function of α1AT and where it is made. α1AT serves as an inhibitor of neutrophil elastase (NE), a powerful, destructive proteolytic enzyme stored in neutrophils (2, 11). The liver is the major site of α1AT gene expression, releasing 2 g of α1AT into the circulation daily. α1AT diffuses into most organs, where it protects extracellular structures from attack by NE released by activated or disintegrating neutrophils. The lower respiratory tract is particularly vulnerable to a deficiency of α1AT, which normally represents > 90% of the anti-NE protective screen of the alveolar walls (13, 14). When serum α1AT levels are < 11 μM, there is insufficient α1AT to protect the lower respiratory tract from its burden of NE, permitting progressive destruction of the alveoli, which culminates in emphysema (14). The pathogenesis of the liver disease is less well understood, but relates to the fact that hepatocytes are the major site of α1AT synthesis, and that certain mutations of the α1AT gene cause derangements in the intracellular processing of α1AT, culminating in hepatocyte injury (8).

α1-Antitrypsin gene and protein

The α1AT protein is a single chain of 394 amino acids with three complex carbohydrate side chains (Fig. 1 *A*). The microheterogeneity of α1AT observed in isoelectric focusing (IEF) analysis of serum at pH 4–5 results mostly from differences in these carbohydrates (1, 6, 9).

The two parental α1AT genes are codominantly expressed. Expression is controlled at the transcriptional level through sequences flanking, and within, the three 5′ non-coding exons (Fig. 1 *B*). Hepatocytes are the major source of α1AT, but the gene is also expressed in mononuclear phagocytes and neutrophils, and possibly megakaryocytes, islet cells, and intestinal epithelial cells (5, 15, 16). In transgenic mice, the α1AT gene is also expressed in kidney and brain (see reference 5 for review).

α1AT levels are increased in trauma, pregnancy, hemorrhage, and neoplasia. It is not known what stimuli actually upregulate the α1AT gene in vivo. In cell culture, α1AT mRNA levels are increased by phorbol esters and interleukin 6 (interferon β2) but is not by interleukin 1 or tumor necrosis factor (15). In vivo administration of typhoid vaccine or estrogens increases α1AT serum levels in normal humans (7, 9), but administration of lipopolysaccharide has no effect, despite the marked increase in other serum "acute-phase reactants."

Most liver α1AT mRNA transcripts start in the middle of exon I_C, a region preceded by typical consensus promoter elements (3). In exon I_B there are two regions capable of binding the nuclear protein AP-1, a protein identical to the *c-jun* proto-oncogene product. Exon I_A does not appear to be important to the modulation of α1AT gene expression, although it is flanked by 5′ consensus promoter elements and mRNA transcripts including exon I_A are found in mononuclear phagocytes and hepatocytes (see reference 5 for review).

α1-Antitrypsin biosynthesis. The biosynthetic pathway of α1AT is relevant to the aberrant intracellular events associated with the different mutations of α1AT coding exons causing α1AT deficiency and to the understanding of the pathogenesis of the liver disease associated with certain α1AT alleles (see below). α1AT synthesis is typical for a secretory glycoprotein (17). The α1AT mRNA codes for a 418-residue precursor protein containing a 24-residue amino-terminal signal peptide that is cleaved as the molecule enters the rough endoplasmic reticulum (RER), leaving the 394-residue mature polypeptide.

The plasma half-life of α1AT is 4–5 d, a process dictated, at least in part, by the carbohydrate side chains. The bare polypeptide disappears from the circulation in hours, and deletion of even one side chain reduces the half-life significantly (18). Most of the α1AT in the body comes from hepatocytes. The

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1. *Abbreviations used in this paper:* α1AT, α1-antitrypsin; ELF, epithelial lining fluid; IEF, isoelectric focusing; NE, neutrophil elastase; RER, rough endoplasmic reticulum.

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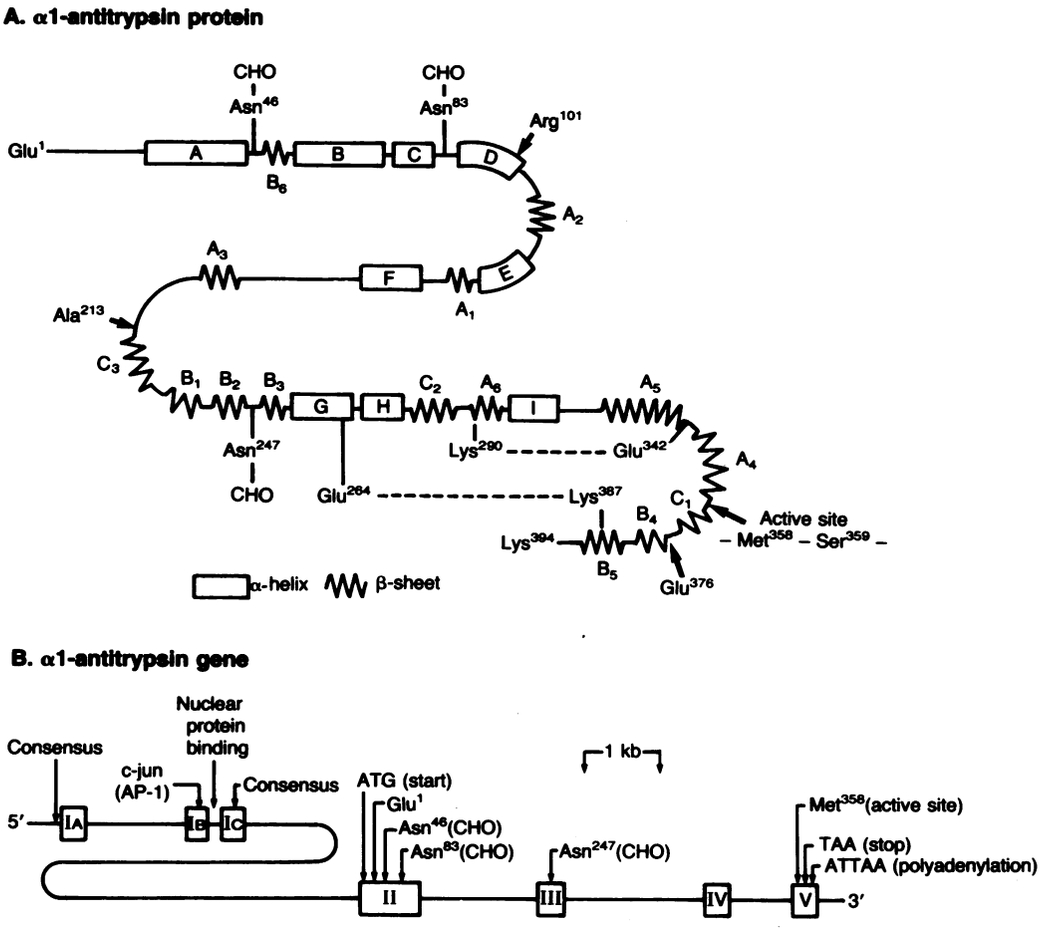

