Heterozygous M3Mmalton α₁-Antitrypsin Deficiency Associated with End-Stage Liver Disease: Case Report and Review

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α₁-Antitrypsin (α1AT) deficiency is an autosomal recessive disorder that can cause pulmonary emphysema and liver disease. We report here the case of a 59-year-old woman who was admitted to hospital for evaluation of jaundice. She had no history of hepatitis or childhood liver disease. She had never received a blood transfusion, nor had she abused drugs or alcohol. Transjugular liver biopsy was then performed and revealed a micronodular cirrhosis. Ten months later, because of persistent liver cell failure and ascites, she underwent an orthotopic liver transplantation. Investigation of α1AT system in the proband revealed a substantial decrease in serum α1AT associated with a low elastase inhibitory capacity. The Pi phenotype revealed a PiM-like profile. Sequencing of exons 1–5 demonstrated the presence of the M3 allele. Moreover, a triple nucleotide deletion was detected in exon 2 of one allele. This caused an “in-phase” frameshift, coding for a protein deficient in a single Phe residue, which corresponded to the Mmalton variant. After liver biopsy, periodic acid-Schiff-positive acidophilic bodies resistant to diastase digestion were observed in the cytoplasm of hepatocytes. These results demonstrated that our patient had a heterozygous M3Mmalton α1AT genotype related to a deficiency phenotype. This observation is the first of a patient with heterozygous Mmalton genotype associated with an α1AT deficiency that induced severe liver disease requiring orthotopic liver transplantation. © 2001 American Association for Clinical Chemistry

α₁-Antitrypsin (α1AT)⁶ deficiency, one of the most common hereditary disorders in Europe, is an autosomal recessive disorder characterized by reduced serum α1AT. Various homo- and heterozygous combinations of α1AT gene mutations are associated with a high risk for development of pulmonary emphysema or liver disease (1). α1AT, also called α₁-proteinase inhibitor, is the major proteinase inhibitor in human plasma. It counteracts the effects of neutrophil elastase and other proteolytic enzymes and diffuses in most organs, where it protects extracellular structures from attacks by activated neutrophils (1). The lower respiratory tract is particularly vulnerable to α1AT deficiency.

The liver is the major site of α1AT gene expression, releasing 2 g of α1AT into the circulation daily. The α1AT protein is extremely pleomorphic, and >90 variants have now been identified (2). The most common allele is PiM, which is associated with serum α1AT concentrations within the reference interval. Other variants are associated with a reduction in serum α1AT concentrations and are so-called deficiency variants. The most common are PiS and PiZ, which are characterized by serum α1AT concentrations between 50–60% and 10–15% of normal, respectively, and for PiZ by early development of pulmonary emphysema. The S variant is considered not associated with clinical disease (3). Homozygous PiZZ is also associated with liver involvement and confers a risk for

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Received February 26, 2001; accepted April 26, 2001.

Nonstandard abbreviations: α1AT, α₁-antitrypsin; RER, rough endoplasmic reticulum; ASAT, aspartate aminotransferase; AB, antibody; PAS, periodic acid-Schiff; and OLT, orthotopic liver transplantation.
liver disease, but only a minority of people with this genotype develop cholestasis and/or cirrhosis (2, 4, 5). The P/V allele is characterized by a single base substitution, which causes replacement of Glu-342 by Lys (6–8). Pathophysiology of liver injury could lead to impaired liver secretion and storage: accumulation of α1AT protein in the rough endoplasmic reticulum (RER) with formation of aggregates could hamper secretion and directly cause liver damage (9, 10). However, other mutated alleles can also be responsible for proteinase inhibitor deficiencies, such as Mmalton, Mduarte, Mheerlen, and Mprocida, which are characterized by a low serum α1AT concentration (3–15% of normal) (11).

There is no specific treatment of α1AT deficiency-induced liver disease. Patients with α1AT deficiency who develop cirrhosis and liver failure, therefore, are candidates for liver transplantation, which treats the liver disease and corrects α1AT deficiency (12–14).

We investigated a patient with an end-stage liver disease treated by liver transplantation and who had a heterozygous M3Mmalton α1AT genotype related to a deficiency phenotype. Association of liver disease in subjects heterozygous for deficiency alleles has not been clearly established. The present study is the first to report a heterozygous Mmalton genotype associated with an α1AT deficiency leading to severe liver disease requiring liver transplantation.

