Increased PiZ gene frequency for α_1 antitrypsin in patients with genetic haemochromatosis

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Abstract

The putative relationship between genetic haemochromatosis and PiZ α_1 antitrypsin deficiency was studied using a monoclonal antibody against the PiZ variant in 67 consecutive patients with genetic haemochromatosis seen at Karolinska Hospital and Huddinge University Hospital, Stockholm over a 10 year period. Three (4.5%) of the patients with haemochromatosis were found to be PiZ homozygotes (odds ratio=82, confidence interval=26, 256; $p<0.0001$). The prevalence of the heterozygous (PiZ) phenotype was similar to that in the general population ($p=0.937$). During the ascertainment period, liver biopsy was performed in 65 (97%) of the patients; 66% (2 of 3) of the PiZ homozygotes were found to have cirrhosis compared with 10% (6/59) of the non-carriers of the PiZ variant ($p=0.039$). None of the homozygous or heterozygous α_1 antitrypsin deficient patients had developed hepatocellular carcinoma compared with 3.4% (2 of 59) of the non-PiZ gene carriers ($p=1.0$). Two of those with the homozygous phenotype had developed severe emphysema. HLA typing was performed in 18 patients, 16 (89%) of whom manifested antigens known to be linked to haemochromatosis. There were no significant differences between the PiZ gene carriers and non-carriers in mean age at onset of disease, sex distribution, or HLA type. Two of the PiZ heterozygotes had plasma α_1 antitrypsin concentrations below the normal range, though the group mean was lower than that of the non-PiZ carriers ($p=0.0003$). The data suggest that the presence of the PiZ allele for α_1 antitrypsin deficiency, in a double dose, is associated with genetic haemochromatosis and may contribute to the earlier onset of cirrhosis in these patients, though it does not increase the risk of hepatocellular carcinoma.

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Keywords: genetic haemochromatosis, PiZ, α_1 antitrypsin deficiency, cirrhosis, hepatocellular carcinoma.

Genetic haemochromatosis is an autosomal recessive disorder characterised by pathological deposition of excessive iron in the parenchymal cells of many organs, especially in the liver, which results in cirrhosis, arthropathy, diabetes, endocrine failure, heart failure, and changes in skin pigmentation.¹

However, the new definition of genetic haemochromatosis does not require the presence of clinical manifestations. An individual is considered to be affected if he or she has inherited two HLA linked haemochromatosis alleles, one from each parent.² Genetic haemochromatosis occurs in association with HLA A3, B7, and B14.³ It seems likely that the haemochromatosis gene and the HLA genes are tightly linked on the short arm of chromosome 6. Necropsy studies in Sweden have reported a frequency of 1 to 2 per 1000,⁴ almost the same frequency as clinically recognised disease.⁵

α_1 Antitrypsin, a glycoprotein synthesised and secreted by the liver, is a major protease inhibitor (Pi) that participates in many inflammatory processes occurring within the body. The gene for α_1 antitrypsin is a single gene of 12.2 kb, located as a pair of codominant autosomal alleles on chromosome 14 at q31-32.3.⁶ The serum α_1 antitrypsin concentration is controlled by a set of 75 different allelic genes, designated by letters denoting their electrophoretic mobility. The PiZ allele is the most important genetic variant responsible for subnormal plasma α_1 antitrypsin concentrations. In the Swedish population, the frequency of PiZ homozygosity has been reported to be 0.00057, but that of PiZ heterozygosity is 0.047.⁷

Severe α_1 antitrypsin deficiency predisposes to emphysema and chronic liver disease associated with a high incidence of hepatocellular carcinoma.⁸ In adults with this deficiency, isolated case reports have suggested a possible relationship between the disease and genetic haemochromatosis,^{9 10} and more recently a significant correlation between heterozygous (PiZ) α_1 antitrypsin deficiency and genetic haemochromatosis was reported from a series of 15 patients referred to a liver transplantation centre in the USA.¹¹ Other studies, however, have failed to show any relationship between these two inborn errors of metabolism.^{12 13}

This study aimed to ascertain whether a relationship exists between heterozygous or homozygous α_1 antitrypsin deficiency of PiZ type and genetic haemochromatosis in a large series of Swedish patients from a major city population (Stockholm).