Figure 1. Structure of the α1AT protein and gene. (*A*) The normal α1AT protein shown in its linear form, a 394-amino acid single chain with three asparaginyl-linked complex carbohydrate side chains at residues 46, 83, and 247. During its synthesis, the molecule folds into a spheroid shape (see cover of this issue of the *Journal*) determined by nine α-helices (A → I) and three β-pleated sheets made up of parallel and antiparallel strands (sheet A, strands 1–6; sheet B, strands 1–6; sheet C, strands 1–3). Two internal salt links (Glu^{342}–Lys^{290}; Glu^{264}–Lys^{387}) help stabilize the molecule and play an important role in the pathogenesis of common deficiency states. The three carbohydrate side chains are on the surface of one half of the spheroid while the Met^{358}-Ser^{359} inhibitory site that combines with NE is localized at the opposite end. Shown are the residues at positions 101, 213, and 376 for the M1(Ala^{213}) allele; residues at these positions define the differences among the four common normal α1AT alleles [M1(Ala^{213}), M1(Val^{213}), M2 and M3; see Table I, Fig. 2]. (*B*) The normal α1AT gene. It spans 12.2 kb and consists of three noncoding exons (I_A, I_B, I_C) and four coding exons (II–V). Exons I_A – I_C are used by a variable degree by α1AT synthesizing cells, with most α1AT transcripts starting in the middle of exon I_C. 5′ to exon I_A and in the middle of I_C are typical *cis*-acting consensus promoter sequences. In the region between I_B and I_C are sequences capable of binding at least two different hepatocyte nuclear proteins and within exon I_B are two sequences capable of binding the *c-jun* protein (AP-1). The start codon (ATG) is in exon II followed by sequences coding for a 24-residue signal peptide. Sequences for the 394-residue mature protein start in exon II (Glu^1) and end in exon V at the TAA stop signal. Also identified are the locations of sequences coding for the carbohydrate (*CHO*) attachment sites (Asn 46, 83, 247), the Met^{358} at the active inhibitor site, and the mRNA polyadenylation signal.

importance of local production of α1AT within organs (e.g., by alveolar macrophages in the lower respiratory tract) is not known. Neutrophils contain α1AT mRNA transcripts and actively synthesize and secrete functional α1AT, but at low levels.

Neutrophil elastase and α1-antitrypsin. NE, a serine protease (EC 3.4.21.37), is a 29-kD single-chain glycoprotein that normally functions as an extracellular protease (19). It destroys elastin, the rubberlike macromolecule that provides elastic recoil to the lung. NE also attacks all other major connective tissue matrix components, and thus can rapidly degrade the matrix providing the supporting architecture of the alveolar walls. When NE is instilled into the lungs of experimental animals, destruction of the lower respiratory tract follows, similar to human emphysema (see reference 11 for review). It is in this context that the concept developed that the emphysema associated with α1AT deficiency occurs because of an imbalance of α1AT (the major anti-NE of the lower respiratory tract) and NE (an enzyme capable of destroying the lower respiratory tract).

A single neutrophil contains ~ 2 pg of NE, but the amount varies among individuals, suggesting genetic variations in expression of the NE gene. Neutrophils disgorge the stored enzyme during phagocytosis, membrane perturbation, or cell lysis. In the normal lung, the burden of neutrophils is low, but chronic (13, 14). After leaving the circulation, neutrophils live for 1–2 d at most. Thus, whether through activation, or by cell lysis, the chronic burden of neutrophils forces the lung to contend with a chronic burden of NE.

α1AT inhibits NE with great avidity with an association rate constant of 10^7 M^{-1} s^{-1} and estimated equilibrium constant of 10^{-14} M (19). Given the choice, NE prefers α1AT to its natural substrates; it is this tight association that allows α1AT to protect the lung from the potent destructive capacity of NE. α1AT diffuses across endothelial and epithelial barriers and is present in lung epithelial lining fluid (ELF) at levels 10–15% of plasma (13, 14). The lung contains other antiproteases capable of inhibiting NE, including α2-macroglobulin and the secretory leucoprotease inhibitor, but α1AT contributes > 90% of the functional anti-NE protection of the alveolar walls (4, 13,

14). In normals, the amount of α1AT in the lower respiratory tract is far in excess of the NE burden, thus protecting the lung.

Genetic basis of α1-antitrypsin deficiency

The α1AT locus is pleomorphic, with approximately 75 alleles identified. The α1AT alleles are conveniently categorized as "normal" and "at risk" (Table I). By convention, a normal allele is one that, when inherited in a homozygous fashion, is associated with serum α1AT levels of 20–53 μM. The at-risk alleles relevant to a risk for lung and liver disease include "deficient" alleles (an allele that when inherited in a homozygous form is associated with serum levels < 20 μM) and "null" alleles (no α1AT in serum attributable to that allele).