Case Report

A 59-year-old housewife was admitted to hospital in March 1995 for evaluation of jaundice. In 1980, she had undergone a cholecystectomy for cholelithiasis and additional surgery. In 1981 and 1982, she had undergone treatments for eventration. At these times, liver function was normal. She had no history of hepatitis or childhood liver disease. She had never received a blood transfusion, nor had she abused drugs or alcohol. In 1990, a checkup revealed abnormal liver functions tests [serum aspartate aminotransferase (ASAT), 45 U/L (reference interval, 0–30 U/L); γ-glutamyltransferase, 60 U/L (reference interval, 5–25 U/L)], but her alkaline phosphatase activities were within the appropriate reference intervals and the physical examination was not remarkable.

At admission, physical examination revealed that she was jaundiced with labored breathing, a distended abdomen, cutaneous collateral circulation, a painless hepatosplenomegaly, and many spider angiomas. There was no digital clubbing or cyanosis, but dyspnea secondary to ascitis was present. Laboratory data revealed anemia (hemoglobin, 111 g/L), thrombocytopenia (50 000/mm³), leukopenia (3500/mm³), reduced prothrombin activity (35% of normal; reference interval, 60–120%), and reduced factor V (32%; reference interval, 60–120%). The results of the patient’s liver function tests were as follows: ASAT, 75 U/L (reference interval, 0–30 U/L); γ-glutamyltransferase, 60 U/L (reference interval, 5–25 U/L); total bilirubin, 84 µmol/L (reference interval, 2–21 µmol/L); serum albumin, 25 g/L (reference interval, 32–52 g/L); and serum cholesterol, 0.64 g/L (reference interval, 1.8–2.6 g/L) were abnormal, whereas her alkaline phosphatases were within the appropriate reference intervals. Serologic markers were negative for anti-nuclear antibodies (ABs), anti-smooth muscle ABs, anti-mitochondrial ABs, and anti-endoplasmic endothelial ABs. Hepatitis B and C virus serologies were also negative for hepatitis B surface antigen, hepatitis B core antigen ABs, and hepatitis C AB. Plasma ferritin and ceruloplasmin were within the appropriate reference intervals. Serum protein electrophoresis revealed a slight decrease of α1-globulin (1.4 g/L; reference interval, 1.8–4.8 g/L). The chest x-ray and spirometry were unremarkable. According to Child-Pugh classification, the cirrhosis was C13. A diuretic treatment rapidly decreased ascites.

Six months later, the jaundice recurred with aggravation of liver failure (prothrombin activity; 23%; factor V, 19%). Transjugular liver biopsy revealed micronodular cirrhosis with periodic acid-Schiff (PAS)-positive acidophilic bodies in the cytoplasm of the hepatocytes. An investigation of the α1AT phenotype and genotype was then performed to search for a rare deficiency allele associated with the liver disease. Because of persistent liver cell failure and ascitis, the patient underwent orthotopic liver transplantation (OLT) in January 1996. Five years after transplantation, the patient was still alive and results of her liver function tests were within the appropriate reference intervals: ASAT, 13 U/L; alanine aminotransferase, 16 U/L; total bilirubin, 12 µmol/L; serum albumin, 42 g/L; and prothrombin activity, 96%.

An investigation of the patient’s family was concomitantly undertaken; plasma samples were obtained from her husband and daughter (who were healthy) for α1AT phenotyping. Two of the patient’s sisters had cryptogenic cirrhosis; one of the two had compensated liver cirrhosis but could not undergo liver transplantation because of her age (68 years). Samples could not be obtained from the patient’s two other sisters and her three brothers.

Materials and Methods

Analytical Methods

Serum α1AT was determined by an automated immunoturbidimetric method on a Cobas Fara II centrifugal analyzer (Roche), using specific commercially available antibodies (Dako). Sera were also analyzed for their elastase inhibitory activity against porcine pancreatic elastase as described previously (13). α1AT phenotypes were determined by isoelectric focusing (16) using Pharmalytes 4.2–4.9 (Pharmacia).

Histochromic and Immunohistochemical Methods

Specimens obtained from transjugular liver biopsy (1-mm length) and from native liver were cut into 3- to 5-µm-thick sections and stained with Hematoxylin-eosin, Mas-
DNA ANALYSIS
Informed written consent was obtained from the patient. Genomic DNA was prepared from peripheral blood samples collected on EDTA according to standard protocols. Sequencing analysis was performed on PCR-amplified exons 1–5. The sequences of primers were obtained from previously published sequences (17, 18) and deduced using the PCR PLAN program from PC GENE Software. The sequences and PCR conditions are given in Table 1. PCR products were purified on Wizard™ PCR prep columns (Promega) before sequencing. Both strands were sequenced using the dRhodamine cycle sequencing ready reaction (Perkin-Elmer) on an ABI Prism 377 XL automated DNA sequencer.