Methods

PATIENTS

The series comprised 67 consecutive, unrelated, white Swedish patients with genetic haemochromatosis. There were 49 men and 18

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women, with a mean age of 55 (14) years (range 22–75 years). All were admitted to Karolinska Hospital or Huddinge University Hospital, Stockholm, over the 10 year period between 1984 and 1993. The diagnosis of genetic haemochromatosis was based on the presence of excessive parenchymal iron accumulation without any indication of secondary iron overload. None of the patients had a history of blood transfusions or dietary iron supplementation. Liver biopsy was obtained in 65 (97%) patients and verified parenchymal cell iron overload (grade 2–4), which could not be explained by secondary forms of haemochromatosis, liver disease, or porphyria cutanea tarda. Liver biopsy was not performed in two cases: in a man aged 65 years, a known case of Hodgkin's lymphoma with abnormal liver function and coagulopathy, whose pretreatment serum iron concentration was 35 $\mu\text{mol/l}$, ferritin 2000 $\mu\text{g/l}$, and transferrin saturation 100%, and whose serum alanine aminotransferase (ALT) was returned to normal by subsequent phlebotomy, and in a 61 year old woman found in family studies of a brother with haemochromatosis, and whose serum iron concentration was 38 $\mu\text{mol/l}$, ferritin 250 $\mu\text{g/l}$, and transferrin saturation 79%, who refused to undergo the procedure.

METHODS

α_1 Antitrypsin phenotyping was performed in all patients using a previously described ELISA procedure.¹⁴ Briefly, a hybridoma cell line, ATZ 11, produced by fusion of spleen cells from BALB/c mice immunised with a purified liver PiZ α_1 antitrypsin and Sp 2/0 Ag 14 mouse myeloma cells, was used to produce monoclonal antibodies (IgG1) that interact specifically with PiZ α_1 antitrypsin. These antibodies were used in an ELISA procedure permitting identification of heterozygous (PiZ) or homozygous (PiZZ) gene carriers, but it cannot distinguish between different types of heterozygotes (PiMZ, PiSZ, or Pi-nullZ). As non-Z phenotypes lack absorbance in this test, they all emerge as negative. Homozygosity was confirmed by isoelectric focusing.¹⁵ Plasma α_1 antitrypsin concentrations were measured with an electroimmunoassay using a monospecific antiserum as described elsewhere.¹⁶ Serum iron concentrations were measured by spectrophotometry using guanidine/ferrozine as the reagent.¹⁷ Serum transferrin concentra-

tions were measured by immunoturbidometry and expressed in forms of iron-binding capacity, and the percentage saturation of transferrin was calculated. Serum ferritin concentrations were measured by immunoradiometry according to a standard technique. The iron content of the liver was determined histochemically as previously described.¹⁸ The hepatic iron concentration was measured by atomic absorption spectrophotometry (normal value <1.0 μg iron/mg protein) as described before.¹⁹ HLA typing was performed with the microlymphocytotoxicity technique, as previously described.²⁰ Liver biopsy was performed according to the Menghini technique, using a biopsy needle with a diameter of 1.6 mm.

STATISTICAL METHODS

The odds ratio was calculated and tested with the χ^2 test to determine the statistical significance of differences between proportions in two by two tables. If the requirements of this test were not fulfilled, Fisher's exact test was used. In comparing mean values, Student's *t* test was used when the distribution was normal and the Mann-Whitney U test when it was skewed.

