

CASE REPORT

Role of alpha-1-antichymotrypsin deficiency in promoting cirrhosis in two siblings with heterozygous alpha-1-antitrypsin deficiency phenotype SZ

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Background: Alpha-1-antitrypsin (A1AT) deficiency is the most common inherited metabolic disorder with the potential to cause injury in the lung and liver. Recent reports suggested that alpha-1-antichymotrypsin (A1AC) deficiency may also be a possible cause of chronic liver disease. However, it has received little attention and is rarely investigated in the clinical setting.

Aims: To assess the role of A1AC deficiency in the pathogenesis of chronic liver disease in two siblings with heterozygous A1AT phenotype Pi SZ.

Patients: Two adult siblings with an A1AT Pi SZ phenotype and reduced levels of A1AC consistent with heterozygosity who developed cirrhosis and underwent liver transplantation.

Methods and results: A1AT and A1AC levels in plasma measured by electroimmunoassay were 74 mg/dl and 90 mg/dl (140–470) and 0.12 mg/ml and 0.14 mg/ml (0.173–0.46), respectively. Immunohistochemistry revealed an apparent accumulation of both A1AT and A1AC in hepatocytes. A previously reported point mutation in exon III (Pro²²⁹ to Ala substitution) of the A1AC gene was not detected by polymerase chain reaction amplification and a single strand conformation polymorphism analysis.

Conclusions: Our report represents the first case of two siblings with A1CA phenotype Pi SZ who developed cirrhosis and underwent liver transplantation. Both siblings were heterozygous for A1AT and A1AC deficiency suggesting that combined deficiency of these two major serine protease inhibitors may enhance the risk of developing liver disease.

Alpha-1-antitrypsin (A1AT) and alpha-1-antichymotrypsin (A1AC) are two closely related proteinase inhibitors.¹ Affected individuals have serum A1AT concentrations 10–20% of levels observed in those with the Pi M phenotype. Pi MZ and SZ heterozygotes have A1AT concentrations of approximately 60% and 40% of those with the Pi M phenotype, respectively.² Whereas the Pi ZZ phenotype is clearly associated with emphysema, neonatal hepatitis, cirrhosis, and hepatocellular carcinoma,^{3,4} a link between the Pi SZ phenotype and liver disease has not been established. Recently, deficiency of A1AC has also been suggested as a possible cause of chronic liver disease. However, despite the apparent importance it has received little attention and is rarely investigated in the clinical setting. Although the precise mechanism(s) leading to the development of liver disease due to deficiencies of these proteinase inhibitors are not fully understood, recent studies indicated that the structural change of the A1AT proteins leads to misfolding of the molecules.⁵ It is conceivable that a similar mechanism is

involved in A1AC deficiency. These misfolded proteins aggregate in cells and probably overwhelm the degradative machinery of the endoplasmic reticulum.

Here we report two siblings with an A1AT Pi SZ phenotype and reduced levels of A1AC, consistent with heterozygosity. Both developed cirrhosis and underwent liver transplantation. Both carried specific inclusions for A1AC suggesting a contributory role of A1AC deficiency in promoting cirrhosis in Pi SZ heterozygotes with near normal levels of A1AT.

CASE REPORTS

Patient No 1

A 47 year old man presented with bleeding from oesophageal varices. He had no known risk factors for liver disease. Laboratory studies revealed the following values: ferritin 560 mg/ml (7–600); iron 195 µg/dl (35–185); total iron binding capacity 224 µg/dl (120–300); albumin 2.8 g/dl (3.5–5.5); total bilirubin 4.2 mg/dl (0.2–1.5); direct bilirubin 2.9 mg/dl (0–0.6); alkaline phosphatase 193 U/l (30–140); lactate dehydrogenase 180 U/l (60–230); aspartate aminotransferase 178 U/l (0–50); alanine aminotransferase 97 U/l (0–55); gamma-glutamyl transpeptidase 70 U/l (4–63); antinuclear antibody negative; antimitochondrial antibody negative; anti-smooth muscle antibody negative; ceruloplasmin 40 mg/dl (15–50); A1AT 0.9 mg/ml (0.98–1.8); A1AT phenotype SZ; hepatitis C antibody negative; hepatitis B core antibody positive; hepatitis B surface antibody positive; hepatitis B surface antigen negative; hepatitis A antibody negative; white blood cell count $10.3 \times 10^3/\text{mm}^3$; mean corpuscular volume 103 (80–100); haemoglobin 15.2 g/dl; platelet count $130 \times 10^3/\text{mm}^3$ (130–400); and prothrombin INR 1.5. He underwent orthotopic liver transplantation but died 10 days after surgery due to cardiac arrhythmia.

Patient No 2

A 42 year old woman, a sister of the first patient, who was diagnosed by an outside physician to have chronic hepatitis was admitted with a one day history of haematemesis and melena. Emergency oesophagogastroduodenoscopy revealed grade IV oesophageal varices with stigmata of recent bleeding. She denied any alcohol abuse, blood transfusion, or illicit drug use.

