Idiopathic Hemochromotosis and Alpha-1-Antitrypsin Deficiency: Coexistence in a Family with Progressive Liver Disease in the Proband

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A patient with coexistent hemochromatosis and alpha-1-antitrypsin deficiency which led to cirrhosis and death despite adequate therapy for hemochromatosis is reported. Evaluation of the family revealed first degree relatives with iron overload and others with alpha-1-antitrypsin deficiency but none with both conditions. The role of family studies in the early recognition and possible prevention of overt clinical disease in individuals with either of these two genetic diseases is discussed.

The term hemochromatosis has been broadened recently to include disorders with a similar clinicopathological picture which result in a progressive increase in total body iron and deposition of iron in hepatic, pancreatic, myocardial, and hypothalamic parenchymal cells (1). The idiopathic variety of hemochromatosis (primary hemochromatosis) is a genetically inherited autosomal recessive disorder linked to the presence of certain histocompatibility antigens (HLA A3; B7 and B14) (2). The incidence of iron overload in first degree relatives of index cases is reported to be 15% (3). The recognition of this association has permitted detection of the abnormality in closely related family members of individuals known to have the disease by demonstrating an HLA haplotype in the individual at risk similar to that of the proband (4).

Secondary hemochromatosis like primary hemochromatosis also occurs in families but is not associated with a common HLA haplotype and is not due solely to excessive iron absorption. Instead, secondary hemochromatosis has been shown to occur in thalassemia major, sideroblastic anemia, alcoholic cirrhosis, and following portocaval shunting and prolonged excessive iron ingestion (1).

Alpha-1-antitrypsin (AAT) is a plasma glycoprotein which functions as a protease inhibitor. The AAT alleles are inherited in an autosomal codominant manner, and the deficiency state is inherited in an autosomal recessive manner with heterozygotes expressing the phenotype when provoked, such as by cigarette smoking (5). AAT deficiency is partially understood with 26 different alleles presently recognized by isoelectric focusing. The most common is Pi MM (homozygous Pi M allele) which results in normal AAT levels. AAT deficiency usually is associated with either Pi ZZ, Pi SZ, or Pi Null haplotypes. The presence of these genotypes results in plasma enzyme deficiency and occasionally liver disease which can progress to cirrhosis (6). In contrast, Pi, MZ, the commonest heterozygous state, is usually not associated with clinical disease. Recently, however, an increased prevalence of the heterozygous state has been reported in patients with cryptogenic cirrhosis and chronic active hepatitis (7). The reported incidence of the genotype Pi ZZ in the general population is 0.13% and that of genotype Pi MZ is 2.74% (8).

Here, we report an individual with both AAT deficiency and primary hemochromatosis who developed progressive liver disease despite adequate therapy for hemochromatosis. In evaluation of the family, relatives with one or the other, but not both, diseases were identified.

CASE HISTORY OF THE PROBAND

P. M. was a 42-year-old white male referred for possible liver transplantation. His medical history began in 1976 when a routine annual physical and laboratory exam showed abnormal enzymes (hypertransaminase-

mia). Additional investigations included a normal oral cholescytogram, normal liver scan, and negative hepatitis B serology. After 6 months of persistently elevated serum enzyme levels, he underwent a percutaneous liver biopsy. The biopsy was interpreted as showing the presence of abundant iron pigment in hepatocytes, mild portal fibrosis but no cirrhosis. Subsequently his serum iron was found to be elevated (283 μg%; n = 42 to 135) with a transferrin saturation of 100% (n = 20 to 40%). A "desferroxamine test" showed an iron excretion of 6.9 mg per 24 hr in the urine (n < 0.15 mg per 24 hr), and a glucose tolerance test documented chemical diabetes mellitus and glycosuria. A diagnosis of hemochromatosis was made, and he was placed on weekly phlebotomy. In January, 1978, after 60 units of blood had been removed, his serum iron was 317 μg with 90% transferrin saturation. Phlebotomy was increased to biweekly and in July, 1978, after removal of approximately 37 mg of stored iron, his serum iron was reduced to 8 μg% and his transferrin saturation was reduced to 2.5%.