THE NORMAL ALLELES

There are four common normal alleles: M1(Ala^{213}), M1(Val^{213}), M2, and M3. Among Caucasians of Northern European descent, M1(Val^{213}) is the most common (allelic frequency 0.44–0.49), with M1(Ala^{213}) (0.20–0.23), M2 (0.14–0.19), and M3 (0.10–0.11) less frequent, respectively (1, 5, 6). These alleles differ by single base mutations and respective amino acids (Figs. 1 and 2). M1(Ala^{213}) is likely the "oldest"

Table I. Sequence Differences among the "Normal" α1-Antitrypsin Alleles and the "At Risk" α1-Antitrypsin Alleles Relevant to Emphysema and Liver Disease

Category	Relative frequency*	Allele	Base allele‡	Mutation site (exon)§	Sequence compared to base allele
Normal	Common	M1(Ala^{213})			
		M1(Val^{213})	M1(Ala^{213})	III	Ala^{213} GCG → Val GTG
		M3	M1(Val^{213})	V	Glu^{376} GAA → Asp GAC
		M2	M3	II	Arg^{101} CGT → His CAT
	Rare	M4‖	M1(Val^{213})	II	Arg^{101} CGT → His CAT
		$B_{alhambra}$	Unknown	Unknown	Lys → Asp
		F	M1(Val^{213})	III	Arg^{223} CGT → Cys TGT
		$P_{saint\ albans}$¶	M1(Val^{213})	V	Asp^{341} GAC → Asn AAC
				III	Asp^{256} GAT → Asp GAC
		V_{munich}	M1(Val^{213})	II	Asp^{2} GAT → Ala GCT
		X	M1(Val^{213})	III	Glu^{204} → Lys
		$X_{christchurch}$	Unknown	V	Glu^{363} → Lys
At risk	Common	Z	M1(Ala^{213})	V	Glu^{342} GAG → Lys AAG
		S	M1(Val^{213})	III	Glu^{264} GAA → Val GTA
	Rare	$M_{heerlen}$	M1(Ala^{213})	V	Pro^{369} CCC → Leu CTC
		M_{malton}	M2	II	Phe^{52} TTC → Delete TTC
		$M_{mineral\ springs}$	M1(Ala^{213})	II	Gly^{67} GGG → Glu GAG
		$M_{procida}$	M1(Val^{213})	II	Leu^{41} CTG → Pro CCG
		$M_{nichinan}$¶	Unknown	II	Phe^{52} TTC → delete TTC Gly^{148} GGG → Arg AGG
		I	M1(Val^{213})	II	Arg^{39} CGC → Cys TGC
		P_{lowell}	M1(Val^{213})	III	Asp^{256} GAT → Val GTT
		$W_{bethesda}$	M1(Ala^{213})	V	Ala^{336} GCT → Thr ACT
		$Null_{granite\ falls}$	M1(Ala^{213})	II	Tyr^{160} TAC → delete C → 5′ shift → $stop^{160}$ TAG
		$Null_{bellingham}$	M1(Val^{213})	III	Lys^{217} AAG → $stop^{217}$ TAG
		$Null_{matawa}$	M1(Val^{213})	V	Leu^{353} TTA → Insert T → Phe^{353} TTT → 3′ shift → $stop^{376}$ TAG
		$Null_{isola\ di\ procida}$	Unknown	II–V	Delete 10 kb including exons II–V
		$Null_{hong\ kong}$	M2	IV	Leu^{318} CTC → delete TC → 5′ shift → $stop^{334}$ TAA
		$Null_{bolton}$	?	V	Pro^{362} CCC → delete C → 5′ shift → $stop^{373}$ TAA
		$Null_{devon}$	Unknown	II	Gly^{115} → Ser
		$Null_{ludwigshafen}$	M2	II	Ile^{92} ATC → Asn AAC

This list includes the 29 α1AT alleles for which a partial or complete sequence is known at the gene or protein level. Details regarding these alleles can be found in the references in reviews 1, 5, 6, and 9 and in reference 45. For a list of alleles identified by IEF of serum but for which the sequence is not known, see reference 1. * Relative frequency for that category. For the "normal" alleles, "common" represents allelic frequencies > 10%; for the "at-risk" alleles, "common" represents allelic frequencies > 1%. ‡ "Base allele" represents the common, normal allele upon which the mutation developed; M1(Ala^{213}) is believed to be the oldest human α1AT allele from which all other alleles derived (see reference 5 for review); unknown, only partial sequence known, and thus base allele cannot be determined. § Unknown, data limited, obviating exact location of mutation. ‖ The DNA sequence data for M4 suggests it could have derived from the M1(Val^{213}) or M2 alleles; since M1(Val^{213}) is the more common allele, it is assumed to be the base allele. ¶ For $P_{saint\ albans}$ and $M_{nichinan}$, there are two mutations distinguishing the allele from its respective base allele, suggesting there may be intermediate, as yet unidentified, alleles.