Results

Three (4.5%) of the patients with genetic haemochromatosis were found to be homozygous for the PiZ gene, a proportion more than 80 fold greater than that expected in the general population (1/1700) (odds ratio=82, confidence interval=26, 256; $p<0.0001$). Only three (4.5%) of the patients were found to be PiZ heterozygotes, a figure almost identical to that of 4.7% in the normal Swedish population ($p=0.977$). The clinical characteristics of the PiZ gene carriers and non-carriers are presented in Table I. There were no significant differences between the two groups in mean age at onset of disease, sex distribution, or HLA type. Liver biopsy was performed in 65 (97%) of the patients; two of three (66%) of the PiZZ homozygotes had cirrhosis, compared with six of 59 (10%) of those not carrying the PiZZ phenotype ($p=0.039$). None of the α_1 antitrypsin deficient homozygotes (PiZZ) or heterozygotes (PiZ) had developed hepatocellular carcinoma, compared with two of 59 (3.4%) non-Z gene carriers ($p=1.0$). The α_1 antitrypsin deficient homozygous patients were not alcohol abusers, nor did they manifest evidence of exposure to hepatitis C or hepatitis B virus infection. Three cirrhosis patients of the non-PiZ carrier group were anti-hepatitis C virus positive and one patient was positive for hepatitis B surface antigen. Overconsumption of alcohol (>50 g/day) was documented in three cirrhosis patients of the non-PiZ carrier group. Thus, we found additional risk factors for the development of liver cirrhosis in this group.

Two of the PiZ heterozygotes had plasma α_1 antitrypsin concentrations below the normal

TABLE I Clinical characteristics of 67 genetic haemochromatosis patients grouped according to carriership of PiZ α_1 antitrypsin allele

	Heterozygous PiZ (n=3)	Homozygous PiZZ (n=3)	Non-PiZ gene carriers (n=61)	p Value
Mean (SD) age (y)	56.3 (11.4)	47 (22.9)	55.5 (13.7)	NS
Sex (male/female)	2/1	1/2	46/15	NS
Mean plasma α_1 antitrypsin concentration (g/l)	0.79 (0.10)	0.17 (0.03)	1.39 (0.27)	0.0003*
Cirrhosis	0	2 (66%)	6 (10%)†	0.039‡
Hepatocellular carcinoma	0	0	2 (3%)†	NS

*Difference between the heterozygotes PiZ and non-PiZ gene carriers.
†Calculation based on 59 non-PiZ carriers in whom the liver biopsy was performed.
‡Difference between the homozygotes PiZZ and non-PiZ gene carriers.

TABLE II Clinical and biochemical features of the PiZ gene carriers with haemochromatosis

No	Age/ sex	Signs*	Pi- type	α_1 AT (g/l)	ALT/ AST (μ cat/l)	SI (μ M/l)	TS (%)	SF (μ g/l)	Liver iron	HIC (μ g/mg protein)	Liver histology†	HLA type
1	52/F	ABEH	PiZZ	0.18	1.52/1.37	39	91	4290	3	ND	C	A3 B27
2	67/F	AEP	PiZZ	0.14	0.83/0.70	36	74	391	4	10.4	C	A2,3 B14,40
3	22/M	N	PiZZ	0.20	1.15/0.78	38	81	161	2	5.3	Fa	A3,32 B7,27
4	47/F	A	PiZ	0.90	0.28/0.27	44	90	205	3	8.7	Fa	A3,28 B7,12
5	53/M	N	PiZ	0.70	1.39/1.15	43	80	2196	3	ND	F	A2,3 B7,12 Cw7
6	69/M	AG	PiZ	0.78	0.95/0.77	32	66	48	3	ND	F	A3 B40,22

*A=arthropathy; B=bronchial asthma; E=emphysema; G=hypogonadism; H=hepatomegaly; P=skin pigmentation; N=none.

†C=cirrhosis; F=fibrosis; Fa=fatty changes.