He did well with continued monthly phlebotomy and maintained normal to low serum iron and transferrin saturation levels. In October, 1981, he reported increasing fatigue, increasing abdominal girth, and was admitted to the hospital for further diagnostic study. For the first time he had abnormal liver function (bilirubin 4.9 mg per dl, protime 17.5 sec per 11 sec control and an albumin level of 3.2 gm per dl). In addition, he demonstrated elevated serum enzyme levels (SGOT, 115 IU per liter; SGPT, 42 IU per liter; alkaline phosphatase, 134 IU per liter). A diagnosis of cirrhosis presumably due to hemochromatosis with portal hypertension was made on clinical grounds and confirmed on liver biopsy (Figure 1C). His subsequent course was rapidly progressive with numerous episodes of spontaneous bacterial peritonitis, sepsis, variceal bleeding, and development of the hepatorenal syndrome.

He was transferred to the Presbyterian-University Hospital for consideration for a liver transplant. Pertinent additional past history revealed no transfusions, no hepatitis exposure, no alcohol abuse, and no family history of pulmonary or hepatic disease. On physical examination, he was alert and cooperative but obviously ill. He had stigmata of chronic liver disease including spider angiomata, decreased body hair, and asterixis. He was jaundiced and had a large right pleural effusion, ascites, and a caput medussa but no clinically apparent splenomegaly. The liver was firm, 4 cm below the right costal margin in the midclavicular line. He had bilateral testicular atrophy. In addition, he had guaiac positive melanic stool.

Additional investigations documented an AAT level of 36 mg per dl (nl = 85 to 213), a homozygous ZZ phenotype and HLA antigens A1, 25; B8, 18; and Dr 3, 4, suggesting a diagnosis of homozygous AAT deficiency in addition to primary hemochromatosis. The progression of his liver disease to cirrhosis and his clinical deterioration in spite of adequate therapy for hemochromatosis suggested that AAT deficiency was the underlying cause of his progressive liver disease.

He received an orthotopic liver transplant 1 month after admission. Severe early rejection and a stormy postoperative course resulted in death. Autopsy confirmed iron overload in the pancreas, testes, spleen, heart, and pituitary. Pathologic examination of the patient's original liver demonstrated evidence of both iron overload and AAT deficiency in addition to advanced cirrhosis (Figure 1).

FAMILY STUDY

A study of 11 immediate family members was performed including testing for histocompatibility antigens, serum iron, TIBC, transferrin saturation, serum ferritin, and AAT phenotyping and quantitation.

MATERIALS AND METHODS

HLA typing was performed using the modified cytotoxicity method of Amos (9). Serum iron was measured after incubation with ascorbic and hydrochloric acid to liberate protein bound iron (10). Total iron binding capacity was measured by saturation of the serum with ferric iron followed by removal of excess iron with magnesium carbonate and then determining the amount of iron bound to protein (11). Serum ferritin was measured utilizing the immunoradiometric assay initially described by Addison et al. (12). AAT phenotyping was accomplished by isoelectric focusing on polyacrylamide gel using established methods and known standards (13).

The surgical and autopsy specimens were fixed in 10% formalin. Sections from the hepatectomy specimen were stained with hematoxylin and eosin, Prussian blue reagent for iron, periodic acid-Schiff reagent with and without diastase treatment, orcein method for hepatitis B antigen, and Hall's method for bilirubin. Paraffin blocks from the referring hospital were obtained, and the above special stains were performed on these specimens. Representative formalin-fixed, paraffin-embedded sections were immuno-stained using the peroxidase antiperoxidase technique (14). Specific antiserum against AAT was employed. All antibodies used were commercially available from ADKO (Accurate Chemical and Scientific Co., Hicksville, NY). Substitution of the primary antibody by saline solution and liver tissue from known cases of AAT deficiency were simultaneously used as controls.