To confirm the nature of the deletion, the PCR products of exon 2.1 were cloned into pCR2.1 vector (Invitrogen). Wild-type and deleted clones were sequenced four times on both strands. The double-stranded plasmid inserts were sequenced manually using the dideoxynucleotide chain termination method (19) with [35S]ATP (Amersham) and Sequenase 2.0 (US Biochemical Corp.), according to the protocol indicated by the manufacturer.

Table 1. Experimental PCR conditions used for amplification of α1AT exons 1–5.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer sequence, 5’ to 3’</th>
<th>Length, bp</th>
<th>MgCl₂, mmol/L</th>
<th>Cyclesa</th>
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</thead>
<tbody>
<tr>
<td>1as</td>
<td>GGGCAGGAATGGGCACCTG</td>
<td>258</td>
<td>0.8</td>
<td>94°C for 2 min; 94°C for 1 min; 66°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
<tr>
<td>1as</td>
<td>GAGCAGCAAGCAGAATTGTCCTC</td>
<td>255</td>
<td>0.8</td>
<td>94°C for 2 min; 94°C for 1 min; 66°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
<tr>
<td>1bs</td>
<td>TCTAACCACCTCTGATCTCCC</td>
<td>150</td>
<td>0.8</td>
<td>94°C for 2 min; 94°C for 1 min; 66°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
<tr>
<td>1as</td>
<td>GTGTACAGCTTCCACTGACCTT</td>
<td>396</td>
<td>2</td>
<td>94°C for 2 min; 94°C for 1 min; 62°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
<tr>
<td>1as</td>
<td>GAGGAGTTCCTGGAAGGCACTT</td>
<td>376</td>
<td>1.5</td>
<td>94°C for 2 min; 94°C for 1 min; 62°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
<tr>
<td>2as</td>
<td>CCCCCATCTCTGTCTTGC</td>
<td>314</td>
<td>1.5</td>
<td>94°C for 12 min; 94°C for 1 min; 63°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
<tr>
<td>3as</td>
<td>CACCCTCAAGGGAAGAATC</td>
<td>195</td>
<td>2</td>
<td>94°C for 2 min; 94°C for 1 min; 63°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
<tr>
<td>4as</td>
<td>AAGGTCGAACGGGGATCTC</td>
<td>296</td>
<td>1.2</td>
<td>94°C for 12 min; 94°C for 1 min; 62°C for 1.5 min; 72°C for 1 min (35); 72°C for 7 min</td>
</tr>
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a Number of cycles in parentheses.

RESULTS
FOLLOW-UP AND CURRENT STATUS
The patient did not experience any medical or surgical complications. During the follow-up after OLT, she never experienced acute rejection episodes. Five years after transplantation, she was still alive and the results of her liver functions tests were within the appropriate reference intervals: ASAT, 13 U/L; alanine aminotransferase, 16 U/L; total bilirubin, 12 μmol/L; serum albumin, 42 g/L; and prothrombin activity, 96%. Immunosuppressive therapy is maintained with cyclosporine (100 mg twice a day) and prednisone (7.5 mg once a day).

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL METHODS
Transjugular liver biopsy revealed micronodular cirrhosis without steatosis. Stained acidophilic bodies (size, 3–10 μm) were observed in the cytoplasm of hepatocytes. These stained acidophilic bodies are highly predictive of α1AT deficiency (Figs. 1 and 2). They were intensively PAS positive and resistant to diastase digestion. The immunoperoxidase technique with specific anti-α1AT antibodies confirmed the presence of α1AT.

Histologic examination of native liver showed micronodular cirrhosis associated with steatosis and stained acidophilic bodies.
**BIOCHEMICAL INVESTIGATIONS**

Investigation of the $\alpha$1AT system in the proband, performed three times before transplantation, revealed a substantial decrease in serum $\alpha$1AT concentration associated with a low elastase inhibitory capacity (Table 2). The Pi phenotype, determined in our experimental conditions, revealed a PiM-like profile. In our system, Pi Mmalton cannot be clearly detected because it comigrates with the M2 allele (Table 2).