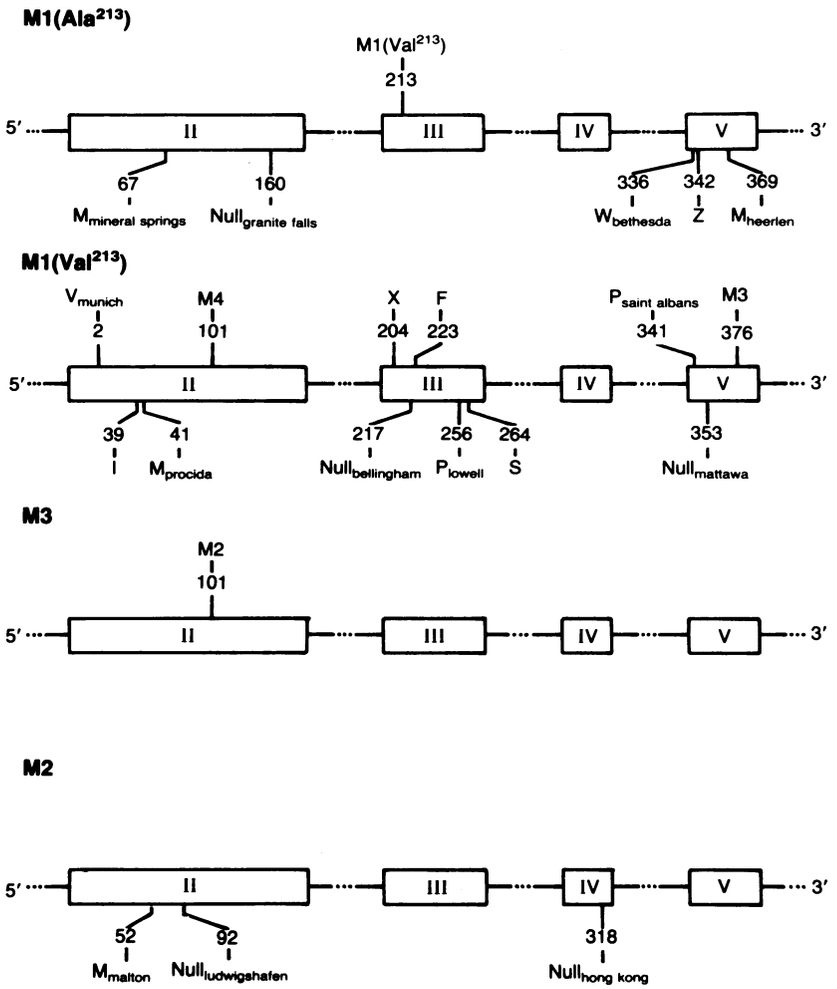

Figure 2. Locations of the mutations of the α1AT gene associated with α1AT deficiency and an increased risk for emphysema and/or liver disease. The four common normal α1AT alleles M1(Ala^{213}), M1(Val^{213}), M2, and M3 serve as the "base" alleles for these mutations; exons II–V of the four normal "base" alleles have identical sequences except for those coding for residues 101, 213, and 376, as indicated. The sequence of M1(Ala^{213}) is the closest to the chimpanzee α1AT gene sequence, and thus M1(Ala^{213}) is likely the "oldest" form of human α1AT, with M1(Val^{213}), M3 and M2 evolving from the M1(Ala^{213}) gene by sequential single base substitutions, respectively (see Table I). Shown above the "base" alleles are the locations of the known "normal" alleles; each resulting from single-base substitutions of their respective base allele. The normal M4 allele may have evolved from the M2 allele, rather than M1(Val^{213}) (5). Shown below the "base" alleles are the locations of a variety of missense, nonsense, deletion and insertion mutations that characterize the "at risk" α1AT alleles (see Table I for details). Null$_{\text{isola di procida}}$ (not shown) results from complete deletion of exons II–V. Also not shown are the α1AT alleles for which only partial coding sequences are known (see Table I).

human α1AT allele, with the others derived from it. Except for M4, M5, and F, most other normal alleles represent single case reports identified by IEF analysis.

AT-RISK ALLELES RELEVANT TO EMPHYSEMA AND LIVER DISEASE

The at-risk alleles almost always occur in populations of European descent, most commonly in Caucasians from Northern Europe (1, 5, 6, 9). α1AT deficiency is rare in Blacks or Asians. Two at-risk alleles must be inherited to confer risk for clinically significant disease (1, 9). For emphysema, this is directly related to the α1AT serum levels (Table II), with the "threshold" level of 11 μM separating the phenotypes at risk and not at risk. Individuals with the null-null and ZZ phenotypes are at high risk, whereas those with MM, MZ, and SS phenotypes (all above 12 μM) have the same risk for emphysema as the general population. SZ heterozygotes, are at mild risk, with only a small proportion having α1AT serum levels < 11 μM. For liver disease, the correlation of risk for disease and serum α1AT levels < 11 μM also holds, but only for certain alleles, and by different mechanisms than those that cause emphysema.

Most at-risk mutations are single-base substitutions causing single amino acid modifications in the mature protein. Others are nonsense mutations inserting a premature stop codon into the normal coding sequence, single- or double-base deletions or insertions causing frameshifts resulting in distal premature stop codons, deletion of one codon in the coding sequence, or deletion of all coding exons. Despite the common denominator of "α1AT deficiency," these various mutations cause α1AT deficiency by at least five different mechanisms (Table III, Fig. 3). Further, there appears to be an association

Table II. Threshold Protective Level Concept Based on the Relationship of Serum α1-Antitrypsin Levels and the Risk for the Development of Emphysema

Phenotype	Serum α1AT levels (μM)	Emphysema risk compared to the general population
MM*	20–53	No increase
MZ‡	12–35	No increase
SS	15–33	No increase
SZ	8–19‖	Mild increased risk
ZZ	2.5–7	High risk
Null-Null§	0	High risk

* Includes all combinations of common normal M-family alleles, including M1(Val^{213}), M1(Ala^{213}), M2, and M3 alleles.
‡ Includes all combinations of common normal M-family alleles with the Z allele.
§ Includes all combinations of Null alleles (see Table I).
‖ The threshold protective serum α1AT level of 11 μM is based on the knowledge that few SZ heterozygotes develop emphysema.

Table III. "At Risk" α1-Antitrypsin Alleles Categorized by the Different Mechanisms Causing the α1-Antitrypsin Deficiency State and the Corresponding Risk for Emphysema and Liver Disease

Dominant mechanism causing the deficiency state*	Allele	Risk for disease	
		Emphysema‡	Liver
Gene deletion	$Null_{isola\ di\ procida}$	Yes	No
mRNA degradation	$Null_{bellingham}$	Yes	No
	$Null_{granite\ falls}$	Yes	No
Intracellular protein accumulation	Z	Yes	Yes
	M_{malton}	Yes	Yes
	$Null_{hong\ kong}$	Yes	?§
Intracellular protein degradation	S	Yes	No
	P_{lowell}	Yes	No
	$W_{bethesda}$	Yes	No
Protein functions poorly on an inhibitor of neutrophil elastase	$M_{mineral\ springs}$	Yes	No

The alleles listed represent only those in which the dominant biological mechanism by which the mutation causes the deficiency state is known; information regarding $Null_{isola\ di\ procida}$ is unpublished (H. Takahashi and R. Crystal) as is $W_{bethesda}$ (M. Holmes, M. Brantly, and R. Crystal); for details regarding other alleles, see references 1, 5, 6, 22, 26, and 27.

* If more than one mechanism is involved for the allele, the dominant mechanism is listed (e.g., the Z allele causes intracellular protein accumulation and poor function of the protein as an inhibitor, but the accumulation mechanism dominates).

‡ The relative risk for each emphysema varies among the different alleles; see Table II and text.

§ Although the mechanism causing the deficiency state is known, the clinical information regarding liver disease is not available.

of the dominant mechanism causing the deficiency state with the relative risk for emphysema and/or liver disease. Whereas all are associated with a risk for emphysema to some degree, only mutations causing intracellular accumulation of the newly synthesized α1AT are associated with a risk for liver disease.