RESULTS

The results of the family study are shown in Figure 2. AAT is seen clearly to be inherited as an autosomal codominant disease with the quantitative level of this plasma protein being dependent on the number of Z alleles present in an individual. The proband (Paul) married a heterozygote (Carol) and had two offspring, both carrying the ZZ phenotype. Both these offspring, although asymptomatic, have low AAT levels.

The presence of significant iron overload was detected in only one family member (Stephen) other than the proband. The well-documented HLA markers for hemochromatosis (HLA A3; B14) were not seen in this family. In contrast, the HLA B7 antigen was present in five family members. The two members with documented iron overload shared two common HLA alleles (HLA A1 and B8). Several other family members, Thomas, Daniel, and Stephen, all in the third generation in Figure 2, also have these alleles. It should be noted, however, that
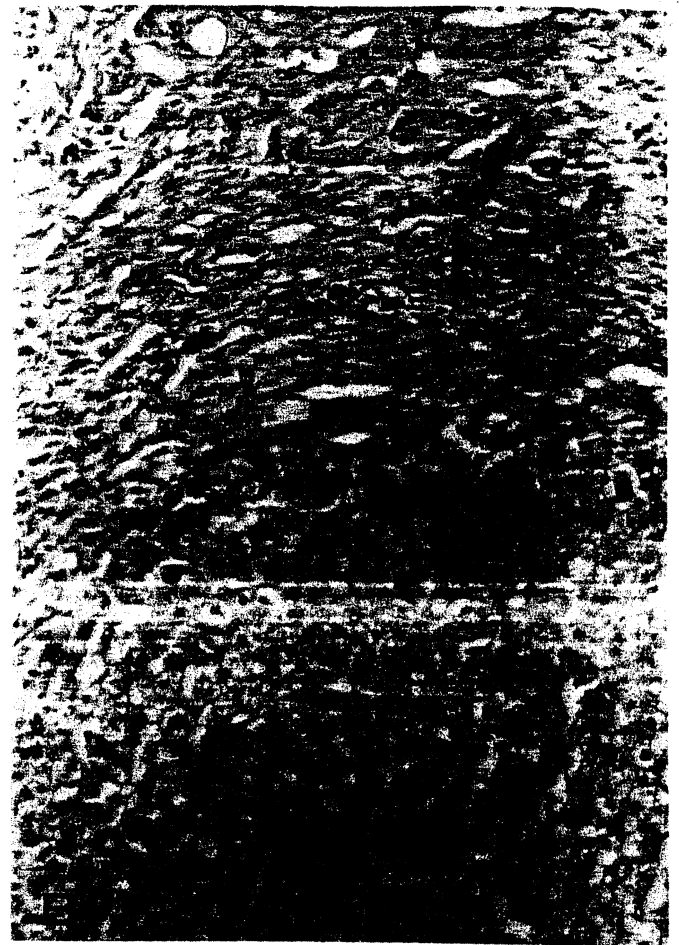