After transplantation, serum $\alpha$1AT and elastase inhibitory capacity were within reference values ($2.76$ g/L and $24 690$ units/L, respectively), and the phenotype was PiMM (Table 2). Serum $\alpha$1AT, elastase inhibitory capacity, and phenotype were also evaluated in the husband and daughter (Table 3). For these subjects, the $\alpha$1AT concentration and inhibitory capacity were both below the lower limit of the reference interval. The husband had an homozygous SS phenotype, and the daughter had the heterozygous MS phenotype. No clinical disease was associated with these phenotypes.

**IDENTIFICATION OF THE Mmalton MUTATION**

Sequencing of exons 1a, 1b, 1c, and 2–5 and of all exon-intron junctions demonstrated two differences from the common $\alpha$1AT gene. The first difference was a single point mutation (GAA$^3\rightarrow$GAC, Glu$^3\rightarrow$Asp) in exon 5 on the two alleles, indicating the presence of the M3 allele. The second was a triple nucleotide deletion in exon 2 of one allele.

The PCR fragments obtained from exon 2 were cloned, and five clones were sequenced. Three of the clones had the common sequence and two had the deletion. The triple nucleotide deletion could correspond to a complete codon for Phe$^{51}$ but also to the two last bases of the codon for Phe$^{52}$ (TC) and to the first base of the codon for Ser$^{53}$ (T; Fig. 3). However, this causes an “in-phase” frameshift that codes for a protein deficient in a single Phe residue.

**Discussion**

$\alpha$1AT deficiency is a common inborn metabolism error and occurs mainly in Caucasians of Northern Europe. Its incidence is 1:1600 to 1:2000 in Western countries (20, 21). Because various events can increase (inflammation) or decrease (liver failure) $\alpha$1AT, quantification of serum $\alpha$1AT may be deceptive and diagnosis of $\alpha$1AT deficiency is based mostly on $\alpha$1AT phenotype analysis.

The $\alpha$1AT locus is pleomorphic, with ~90 alleles identified (2). These $\alpha$1AT alleles are categorized as “normal” or “at risk”. There are four common wild-type alleles: M1 (Ala$^{213}$), M1 (Val$^{213}$), M2, and M3. Among

<table>
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<th>Table 2. Serum $\alpha$1AT concentration, elastase inhibitory capacity, and $\alpha$1AT phenotype before and after OLT.</th>
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<tr>
<td>Elastase inhibitory capacity (17 500–31 500 units/L)$^*$</td>
</tr>
<tr>
<td>Phenotype</td>
</tr>
<tr>
<td>Before OLT</td>
</tr>
<tr>
<td>Sept. 12, 1995</td>
</tr>
<tr>
<td>Sept. 22, 1995</td>
</tr>
<tr>
<td>Oct. 10, 1995</td>
</tr>
<tr>
<td>After OLT</td>
</tr>
<tr>
<td>April 30, 1996</td>
</tr>
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<td></td>
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<td>$^*$ Reference interval.</td>
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<tr>
<th>Table 3. Serum $\alpha$1AT concentrations, elastase inhibitory capacity, and phenotypes of the proband’s daughter and husband.</th>
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<tr>
<td>Elastase inhibitory capacity (17 500–31 500 units/L)$^*$</td>
</tr>
<tr>
<td>Phenotype</td>
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<tr>
<td>Daughter</td>
</tr>
<tr>
<td>Husband</td>
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<td>$^*$ Reference interval.</td>
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</table>
Caucasians of Northern Europe, MI (Val213) is the most common allele (1). The at-risk group includes “deficient” alleles, which when inherited in homozygous form are associated with serum α1AT <20 μmol/L, and “null” alleles (no α1AT in serum attributable to that allele) (22). According to the literature, two at-risk alleles must be inherited to confer a real risk for clinically significant disease. These mutations are probably directly relevant to the pathogenesis of the liver disease. In addition to inflammation and liver cirrhosis, all cases of liver disease associated with α1AT deficiency are also characterized by an accumulation of α1AT in the hepatocytes.

Liver disease associated with α1AT deficiency, first described by Sharp et al. in 1969 (4), is discovered mainly in childhood, with jaundice as the first symptom a few weeks after birth (14). Ten percent of neonates with α1AT deficiency will develop cholestasis and hepatitis. This prolonged neonatal cholestasis often is associated to further spontaneous regression and is not considered an indicator of poor prognosis because subsequent evolution is variable, except for patients with paucity of intralobular bile ducts, which rapidly leads to liver cell failure (23). Whereas most of these children remain free of any symptoms, others progress toward cirrhosis (14). The second pattern of evolution includes portal hypertension with esophageal varices and gastrointestinal bleeding, which constitute criteria for an unfavorable prognosis requiring liver transplantation. In adults >40 years, hepatitis and cirrhosis can also rarely develop (5), with a minority of patients requiring OLT. These patients have a survival rate of 80–94% at 3 years, and transplantation is the best therapy (14, 24).