Z mutation. The Z allele is the most common cause of the major clinical manifestations of α1AT deficiency, with Z homozygotes at risk for both emphysema and liver disease (6). With an allelic frequency of 1–2% in Caucasians of Northern European descent, there are 20,000–40,000 Z homozygotes in the United States (1, 9). The Z mutation is a single-base substitution in exon V of the normal M1(Ala^{213}) allele causing a $Glu^{342} \rightarrow Lys$ substitution in the molecule (6, 20). The number of α1AT mRNA transcripts in the α1AT synthesizing cells of Z homozygotes is normal, but the cells secrete 10–15% of that of normal cells (16). The deficiency in secretion results from an accumulation of α1AT molecules in the RER of α1AT synthesizing cells, a phenomenon observed in liver biopsies of Z homozygotes, frog oocytes injected with Z-type α1AT mRNA, heterologous cells transfected with a Z-type α1AT cDNA, and transgenic mice receiving the Z cDNA (16, 21, 22).

The mechanisms responsible for the intracellular accumulation of α1AT in association with the Z gene are unclear. The $Glu^{342} \rightarrow Lys$ mutation causes a loss of a normal internal salt bridge between Glu^{342} and Lys^{290} (23) (Fig. 2 *B*). Site-directed mutagenesis studies suggest that the effects of the Z mutation, a positively charged Lys at 342, can be counteracted by changing the normal Lys^{290} to the negatively charged Glu (22), consistent with the concept that this salt bridge stabilizes the molecule. Other data suggest that the insertion of a positive charge at residue 342 per se causes the α1AT to accumulate, independent of the charge of residue at 290 (24). It is hypothesized that the Z form of α1AT folds into its three-dimensional form at a slow rate, allowing the α1AT molecules to aggregate through

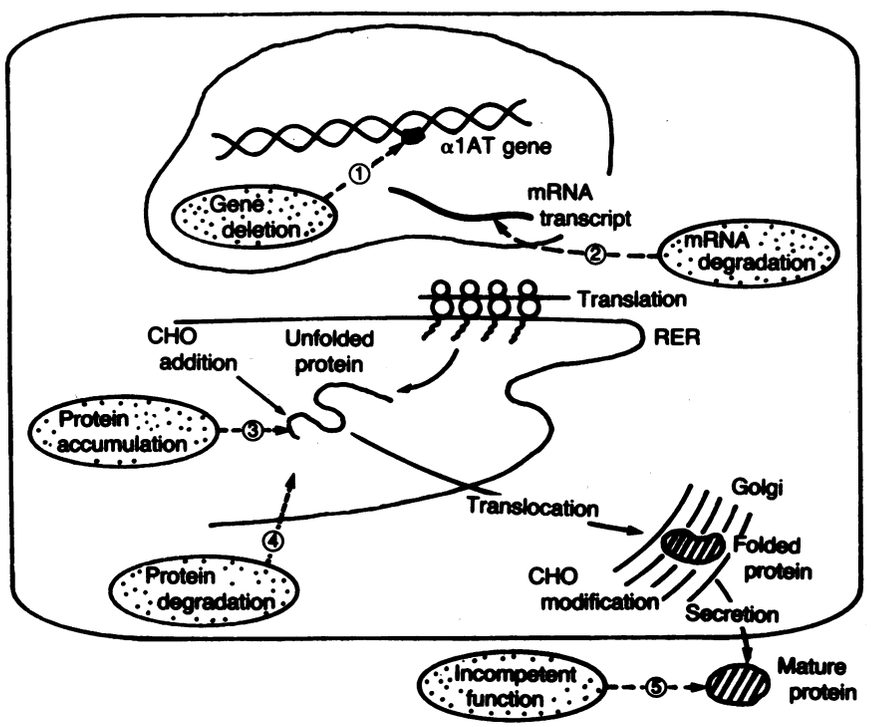

Figure 3. Classifications of the mutations of the α1AT gene by the mechanisms by which they cause α1AT deficiency. Shown is a hepatocyte synthesizing and secreting α1AT through the normal pathways of transcription of the α1AT gene, translation of the α1AT mRNA on the rough endoplasmic reticulum (*RER*), addition of carbohydrate (*CHO*) side chains in the RER, translocation to the Golgi, modification of the CHO side chains, and secretion. Mutations of the α1AT gene cause α1AT deficiency by one or more mechanisms, including: (*1*) deletion of the α1AT gene; (*2*) degradation of unstable α1AT mRNA; (*3*) intracellular accumulation of the newly synthesized α1AT protein; (*4*) intracellular degradation of the newly synthesized α1AT protein; and (*5*) incompetent function of the mature secreted protein as an inhibitor of neutrophil elastase.

interactions of normally hidden internal hydrophobic residues, preventing α1AT translocation to the Golgi. Alternatively, the Z mutation might cause a loss of translocation signal within the primary or tertiary structure of the molecule, i.e., the accumulation of the α1AT could result from a lack of recognition of the α1AT to be vectored to the Golgi.

There is also evidence that the Z molecule does not function normally as an inhibitor of NE; at the concentrations found in the lung, inhibition of NE by the Z form of α1AT takes 12-fold longer to inhibit an equivalent amount of NE than does normal α1AT (25). Thus, the actual "deficiency" is a combined defect of reduced amounts of a partially incompetent molecule, leaving the lung of the Z homozygote almost defenseless against NE.

S mutation. The S allele is more common than Z, with an allelic frequency of 2–4% in Caucasians of Northern European descent (1, 5, 9). Because the deficiency is relatively mild, and since the S molecule functions reasonably well as an inhibitor of NE, the S homozygote is not at increased risk. However, inheritance of the S allele with an allele causing a profound deficiency of α1AT (e.g., Z) puts the affected individual at mild risk. The S mutation ($Glu^{264} \rightarrow Val$) affects a salt bridge (Glu^{264}--Lys^{387}), i.e., like the Z mutation, the S mutation modifies the internal architecture of α1AT, a concept supported by mutagenesis studies (Fig. 1 *A*). However, the consequences of the S mutation are very different from the Z mutation. In α1AT synthesizing cells of S homozygotes, α1AT mRNA is transcribed in normal form and amount, but some of the newly synthesized S molecule is degraded, probably shunted from the RER into lysosomes (26).