FIG. 1. (A) Photomicrograph of original liver biopsy on which the diagnoses of hemachromatosis and cirrhosis were made. Note iron (black pigment) in hepatocytes and bile duct. Prussian blue, × 193. (B) Photomicrograph of liver removed at time of liver transplant which demonstrated only minimal iron (*arrows*) in bile ductules and hepatocytes. Prussian blue, × 193. (C) Photomicrograph of liver removed at time of liver transplant which demonstrated positive immunoperoxidase staining (black pigment) for AAT in hepatocytes. Note most densely stained hepatocytes are at the periportal margin. × 386.
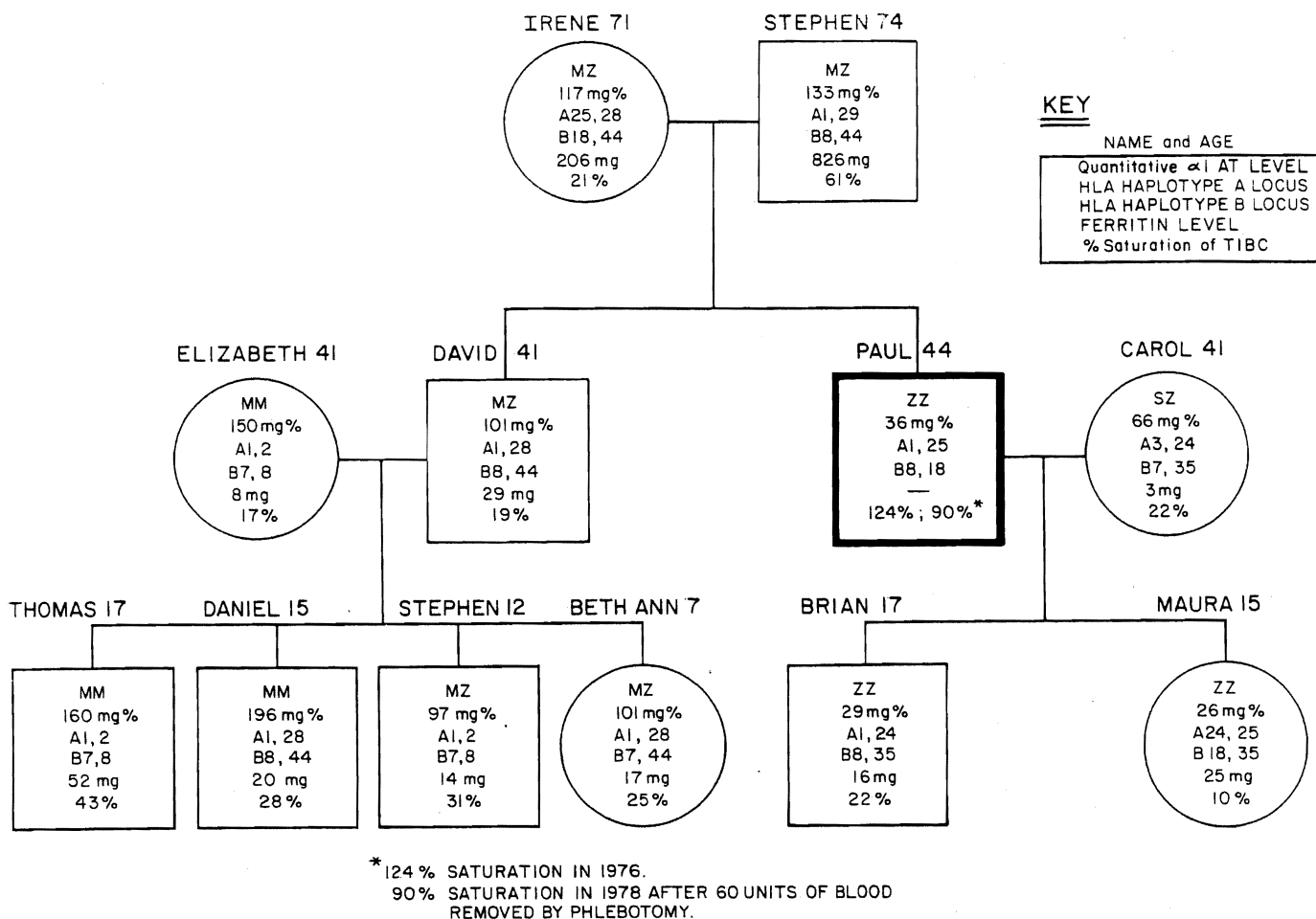
FIG. 2. Family tree of proband (marked by *dark square in second row*) and his family. Results of special studies performed on family members are presented for each member as shown by the key in the *right upper corner* of the figure.

Daniel inherited the *A*1 *B*8 haptotypes from his mother (Elizabeth), not his father (David). Three of these children also possess the HLA *B*7 antigen inherited from their mother and are therefore possibly at risk for iron overloading (presently negative).

