

'Duarte variant with clinical signs' has alpha₁-antitrypsin genotype ZZ

L. R. WEITKAMP, J. W. SAYRE, R. H. SCHWARTZ, R. DOHERTY, and S. A. KHERA

From the Departments of Psychiatry, Pediatrics, and Division of Genetics of The University of Rochester School of Medicine and Dentistry, Rochester, New York, and the Corning Hospital, Corning, New York, U.S.A.

Summary. A patient with neonatal jaundice and cirrhosis who was previously reported homozygous for the Duarte variant of galactose-1-phosphate uridyl transferase has the ZZ genotype for alpha₁-antitrypsin. A sister of the patient, also with ZZ genotype, is less severely affected with liver disease and is a heterozygote for the Duarte variant. Since a number of patients with ZZ genotype of alpha₁-antitrypsin have been previously reported to have liver disease, the latter genotype is the more probable explanation for the patients' clinical state. A question is raised, however, whether the Duarte variant may be specifically associated with the development of liver disease in ZZ individuals.

Kelly, Desjardins, and Khera (1972) have associated homozygosity for the Duarte variant of galactose-1-phosphate uridyl transferase with neonatal jaundice and cirrhosis in one patient. Here we report that this patient is also homozygous for the Z allele of alpha₁-antitrypsin and suggest that either the antitrypsin genotype alone or the co-occurrence of the antitrypsin ZZ genotype with the Duarte variant in the patient and his sister may be associated with the development of liver disease in the two sibs.

Case report

The clinical history of the propositus for the first 9 months of life, obtained by one of us (J. W. Sayre), has been presented previously (Kelly *et al*, 1972). At 3 to 4 months of age serum aldolase was 504, 790, and 580 IU (normal range 171-473 IU); serum alanine aminotransferase (ALT) was 125, 149, and 154 IU (normal range 0-50 IU); and serum alkaline phosphatase was 197, 259, and 182 IU (normal range 30-85 IU). Three months later aldolase (440 IU) was within normal limits, but ALT (193 IU) and alkaline phosphatase (175 IU) were still raised.

At 15 months of age the patient was seen again. Interim growth and development were normal. Icterus had not recurred; the stools had been of normal colour. The liver, however, was enlarged to 7 cm below the right costal margin and had a firm consistency. Three months

later the patient was readmitted for a galactose tolerance test. After an intravenous dose of galactose (1.00 g/kg) blood galactose concentrations were determined during a 90-minute interval by the galactose oxidase method (Galactostat, Worthington). The blood galactose half-time (t_{1/2}), calculated by least squares linear regression, was 21.5 min (95% confidence interval=17.6 to 27.4 min). Before and after this test, serum aldolase remained normal (470 and 450 IU) and ALT remained high (118 and 113 IU; normal range 10-30 IU). Slit-lamp ophthalmological examination revealed no abnormalities. The electrophoretic pattern of galactose-1-phosphate uridyl transferase, determined without knowledge of the results of Kelly *et al* (1972), indicated that the patient was homozygous for the Duarte variant of galactose-1-phosphate uridyl transferase and that his sister and parents were heterozygotes.

Two years later the blood samples which had been previously obtained from the family and stored were included in a screening of samples from diverse sources for alpha₁-antitrypsin variants. On crossed antigen-antibody electrophoresis the patient and his sister were homozygotes for the 'Z' variant. Both parents were MZ heterozygotes.

Re-examination of fresh blood samples from family members confirmed the alpha₁-antitrypsin phenotypes. The patient, now age 4, has had normal weight gain and development. There has been no further jaundice or history of chronic or recurrent respiratory infection. Physical examination was normal except that the liver was firm and palpable 4 cm below the right costal margin. The sister has had no recurrent respiratory infections, asthma, or symptoms of liver disease. On examination

Received 12 June 1975.

at age 6 her liver was 1 cm below the right costal margin and of a soft consistency. Both children had normal chest x-rays at 4 years and 6 years of age, respectively. However, the patient and his sister did have a rise in some serum enzyme levels (see Table).

Methods

Vertical starch gel electrophoresis of galactose-1-phosphate uridyl transferase was run for 7 hours at 8 volts per cm, using the histidine-citrate buffer system of Fildes and Harris (1966). The gel was sliced, and cellulose acetate strips soaked in 4 ml 'developing solution' were overlaid on the freshly exposed surface. The developing solution contained 30 mg gal-1-P, 12 mg UDPG, 8 mg $MgCl_2$, 40 units G6PD, 60 units PGM, and approximately 1 mg each of MTT tetrazolium and phenazine methosulfate in 0.1M tris-HCl at pH 8.0. Multiple bands of enzyme activity appear after 30 to 60 minutes' incubation at 37° C (cf. Weitkamp, 1973). Under these conditions the usual homozygous type of galactose-1-phosphate uridyl transferase appears as two equally intense bands. Heterozygotes have one or two bands which migrate anodal to the usual bands and can often be subclassified as having either a weak anodally migrating band relative to the usual band (Duarte heterozygote) or a more intense anodally migrating band relative to the usual band (Los Angeles heterozygote).