Other "at-risk" mutations (Tables I and III). Like the Z mutation, M_{malton} and $Null_{hong\ kong}$ are associated with accumulation of α1AT in the α1AT synthesizing cell. Like the S mutation, P_{lowell} and $W_{bethesda}$ cause intracellular degradation of the newly synthesized α1AT. $Null_{isola\ di\ procida}$ results from deletion of a 17-kb fragment that includes exons II–V of the α1AT gene. Most of the other known "null" α1AT at-risk alleles result from the formation of stop codons in coding exons of the α1AT gene. For $Null_{granite\ falls}$ and $Null_{bellingham}$, evaluation of α1AT-synthesizing cells shows an absence of α1AT mRNA transcripts. One α1AT allele, ($M_{mineral\ springs}$ [$Gly^{67} \rightarrow Glu$]) causes a profound deficiency state primarily because the α1AT protein functions poorly as an inhibitor of NE (27).

LABORATORY DIAGNOSIS OF α1-ANTITRYPSIN DEFICIENCY

Detection of the deficiency state is a simple measurement of the serum α1AT level, usually by radial immunodiffusion or nephelometry. Identification of the α1AT phenotype or genotype provides important information relevant to the relative risk for emphysema and/or liver disease, and thus plays a critical role in the laboratory diagnosis of α1AT deficiency. Phenotyping is conventionally done by IEF of serum (6, 9). α1AT genotyping can be accomplished by a variety of methods, but most laboratories either use the polymerase chain reaction (PCR) to amplify individual segments of the coding exons which are then evaluated with labeled specific oligonucleotide probes or by gradient gel analysis, or with the technique of "allele-specific amplification", where only specific alleles are amplified depending on the specificity of the primer (28).

α1-Antitrypsin deficiency and emphysema

Emphysema is the most common manifestation of α1AT deficiency, and was the clinical disorder recognized by Laurell and Eriksson (29) when they discovered the deficiency state. Longitudinal studies of adults with α1AT deficiency suggest they have a shortened life span of 10–15 years compared to the normal population. In the United States, individuals with α1AT deficiency known to have emphysema who are at least 18 yr old, have a 52% chance of being alive at age 50 (compared with 93% for the general population) and a 16% chance of being alive at age 60 (compared with 85%). A Scandinavian study showed similar mortality statistics with the important addition that, if the individual with α1AT deficiency had a history of cigarette smoking, life expectancy was reduced at least by 10 yr, i.e., an acquired factor (smoking) markedly modulates the response to the deficiency state (see reference 30 for review).

The fact that the α1AT molecule has a Met^{358} residue at its active site provides a direct explanation for why cigarette smoking accelerates the development of emphysema. With smoking, the anti-NE defenses of the lung are further compromised because the Met^{358} is vulnerable to free radicals, in cigarette smoke, and released by inflammatory cells recruited to the lung in response to smoking. When the Met^{358} is oxidized, the association rate constant defining the inhibition of NE by α1AT decreases more than 1,000-fold (19); i.e., cigarette smoking renders an already poorly defended lung completely defenseless.

One unanswered question relating to the pathogenesis of the emphysema is the variability of the extent of the disease even when affected individuals are matched by age, α1AT serum levels, α1AT phenotype, and smoking history. One possibility is genetic variations in the expression of the NE gene. Consistent with this concept, Z homozygotes between ages 30 and 45 yr matched for α1AT serum levels and smoking history demonstrated higher levels of NE in neutrophils in those with severe disease compared with those with minimal or no emphysema (31).

α1-Antitrypsin deficiency and liver disease

Liver disease in association with α1AT deficiency was first recognized by Sharp et al. (32). It occurs in two distinct age groups. Approximately 10% of neonates with α1AT deficiency develop cholestasis with hepatitis, occasionally progressing to cirrhosis (32). In the age group > 40 yr, hepatitis and cirrhosis can develop, but in a small proportion of α1AT-deficient individuals (8). This can proceed to liver failure. Significant lung and liver disease rarely coexists in the same individual.

The association of α1AT deficiency and liver disease is incontestable, but why α1AT deficiency is associated with liver disease is controversial. Two facts dominate the current thinking in the field. First, no liver disease has been observed in individuals with the Null-Null phenotype. Since such individuals have no serum α1AT, it is not likely the deficiency of α1AT per se that causes the liver disease; i.e., the liver disease is not secondary to a NE-α1AT imbalance as is the pathogenesis of the emphysema. Secondly, although a variety of mutations of the α1AT gene cause α1AT deficiency, only two mutations, Z and M_{malton}, have been clearly associated with an increased risk for liver disease (Table III); i.e., there is something about these mutations directly relevant to the pathogenesis of the liver disease.

One clue comes from the morphologic features of the liver disease. In addition to inflammation, and later cirrhosis, all cases of liver disease associated with α1AT deficiency are also characterized by an accumulation of α1AT in hepatocytes (8,