SI=serum iron (normal=12–32); TS=transferrin saturation (normal=62); SF=serum ferritin (normal=11–120 in women and 25–400 in men); HIC=hepatic iron concentration; ND=not done.

range (0.9–1.7 g/l), and the group mean was differed from that of the non-PiZ carriers (0.79 (0.10) *v* 1.39 (0.27); *p*=0.0003). The mean plasma α_1 antitrypsin concentrations in the homozygotes (PiZZ) was 0.17 (0.03). HLA typing was performed in 18 patients, of whom nine (50%) had at least one histocompatibility antigen (HLA A3), and seven (39%) had two antigens (HLA A3, B7, or B14) known to be linked to genetic haemochromatosis. All the PiZ gene carriers were carriers of the HLA type known to be linked to genetic haemochromatosis. The clinical and biochemical data of the homozygous and heterozygous α_1 antitrypsin deficiency patients are shown in Table II.

The first α_1 antitrypsin homozygote (patient no 1) presented to the family doctor with a history of haematemesis. Gastroscopy showed gastritis but no ulcer or oesophageal varices. Abdominal ultrasound showed moderate ascites. Routine laboratory investigations revealed abnormal liver function tests. The patient was referred to the gastroenterology department at Karolinska hospital for further investigation. The second α_1 antitrypsin homozygote (patient no 2) was a known case of lung emphysema referred from the family doctor to the Department of Gastroenterology and Hepatology at Huddinge hospital because of arthralgia, abnormal liver function tests, and high level of serum iron and serum ferritin. The third α_1 antitrypsin homozygote (patient no 3) presented to the family doctor with a history of tiredness. Laboratory investigations showed high levels of serum transaminases and serum iron. He was referred to the gastroenterology department of Karolinska hospital for further investigations, including liver biopsy. In all three PiZ homozygotes the referring family doctor was unaware of the α_1 antitrypsin deficiency state; this was detected by serum protein electrophoresis, included as a routine investigation for all patients with liver diseases. Two of the homozygotes (PiZZ) patients were women and had a liver histology consistent with macronodular cirrhosis, whereas the third and youngest patient had fatty changes. None of the homozygotes had hepatocellular carcinoma, diabetes mellitus, or cardiomyopathy; two of them had severe emphysema ($FEV_1 < 0.5$ l in both cases) one of whom was a non-smoker. One heterozygote (patient no 6) had been treated regularly with phlebotomy before he was referred to the Karolinska Hospital and included in the study.

Discussion

The frequency of homozygous (PiZZ) α_1 antitrypsin deficiency in the Swedish population is well established as 1 in 1700 adults,⁷ a figure that agrees with that of 1 in 1666 (0.06%) found by screening 200 000 newborns.²¹ Based on the prevalence of genetic haemochromatosis in the normal population (that is, 1 in 1000),⁵ the expected frequency of a chance combination of both diseases can be calculated as $1/1666 \times 1/1000$ (or 0.6 of 10^6). It is noteworthy that we found three α_1 antitrypsin homozygotes in 67 consecutive patients with genetic haemochromatosis, a prevalence of 4.5% compared with that of about 0.06% in the general population (*p*<0.0001). To the best of our knowledge, this is the first report of an unequivocal increase in the prevalence of α_1 antitrypsin homozygosity in patients with genetic haemochromatosis, though both Anand *et al*⁹ and Eriksson *et al*¹⁰ suggested that such a relationship might exist by showing the coexistence of the two disorders in one family member in each study.

We have no explanation for this phenomenon. All of our PiZ allele carriers had the expected HLA type usually seen in haemochromatosis patients. Presence of an unknown gene associated in some way to the α_1 antitrypsin gene, known to be located on chromosome 14 and to the haemochromatosis gene on chromosome 6 has been suggested, though further research will be needed to test this. A careful study of the homozygotes showed no evidence of consanguinity, admission bias, or clustering effect.

Hepatocyte iron overload was found in three men with chronic liver disease and severe α_1 antitrypsin deficiency.²² In adults with severe α_1 antitrypsin deficiency, the prognosis of the liver disease is worse in men than women.²³ Crowley *et al* suggested that this might be related to the higher iron load found in men.²⁴ Our data are insufficient to settle this issue, as two of our three PiZZ patients were women.