The pathogenesis of liver disease related to α1AT deficiency is poorly understood but is related 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 (5). The pathophysiology of liver injury does not seem to be related to an uninhibited proteolytic attack because it is well established in lung injury. Most evidence favors the concept that the accumulation of α1AT in the endoplasmic reticulum is directly related to liver cell damage (11). This “accumulation theory” is directly supported by studies by Carlson et al. (25) and Dycaico et al. (26). The mechanisms responsible for intracellular accumulation of α1AT in association with Z and Mmalton alleles are not fully understood. It has been suggested that in the Pi Z phenotype, the Glu342→Lys mutation slows the rate of folding of the α1AT polypeptide and/or changes the tertiary structure of the α1AT molecule within the RER (27). Abnormal folding might expose a site molecule that is recognized by a protein in the RER. According to Birrer et al. (27), three mechanisms have been proposed: (a) this interaction may cause specific intracellular retention of the molecule; (b) improperly folded α1AT may alter the affinity of α1AT for a receptor that is involved in the translocation of α1AT from the RER to the Golgi apparatus, with consequent trapping and accumulation of α1AT in the RER; and (c) specific intracellular systems have been identified in the RER that degrade improperly folded proteins. One such system is the ubiquitin pathway, in which selectivity for degradation depends on the conjugation of the protein to ubiquitin. Ubiquitin is increased in monocytes and hepatocytes of PiZ homozygotes, suggesting that the accumulation of the Z form of α1AT in the hepatocytes may be the result of a relative increase in binding to heat-shock proteins combined with a malfunctioning degrading system (27).

Pi Mmalton was initially described in a family exhibiting Pi MmaltonZ and Pi MnaltonM phenotypes, but without liver or lung involvement (28). The Pi Mmalton allele has been also reported as Pi MmaltonZ in a few patients with severe emphysema and as Pi MmaltonM in
patients with intrahepatic globules positive for PAS staining after diastase digestion (29). These diastase-resistant globules are always found in the livers of PI Z, Mduarte, and Mmalton subjects. The distribution, number, and size vary greatly. Usually they are situated in the periportal hepatocytes, but in cirrhotic nodules, they are spread throughout (30). Globules can be difficult to find, especially in infancy. In liver biopsy specimens taken with Menghini or trocut, the globules can be almost undetectable, especially in children <12 weeks of age. In cirrhotic adults, globules can be irregularly distributed, and some resistant globules are always found in the livers of MMalton effect of the mutation is apparently attributable to a decrease in glycoprotein in isoelectric focusing gels (31). Like Z α1AT, Mmalton α1AT has the wild-type promoter region and signal sequence, and is stable in vivo. The decreased serum concentration of Mmalton α1AT is therefore likely attributable to the self-aggregation phenomenon, which is observed in vitro (11). The Mmalton allele shows, like the Z allele, the association of liver disease with the same type of abnormalities of α1AT biosynthesis. Sequence analysis demonstrated that the Mmalton allele differs from the wild-type α1AT M2 allele by deletion of a triplet TCT that straddles two consecutive codons. The Mmalton and the M2 proteins are similar in charge; the isoelectric point of Mmalton protein is slightly more cathodal than the M2 proteins are similar in charge; the isoelectric point of Mmalton protein is slightly more cathodal than the M2 protein in isoelectric focusing gels (11). The mutation causes a deleted residue (Phe) in a hydrophobic region within the center of the molecule. The concentrations of α1AT mRNA transcripts in the liver biopsies were normal, but there was abnormal intracellular accumulation of newly synthesized α1AT in the RER with consequent reduced α1AT secretion. This abnormal accumulation of the newly synthesized Mmalton α1AT derives from perturbations of the molecular conformation, which impairs maturation from the RER to the Golgi (22).

In conclusion, this case provided information on the following: (a) the possibility of heterozygous α1AT deficiency associated with Mmalton phenotype; (b) end-stage liver disease without any pulmonary disease; and (c) late symptomatic liver disease, although α1AT deficiency is discovered mainly in childhood.

We thank Dr. M. Crepin ("Plateau Technique de Séquençage du CH et U de Lille") for excellent technical assistance and Prof. A. Cortot for reviewing the manuscript.

References