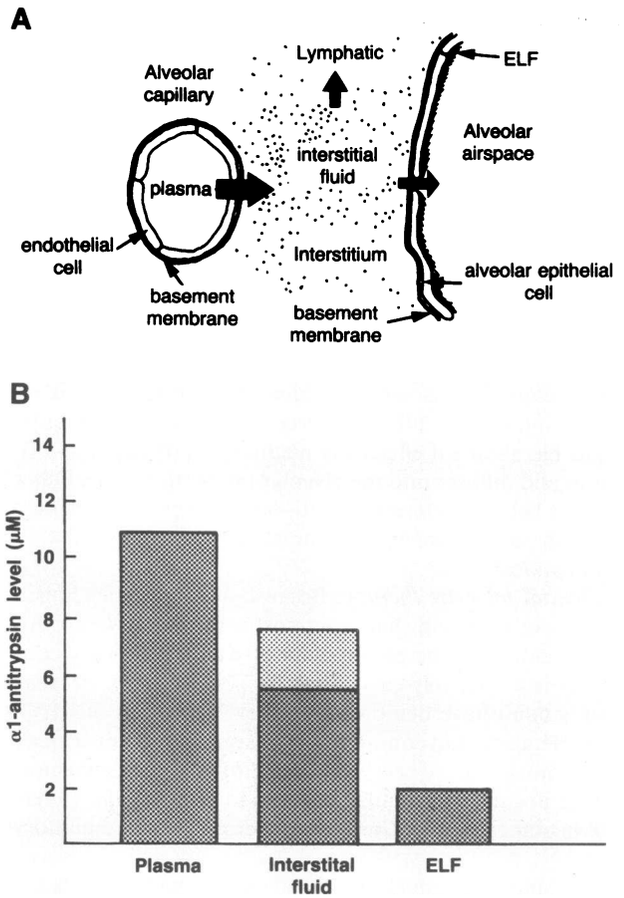

Figure 4. Equivalent α1AT levels necessary for "biochemical efficacy" of therapy to augment the anti–NE screen of the lower respiratory tract in α1AT deficiency. (*A*) α1AT protection of the alveolar walls in normal individuals. α1AT in plasma normally diffuses across the alveolar capillary endothelial barrier into the interstitium. The process is dynamic, with most α1AT in the interstitial fluid leaving via the lymphatics, and with some moving across the epithelial barrier into the alveolar epithelial lining fluid (*ELF*). (*B*) Minimal levels of α1AT required to protect the alveolar walls from its usual burden of NE. In normal individuals, plasma α1AT levels are 20–53 μM, interstitial fluid levels are 10–40 μM, and ELF levels are 2–5 μM. Studies correlating α1AT phenotypes, α1AT levels and risk for emphysema suggest a "threshold" of plasma levels of 11 μM that is necessary to provide sufficient α1AT to protect the alveolar walls. This translates to minimal interstitial fluid levels of 5–7 μM and minimal ELF levels of 1.7 μM. For any form of anti-NE augmentation therapy, the levels of protection in the interstitial fluid and ELF must be equivalent of these minimal amounts of α1AT. With intravenous weekly or monthly α1AT augmentation therapy, such levels can be readily achieved.

33). Consistent with this, transfer of the Z gene to transgenic mice causes an accumulation of α1AT in hepatocytes and hepatitis (21). In contrast, homozygous inheritance of the S gene is associated with no hepatocyte α1AT accumulation and no liver disease. Likewise, for all the rare alleles in which the dominant mechanism causing the deficiency state is other than intracellular α1AT accumulation, no liver disease has been observed.

From these observations, it is reasonable to conclude that if the mutation causes intracellular accumulation of α1AT, the individual is at risk for liver disease. However, while compelling, this concept is based on relatively few cases other than those in association with the Z mutation. Furthermore, even if true, it is not clear how the accumulation of α1AT causes injury to hepatocytes, and inflammation in the local milieu. One interesting possibility relates to the observation that cells with the Z mutation also exhibit increased levels of heat-shock proteins (34). Although this may be unrelated to the liver disease, it is of interest that a member of the HSP 70 heat-shock gene product family facilitates intracellular protein translocation, perhaps related to protein unfolding (17). In the context that the newly synthesized Z and M_{malton} proteins accumulate in the RER, it is conceivable that abnormalities in translocation processes and hepatocyte injury are intimately linked.

It is not understood why only a minority of Z homozygotes ever develop liver disease or why only a portion of those infants affected go on to develop cirrhosis (see references 1 and 7–9 for review). It has been suggested that another gene may be involved, but the evidence for this is not convincing.

Therapy for the emphysema associated with α1-antitrypsin deficiency

With the overwhelming evidence that the emphysema associated with α1AT deficiency results from an insufficient anti–NE protective screen for the alveolar walls, strategies to prevent the progressive lung destruction have focused on reestablishing the NE/anti–NE balance in the lower respiratory tract in favor of the anti–NE defenses. The central question is: how much anti–NE defense is required to take the individual out of the at-risk group? Normally, > 90% of the anti–NE protection to the alveolar wall comes from plasma α1AT that diffuses across the alveolar wall (Fig. 4). With the knowledge that serum levels of 11 μM are sufficient to protect the lung, the target levels of equivalent anti–NE protection for the alveolar interstitium can be determined from the knowledge of α1AT levels in the lung in normal and α1AT deficiency states. For the epithelial surface of the lung, the minimum protective level is 1.7 μM. For the interstitium the threshold α1AT levels are estimated to be 5–7 μM. Thus, for an anti–NE therapy to be effective, it must provide the equivalent protection.

Augmentation therapy with intravenous α1-antitrypsin purified from plasma. We initially approached the problem of preventing the emphysema associated with α1AT deficiency with the same strategy used to treat hemophilia: i.e., since the affected individual is α1AT "deficient," the clinical manifestations of the disease should be preventable by augmenting α1AT levels in the lung by administering intermittent intravenous infusions of α1AT. In 1979, Gadek et al. (35) demonstrated this was feasible with weekly administrations of purified α1AT. When sufficient amounts of purified α1AT became available to mount larger-scale clinical trials, Wewers et al. (14) showed that once weekly infusions with 60 mg/kg α1AT resulted in the chronic maintenance of α1AT serum levels above levels sufficient to bring the individual out of the at-risk group for emphysema, the infused α1AT diffused into the lower respiratory tract, and the α1AT reaching the lung was functional, chronically providing a sufficient anti–NE screen to protect the lower respiratory tract from its burden of NE. On the basis of this demonstration of the "biochemical efficacy" of augmentation therapy, administration of weekly intravenous α1AT was approved for general use, and more than 1,000 α1AT-deficient individuals are receiving this therapy worldwide. In an attempt to reduce the frequency of infusions, Hubbard et al. (36) hypothesized that fourfold more α1AT

(250 mg/kg) could be given once monthly. This approach works, with similar biochemical efficacy in the lung. However, 250 mg/kg apparently is the limit that the body will accept, since administration of larger amounts by plasma exchange failed to extend beyond 1 mo the time at which plasma levels remained above the threshold level of 11 μM (unpublished observations).

Importantly, augmentation therapy is safe, with minor adverse reactions and no evidence of anti-α1AT antibodies or immune complexes, and no evidence of HIV seroconversion or development of hepatitis B (4, 14). Despite the fact that the plasma exchange approach to therapy is not effective, it does demonstrate that administration of up to 40 g of α1AT can be administered without adverse effects, an observation important to the development of strategies for gene therapy (see below).