No relationship was found between heterozygous (PiZ) α_1 antitrypsin and genetic haemochromatosis in this study, the prevalence of α_1 antitrypsin heterozygosity was not significantly higher in our patients with genetic haemochromatosis than in the general population (*p*=0.937). This finding is at variance with previous reports suggesting that a relationship exists between heterozygosity for α_1 antitrypsin and genetic haemochromatosis,^{11 25 26} but is

consistent with the lack of any such relationship found in other studies.^{12,13} We have, at present, no explanation for this lack of association but further studies in larger series may settle this issue.

Both α_1 antitrypsin deficiency and untreated genetic haemochromatosis are known to cause liver cirrhosis and predispose to hepatocellular carcinoma. Hepatocellular carcinoma was present in only two of our patients, both of whom were non-PiZ allele carriers. The pathogenesis of liver disease in homozygous α_1 antitrypsin deficiency may be related to an accumulation of the abnormal PiZZ protein in the rough endoplasmic reticulum, seen histologically as PAS-positive inclusion bodies.²⁷ The gene for the Z α_1 antitrypsin variant contains a mutation altering its amino acid sequence and thus affecting the secretion of α_1 antitrypsin, which is finally trapped in the endoplasmic reticulum as a result of incorrect protein folding²⁸ or secretion.²⁹ Inclusion bodies of the endoplasmic reticulum were also found in hepatocytes of transgenic mice able to synthesise the Z protein, and which eventually developed liver disease.³⁰ The theory that a protease-antiprotease imbalance or a secretion defect, or both, renders the hepatocytes immunogenic, thus triggering an autoimmune cascade leading to the liver damage has also been proposed.³¹ Moreover, the deficiency of circulating or locally secreted α_1 antitrypsin would make the hepatocytes vulnerable to lysosomal proteolytic enzymes leakage. However, the fact that the liver disease is not universal in homozygotes suggests a complex pathogenesis, possibly involving other genetic and environmental factors. In our patients, accumulation of iron, in addition to the presence of the PiZ allele, may have hastened the progression of liver injury and contributed to the earlier onset of cirrhosis.

The mechanism by which iron induces hepatic cirrhosis remains elusive. Several mechanisms have been postulated whereby excess hepatic iron might cause cellular injury with resultant cirrhosis. The key pathogenetic factor may be iron induced peroxidative decomposition of organelle membrane phospholipids, in particular of lysosomes³² and mitochondria,³³ with the consequence of cell degeneration and cell death. It is well established that ionic iron forms highly reactive free radicals, especially from oxygen. These are capable of initiating numerous free radical cascades and oxidations.³⁴ Abnormally high quantities of oxygen free radicals may have contributed to oxidative inactivation of α_1 antitrypsin in the homozygotes, thus promoting development of cirrhosis as well as severe emphysema in the non-smoker. In genetic haemochromatosis, Bassett *et al*³⁵ showed a threshold level of hepatic iron concentration, above which there is an increased risk for the development of liver fibrosis. The threshold corresponds to a level of approximately 19 μg iron/mg protein in our studies. In patient no 2, who was a homozygous PiZZ carrier and had liver cirrhosis, the hepatic iron concentration of 10.4 μg iron/mg protein

lies below this level. Thus, in this case, the coexistence of the two inherited diseases may have speeded up the process of fibrogenesis, since it seems unlikely that either disease alone could fully explain the degree of the cirrhotic changes found in the liver biopsy specimens. This view is also supported by the presence of cirrhosis in two women, one only 52 years old. α_1 Antitrypsin deficiency is predominantly complicated by cirrhosis in elderly men. In a case-control study a significant association between α_1 antitrypsin deficiency and cirrhosis could only be shown in men.⁸ In a Swedish autopsy study of 94 cases, cirrhosis was found in 35 men but in only seven women.³⁶ Similarly, in genetic haemochromatosis, women are affected less and later than men because of a much smaller rate of hepatic iron accumulation.³⁷

In conclusion, we have shown a relation between two distinct genetic disorders, α_1 antitrypsin deficiency and genetic haemochromatosis. Their coexistence may contribute to the earlier development of cirrhosis, though it does not increase the risk of hepatocellular carcinoma. At the present time, there seems to be no evidence of a genetic linkage between the two diseases. Further studies are necessary to explain the putative relationship between these two different metabolic disorders.