Laboratory studies revealed the following values: ferritin 58 mg/ml (7–600); iron 89 µg/dl (35–185); total iron binding capacity 143 µg/dl (120–300); albumin 2.4 g/dl (3.5–5.5); total bilirubin 3.4 mg/dl (0.2–1.5); direct bilirubin 2.2 mg/dl (0–0.6); alkaline phosphatase 181 U/l (30–140); lactate dehydrogenase 285 U/l (60–230); aspartate aminotransferase 67 U/l

Abbreviations: A1AT, alpha-1-antitrypsin; A1AC, alpha-1-antichymotrypsin.

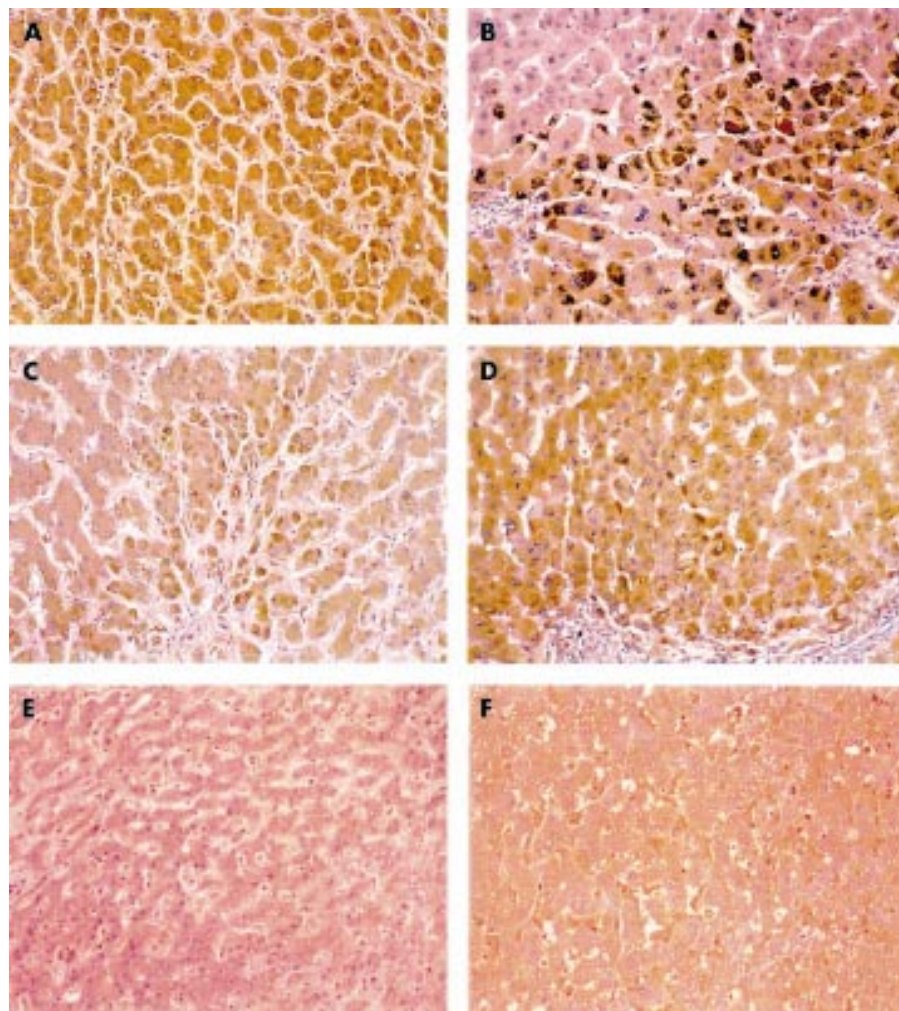


Figure 1 (A, B) Staining with specific monoclonal alpha-1-antitrypsin (A1AT) antibody shows A1AT granules within the cytoplasm of the hepatocytes in the first (A) and second (B) sibling. (B) Deeply stained A1AT granules within the cytoplasm of periseptal hepatocytes in the second patient. (C, D) Staining with specific monoclonal alpha-1-antichymotrypsin (A1AC) antibody shows A1AC granules in the first (C) and second sibling (D). (E, F) Negative staining of control specimens with specific monoclonal A1AT (E) and A1AC antibodies (F) (magnification $\times 100$)

(0–50); alanine aminotransferase 54 U/l (0–55); gamma-glutamyl transpeptidase 60 U/l (4–63); antinuclear antibody negative; antimitochondrial antibody negative; antismooth muscle antibody negative; ceruloplasmin 18.3 mg/dl (15–50); A1AT 0.74 mg/ml (0.98–1.8); Pi phenotype SZ; hepatitis C antibody negative; hepatitis B core antibody negative; hepatitis B surface antibody negative; hepatitis B surface antigen negative; hepatitis A antibody negative; white blood cell count $7.4 \times 10^3/\text{mm}^3$; mean corpuscular volume 95 (80–100); haemoglobin 12.3 g/dl; platelet count $162 \times 10^3/\text{mm}^3$ (130–400); and prothrombin INR 1.6. She underwent orthotopic liver transplantation and is doing well.