Aerosol augmentation therapy. Since some α1AT normally diffuses from plasma through the alveolar wall, it is reasonable to assume that the process could work in reverse: i.e., that plasma α1AT might be administered by aerosol as a means of augmenting anti-NE lung defenses in α1AT deficiency (37). While simple in concept, there are a number of hurdles that must be overcome for such an approach to be effective, including: (*a*) the α1AT must be able to tolerate being aerosolized; (*b*) the droplets containing α1AT must be 0.2–3 μm in diameter, a size optimal to delivery to the lower respiratory tract; (*c*) the anti-NE defenses on the alveolar epithelial surface must be augmented above the threshold level; (*d*) the interstitial threshold levels must be surpassed; and (*e*) the therapy must be safe. Hubbard et al. (37) have shown such an approach is feasible in humans, demonstrating that aerosolized plasma α1AT can be recovered from the alveolar surface in an intact, functional form that reestablishes the anti-NE protective screen of the epithelium in a safe fashion. The intact molecule can be detected in blood of treated individuals, and studies in experimental animals have shown the α1AT is present in lung lymph, indicating that it is present in alveolar interstitial fluid (37, 38). The interstitial levels, however, appear to be lower than that required for effective reestablishment of the anti-NE protective screen, and if aerosol therapy is to become a reality, this obstacle will have to be overcome.

The development of the aerosol route has opened up the possibility that recombinant DNA produced forms of α1AT may be used for α1AT augmentation therapy. Human α1AT has been produced in *E. coli* and yeast directed by the normal M1(Val^{213}) cDNA (39, 40). Despite the fact that the recombinant molecules are not glycosylated, they function normally as inhibitors of NE. However, the lack of carbohydrate side chains on the molecule causes the plasma half-life to be severalfold reduced, obviating the intravenous route to treat α1AT deficiency on a chronic basis. However, aerosolization of recombinant α1AT to experimental animals and to humans (see reference 40 for review) has demonstrated that it augments epithelial anti-NE defenses, and the molecule diffuses across the alveolar epithelium in an intact fashion. However, as with aerosolization of plasma α1AT, there is as yet no proof that the interstitial threshold levels can be achieved by this route.

If aerosol therapy can be effectively developed, the recombinant molecule offers the advantage that it can be modified to form a "better" α1AT. Using site-directed mutagenesis, the α1AT inhibitory site centered at Met^{358} has been modified, to prevent it from being rendered impotent by oxidants, such as those from inflammatory cells (10, 39). The best substitution is Leu^{358}; such recombinant α1AT molecules are oxidant resistant, have excellent anti-NE capacity, and also inhibit cathepsin G, another neutrophil protease capable of modulating connective tissue injury. Such a molecule may be useful for conditions in which there is a NE burden together with an oxidant burden in the lung, e.g., adult respiratory distress syndrome, cigarette smoking–induced lung disease, and cystic fibrosis.

In addition to recombinant α1AT, a recombinant form of the upper airway secretory leucoprotease inhibitor (rSLPI) has been developed. This inhibitor can be aerosolized, it is an excellent inhibitor of NE, and aerosolization to experimental animals has shown it effectively augments epithelial anti-NE defenses and diffuses into the alveolar interstitium (41). Other molecules being developed for anti-NE augmentation therapy include pharmacologic NE inhibitors, including β-lactams and small peptides.

Potential for gene therapy. Because α1AT deficiency is a deficiency of a protein that functions in the extracellular milieu, prevention of the emphysema of α1AT deficiency conceptually is a relatively easy target for gene therapy. Because human studies have demonstrated very high levels of α1AT can be tolerated, tight control of gene expression is not critical. Several strategies have been considered to safely place the normal gene in sufficient numbers of cells to produce and secrete α1AT in sufficient quantities to protect the lower respiratory tract.

First, since mononuclear phagocytes normally express the α1AT gene (15, 16), and since alveolar macrophages (the pulmonary representative of the mononuclear phagocyte system) are derived from circulating blood monocytes and hence bone marrow, it should be possible to provide augmentation of local α1AT production by inserting the normal α1AT cDNA into bone marrow precursor cells, with consequent eventual repopulation of alveolar macrophages producing α1AT in the lower respiratory tract.

Secondly, by inserting the α1AT cDNA with appropriate controlling elements into cells that do not normally produce α1AT, and transplanting these cells in the recipient, a mass of α1AT-producing cells could be established in affected individuals sufficient to adequately augment α1AT levels. This approach has been evaluated using a retroviral vector containing the human α1AT cDNA driven by a constitutive promoter (42). After packaging into an infectious virus, the provirus was permanently integrated into murine fibroblasts, directing the cells to produce and secrete human α1AT that was glycosylated, reacted with human NE in a normal fashion, and had a normal half-life in plasma. When these cells were transplanted into the peritoneal cavity of nude mice, evaluation 1 mo later demonstrated human α1AT in plasma and, most importantly, in lung epithelial lining fluid. With a similar construct, human cells that do not normally express the α1AT gene can be easily converted into α1AT-producing cells. The cell target being considered for human studies is the T lymphocyte. The T cell not only will produce α1AT when appropriately modified by gene transfer (43), but it has the advantage that it can be grown rapidly in vivo with interleukin 2, and the presence of the T cell antigen receptor may permit in vivo expansion for the numbers of α1AT-secreting cells, as well as possible localization of the α1AT producing cells to specific sites.

Finally, the epithelial cells lining the lower respiratory tract are an inviting target for gene therapy for α1AT deficiency. In vitro studies have shown that lung epithelial cells can be modified by gene transfer to produce and secrete α1AT (44).

Therapy for the liver disease associated with α1-antitrypsin deficiency

Other than transplantation, there is no available therapy for the liver disease associated with α1AT deficiency. If the liver disease is secondary to the accumulation of α1AT in the RER, then it may be possible to reduce α1AT synthesis and/or increase the rate of its translocation from the RER. Consistent with the concept that the liver disease does not develop because of the lack of anti–NE protection, our experience with intravenous augmentation therapy with plasma α1AT suggests that the mild liver function abnormalities in some adults with emphysema are unaffected by therapy.

The strategies for gene therapy of the liver disease are simple in concept but difficult to achieve in vivo. Since Z homozygotes are at risk for developing clinically significant liver disease, but MZ heterozygotes are not (8), the strategy would be to insert the normal cDNA into hepatocytes, converting them into the equivalent of MZ heterozygote cells. The normal gene can be put into hepatocytes in culture and can be made to express in the liver (using an α1AT promoter) in transgenic mice. How this will be achieved in vivo in man, however, is not clear, although a variety of targeting vehicles are being considered.

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