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- 1 Brissot P, Deugnier Y. Genetic hemochromatosis. In: McIntyre N, Benhamou J-P, Bircher J, Rizzetto M, Rodes J, eds. *Oxford textbook of clinical hepatology*. Oxford University Press, 1991: 948–58.
- 2 Edwards CQ, Griffen LM, Kushner JP. Southern blood club symposium: An update on selected aspects of hemochromatosis. *Am J Med Sci* 1990; **300**: 245–50.
- 3 Simon M, Fauchet R, Hespel JP, Beaumont C, Brissot P, Hery B, *et al*. Idiopathic hemochromatosis: A study of biochemical expression in 247 heterozygous members of 63 families: Evidence for a single major HLA linked gene. *Gastroenterology* 1980; **78**: 703–8.
- 4 Lindmark B, Eriksson S. Regional differences in the idiopathic hemochromatosis gene frequency in Sweden. *Acta Med Scand* 1985; **218**: 299–304.
- 5 Hallberg L, Björn-Rasmussen E, Jungner I. Prevalence of hereditary hemochromatosis in two Swedish urban areas. *J Intern Med* 1989; **225**: 249–55.
- 6 Brantly M, Nukiwa T, Crystal RG. Molecular basis of alpha-1-antitrypsin deficiency. *Am J Med* 1988; **84** (suppl 6A): 13–31.
- 7 Eriksson S. Studies in alpha 1-antitrypsin deficiency. *Acta Med Scand* 1965; **177** (suppl 432): 38–40.
- 8 Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha 1-antitrypsin deficiency. *N Engl J Med* 1986; **314**: 736–9.
- 9 Anand S, Schade RR, Bendetti C, Kelly R, Rabin BS, Krause J, *et al*. Idiopathic hemochromatosis and alpha 1-antitrypsin deficiency: Coexistence in a family with progressive liver disease in the proband. *Hepatology* 1983; **3**: 714–8.
- 10 Eriksson S, Lindmark B, Hanik L. A Swedish family with alpha 1-antitrypsin deficiency, hemochromatosis, haemoglobinopathy D and early death in liver cirrhosis. *J Hepatol* 1986; **2**: 65–72.
- 11 Rabinovitz M, Gavalier JS, Kelly RH, vanThiel DH. Association between heterozygous alpha 1-antitrypsin deficiency and genetic hemochromatosis. *Hepatology* 1992; **16**: 145–8.
- 12 Eriksson S, Lindmark B, Olsson S. Lack of association between hemochromatosis and alpha-1-antitrypsin deficiency. *Acta Med Scand* 1986; **219**: 291–4.
- 13 Kaserbacher R, Propst T, Propst A, Graziadei I, Judmaier G, Vogel W. Association between heterozygous alpha 1-antitrypsin deficiency and genetic hemochromatosis. *Hepatology* 1993; **18**: 707–8.
- 14 Wallmark A, Alm R, Eriksson S. Monoclonal antibody specific for the mutant PiZ α_1 -antitrypsin and its application in an ELISA procedure for identification of PiZ gene carriers. *Proc Natl Acad Sci USA* 1984; **81**: 5690–3.
- 15 Jeppsson JO, Laurell CB, Fagerhol M. Properties of isolated human α_1 -antitrypsin of Pi type M, S and Z. *Eur J Biochem* 1978; **53**: 143–53.