DISCUSSION

To our knowledge, there are no reported cases in the English literature of (AAT) deficiency and idiopathic hemochromatosis in the same patient and in the same family. The prevalence of the homozygous AAT deficiency state (Pi ZZ) is reported to be 0.13% and that of the heterozygous deficiency state (Pi MZ) to be 2.74% as determined in a group of patients evaluated for lung disease (8). As might be expected, autopsy series report a somewhat higher figure for the prevalence (6.3%) of the Pi Z allele (15).

In general the expression of AAT deficiency is inherited as an autosomal codominant trait as seen in the present family (5). Both parents of our proband were heterozygotic for the Z allele. Interestingly, this combination has been reported to be associated with a higher incidence of offspring who are homozygotic for the Z allele than would be expected otherwise by simple Mendelian genetics (16).

Unfortunately, our proband married a heterozygote (Pi SZ) and had two homozygotic (Pi ZZ) offspring

(Figure 2). Again, following a classical pattern of codominant genetic expression, quantitative AAT levels in the homozygotes (Pi ZZ) were about one-quarter those seen in normals (Pi MM). The homozygotes are at definite risk for developing clinical pulmonary and/or hepatic disease, and heterozygotes are at a somewhat smaller risk of developing clinical disease, especially if other cofactors are present (17).

Early diagnosis of such a condition in a specific individual can be quite difficult and is made considerably easier when based upon data obtained from a family study. Once detected, immediate careful testing for occult disease in family members is mandatory, and the initiation of preventive measures to either reduce the morbidity or prevent the expression of disease can be instituted. For example, abstinence from smoking has been established to be clearly useful (17) and abstinence from alcohol might be prudent, although not established in such individuals. Further, genetic counseling is important, especially pertaining to the likelihood of bearing children with AAT deficiency (18, 19).

The proband in our study had good evidence for the diagnosis of idiopathic hemochromatosis documented by the extreme amounts of parenchymal iron demonstrated by both pathologic (4+ staining with Prussian blue) and biochemical data (approximately 37 gm of iron removed by phlebotomy without development of anemia). The

fact that the proband had continued progression of liver disease in spite of active phlebotomy therapy suggested that a second condition might be present. Based upon such reasoning, other causes for progressive liver disease were investigated and AAT deficiency was discovered.

The HLA alleles (A3, B7, and B) classically linked with hemochromatosis (2) were not found in this family except in the wife of the proband (Carol) (Figure 2). In this regard, it has been shown previously that the presence of hemochromatosis in family members of a proband is not linked solely to the presence of these specific HLA alleles, but is linked to the possession of a similar HLA haplotype as is present in the proband (3, 20, 21). In the present family, six members share the same HLA haplotype (A1, B8) as the proband (Figure 2) and might therefore be hemochromatosis heterozygotes. One (Stephen in the first generation) is clearly iron overloaded. Four others (all in the third generation) are not presently iron overloaded but are too young to conclusively exclude the disease (3). One adult member in the second generation (David), although possessing the same (A1, B8) haplotype as the proband, has no evidence for iron overloading and presumably is a heterozygote. It should be noted, however, that five family members have the HLA B7 (all in the second and third generation, Figure 2) and that one (Carol) has both the A3 and B7 antigens and may also be heterozygous for hemochromatosis. It must be remembered, however, that both haplotypes are not uncommon in the population at large (28.4M and 25.8M, respectively) and each may occur in the absence of the hemochromatosis antigen.

Liver transplantation has been shown to correct the metabolic defect in AAT deficiency, and as in our case, results in the conversion of the patients' phenotype from ZZ to MM after successful transplantation (22).

In conclusion, we have presented a unique association of two distinct genetic disorders coexisting in the same individual and occurring in other members of his family. At this time, there seems to be no evidence to believe or suspect that the two diseases are genetically linked. Apparently, their coexistence in the proband has to be attributed to a rare chance event.

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