METHODS AND RESULTS

Plasma concentrations of A1AT and A1AC were determined by radial immunodiffusion and electroimmunoassay using highly purified preparations as standards.⁶ Normal concentrations based on healthy blood donors are 1.34 (0.18) mg/ml (95% confidence interval 0.98–1.8 mg/ml) for A1AT and 0.316 (0.073) (SD) (95% confidence interval 0.17–0.46 mg/ml) for A1AC. A1AT levels were 0.90 mg/ml for patient No 1 and 0.74 mg/ml for patient No 2, both consistent with heterozygosity. A1AC levels were also in the range consistent with heterozygosity for a deficiency gene in both patients: 0.12 and 0.14 mg/ml, respectively.

The most common type of A1AC deficiency is caused by a mutation in exon III leading to a ²²⁹Pro to Ala substitution.¹ This substitution apparently causes a major conformational change and aggregation and retention of the A1AC protein in the endoplasmic reticulum. However, when polymerase chain reaction amplification and single strand conformation polymorphism analysis was performed on the available DNA from one of the two siblings, no new alanine site was present in exon III of the A1AC gene (data not shown). This finding does not preclude that another deficiency or mutation may be present in our patients. It is conceivable that other structural mutations can lead to aggregation and intracellular accumulation of the altered protein.

The liver explants from both siblings revealed similar histopathological features. They showed multiple regenerative nodules of varying size separated by thick fibrous bands containing residual vascular and bile duct structures. There was also mild to moderate inflammation, consisting primarily of lymphocyte infiltrates. The hepatocytes showed focal periportal cytoplasmic accumulation of periodic acid-Schiff positive, diastase resistant globules. Because of the patient's heterozygosity for A1AT, we performed immunohistochemical studies of the liver specimens using primary monoclonal antibodies specific to human A1AC and A1AT obtained from CalBiochem (San Diego, California, USA). Specimens from both patients showed intensely stained A1AT intracytoplasmic globules,

mostly in periportal hepatocytes (fig 1A, B); staining with specific AIAC also demonstrated intense staining in the same areas (fig 1C, D). However, the globules were smaller and fewer than the A1AT globules. These findings are consistent with previous reports.⁷⁻⁹ Four specimens from patients with ZZ phenotypes and eight from subjects with other liver conditions were negative for AIAC staining (representative staining of control specimens are shown in fig 1E, F).

DISCUSSION

The association of homozygous A1AT deficiency Pi Z and liver disease is well established.²⁻⁴ The structural change of the A1AT-Z protein leads to misfolding of the molecule. This misfolded protein aggregates in the endoplasmic reticulum of liver cells, possibly overwhelming the degradative machinery of the endoplasmic reticulum.⁶ At present, it is not clear whether heterozygotes who carry a single Pi *Z allele are also at increased risk of developing chronic liver disease. There are only two case reports of liver diseases in patients with the Pi SZ phenotype.^{10,11} Our report presents the first case of two siblings with the A1AT phenotype Pi SZ and end stage liver disease.

Recent studies have evaluated the prevalence of chronic liver disease in adult heterozygotes for A1AT deficiency. These studies have suggested that the Pi MZ phenotype may be a potential independent risk factor for the development of cirrhosis and chronic liver failure requiring liver transplantation.^{12,13} Factors responsible for progression of liver disease in heterozygous A1AT deficient patients remain to be identified. Other investigators have failed to find any significant relationship between heterozygous A1AT deficiency and chronic liver disease.¹⁴ There is ongoing speculation on the contribution of coexisting liver disease to the pathogenesis of cirrhosis in patients with heterozygous A1AT deficiency. Recent studies speculated that carriers of at least one Pi *Z allele are more susceptible to hepatic viral infections and consequently to the development of chronic liver disease.¹⁵ However, no overrepresentation of chronic hepatitis B or C virus in heterozygous A1AT deficiency was found in a recent study.¹ Some investigators suggested that A1AT deficiency may play a contributing role in the development of liver disease in subjects with hepatitis C or alcohol abuse.^{17,16,17} Our two siblings had negative markers of active hepatitis B and C virus infections and denied alcohol intake, indicating that these factors did not play any contributing role in the pathogenesis of their liver disease.

Deficiency of AIAC has also been suggested as a possible cause of chronic liver disease but has received little attention and is rarely investigated in the clinical setting. Lindmark and Eriksson found that partial AIAC deficiency was associated with cryptogenic cirrhosis.¹⁸ As our patients were heterozygotes for the A1AT deficiency alleles (Pi *SZ) and were also heterozygotes for an AIAC deficiency gene, it is conceivable that the combination resulted in intrahepatic inclusions of the abnormal protein products. It is tempting to assume that this doubling of the load may be a precipitating factor for liver disease eventually leading to cirrhosis. Screening of more patients with liver disease for deficiencies of A1AT and AIAC will show more definitely if these conditions can cause or contribute to cirrhosis.

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