- 16 Laurell CB. Electroimmuno assay. *Scand J Clin Lab Invest* 1972; 124 (suppl): 21–37.
- 17 Williams HL, Johnson DJ, Haut MJ. Simultaneous spectrophotometry of Fe²⁺ and Cu²⁺ in serum denatured with guanidine hydrochloride. *Clinical Chemistry* 1977; 23: 237–40.
- 18 Scheuer PJ. *Liver biopsy interpretation*. 4th ed. London: Bailliere & Tindall, 1989.
- 19 Hultcrantz R, Glaumann H. Studies on the rat liver after iron overload. Biochemical studies after iron mobilization. *Lab Invest* 1982; 46: 383–92.
- 20 Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A, -B, -C and -D antigen. *Am J Clin Pathol* 1978; 69: 103–20.
- 21 Sveger T. Liver disease in alpha 1-antitrypsin deficiency detected by screening of 200 000 infants. *N Engl J Med* 1976; 294: 1316–21.
- 22 Berg NO, Eriksson S. Liver disease in adults with alpha-1-antitrypsin deficiency. *N Engl J Med* 1972; 287: 1264–7.
- 23 Carlson J, Eriksson S. Chronic 'cryptogenic' liver disease and malignant hepatoma in intermediate alpha 1-antitrypsin deficiency identified by PiZ-specific monoclonal antibody. *Scand J Gastroenterol* 1985; 20: 834–42.
- 24 Crowley JJ, Sharp HL, Freier E, Ishak KG, Schow P. Fatal liver disease associated with α_1 -antitrypsin deficiency PiM₁/Pi_{MD} variant. *Gastroenterology* 1987; 93: 242–4.
- 25 Rigas DA, Finch SC. Electrophoretic studies of serum proteins in hemochromatosis. *Am J Med Sci* 1959; 237: 566–73.
- 26 Janus ED, Carrell RW. α_1 -antitrypsin deficiency and adult liver disease. In: Peeters H, ed. *Proteins and related subjects*. Oxford: Pergamon Press, 1975: 383–5.
- 27 Hultcrantz R, Mengarelli S. Ultrastructural liver pathology in patients with minimal liver disease and α_1 -antitrypsin deficiency: A comparison between heterozygous and homozygous patients. *Hepatology* 1984; 4: 937–45.
- 28 Lebermann H, Tokuoka R, Deisenhofer J, Huber R. Human α_1 -antitrypsin inhibitor: crystal structure analysis of two crystal modifications, molecular model and preliminary analysis of the implications for function. *J Mol Biol* 1984; 177: 531–56.
- 29 McCracken AA, Kruse KB, Brown JL. Molecular basis for defective secretion of the Z variant of human alpha-1-proteinase inhibitor: secretion of variants having altered potential for salt bridge formation between amino acids 290 and 342. *Mol Cell Biol* 1989; 9: 1406–14.
- 30 Carlson JA, Rogers BB, Sifers RN, Finegold MJ, Clift SM, DeMayo FJ, et al. PiZ α_1 -antitrypsin causes liver disease in transgenic mice. *J Clin Invest* 1989; 83: 1183–90.
- 31 Perlmutter DH. The cellular basis for liver injury in α_1 -antitrypsin deficiency. *Hepatology* 1991; 13: 172–85.
- 32 Myers BM, Prendergast FG, Holman R, Kuntz SM, LaRusso NF. Alterations in the structure, physiochemical properties, and pH of hepatocyte lysosomes in experimental iron overload. *J Clin Invest* 1991; 88: 1207–15.
- 33 Bacon BR, Park CH, Brittenham GM, O'Neill R, Tavill AS. Hepatic mitochondrial oxidative metabolism in rats with chronic dietary iron overload. *Hepatology* 1985; 5: 789–97.
- 34 Stremmel W, Riedel HD, Niederau C, Strohmeyer G. Pathogenesis of genetic haemochromatosis. *Eur J Clin Invest* 1993; 23: 321–9.
- 35 Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology* 1986; 6: 24–9.
- 36 Eriksson S. Alpha 1-antitrypsin deficiency and liver cirrhosis in adults. An analysis of 94 Swedish autopsied cases. *Acta Med Scand* 1987; 221: 461–7.
- 37 Summers KM, Halliday JW, Powell LW. Identification of homozygous haemochromatosis subjects by measurement of hepatic iron index. *Hepatology* 1990; 12: 20–5.