Alpha₁-antitrypsin phenotyping was performed using a modification of Laurell's (1965) antigen-antibody crossed electrophoresis in which the initial electrophoresis is done in an acid starch gel (Fagerhol, 1968).

Results

The results are shown in the Table. The patient and his sister are homozygous for the Z allele of alpha₁-antitrypsin; both parents are heterozygotes. On electrophoresis the sister and both parents were classified as having the Duarte variant (i.e. an anodally migrating variant band with less activity than the usual band in the same individual). Previously Kelly *et al* have established through quantitative assay as well as electrophoresis that the variant of galactose-1-phosphate uridyl transferase inherited through both parents is the Duarte type.

Serum activity of alkaline phosphatase, lactic dehydrogenase (LD), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and aldolase are in the normal range for both parents, but indicate liver disease in the patient and his sister as well. The slight prolongation in the t_{1/2} for the galactose tolerance test in the patient is consistent with cirrhosis (Colcher, Patek, and Kendall, 1946; Vink and Kroes, 1959; Tengström, 1966). The history of neonatal jaundice, persistent hepatomegaly, and evidence of cirrhosis in the patient (see also Kelly *et al*, 1972) indicates that he has had more severe liver injury than his sister.

Discussion

The Duarte variant of galactose-1-phosphate uridyl transferase has about one half of the activity of the more commonly encountered type of this enzyme (Beutler *et al*, 1966). Thus heterozygotes have three-quarters and homozygotes half of the usual level of red cell transferase activity. Individuals homozygous for the Duarte variant have a frequency in the Caucasian population of 1 in 200 to 1 in 400 (Beutler, 1973). Despite the high prevalence of such homozygotes, cirrhosis has not been associated with this genotype except in the patient, as previously reported by Kelly *et al* (1972). Heterozygotes for the galactosaemia gene, who also have 50% of the normal level of transferase activity, do not show signs of galactosaemia. Further, Beutler *et al* (1966) found no clinical evidence of abnormality in an individual heterozygous for the Duarte variant and the galactosaemia gene (with a quarter of the normal transferase activity). Several such individuals have since been observed, with no apparent clinical manifestations (E. Beutler (1975), personal communication). Two rare electrophoretic variants of galactose-1-phosphate uridyl transferase have been associated with galactosaemia (Schapira and Kaplan, 1969; Chacko, Christian, and Nadler, 1971). However, in the first instance the enzyme activity in the

TABLE
ALPHA₁-ANTITRYPSIN AND GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE PHENOTYPES AND VARIOUS SERUM ENZYME ACTIVITIES IN PROPOSITUS AND HIS FAMILY

Individual	Age	α_1 -antitrypsin genotype	Gal-1-P uridyl transferase genotype	Serum enzyme activity				Aldolase
				Alk. phos.	LD	AST	ALT	
Patient	4	ZZ	DD	165	240	72	54	310
Sister	6	ZZ	ND	180	210	75	46	250
Father	26	MZ	ND	37	127	26	27	210
Mother	26	MZ	ND	28	165	17	17	230

Normal values are: alkaline phosphatase 51-153 IU (children) and 34-85 (adult), LD 100-225 IU, AST 7-40 IU, ALT 5-30 IU, and aldolase 171-473 IU.

two patients was 1/14 of normal and in the second the variant transferase manifested storage instability. In each instance the variant enzyme migrated more slowly than normal, whereas the Duarte variant migrated more rapidly than normal on alkaline electrophoresis. We conclude that it is unlikely that homozygosity for the Duarte variant is the sole or primary cause of liver disease in the patient.

Sharp *et al* (1969) first noted that juvenile cirrhosis was associated with near absence of serum alpha₁-antitrypsin in 7 propositi from 6 kindreds, though cirrhosis was not present in the 2 related individuals who also had very low serum antitrypsin levels. Further study of the families of a total of 13 unrelated probands revealed that the deficiency was a result of homozygosity for the Z allele of alpha₁-antitrypsin and that most homozygous sibs were showing evidence of cirrhosis (Sharp, 1971). Several reports of association of the ZZ genotype with liver disease in children (*inter alia* Johnson and Alper, 1970; Aagaens *et al*, 1972; Ostergaard, 1973; Leung, Gilly, and Valancogne, 1973; Glasgow *et al*, 1973; Talamo and Feingold, 1973) and adults (Berg and Eriksson, 1972; Campa *et al*, 1973; Cohen *et al*, 1973) have followed. Sibs with the ZZ genotype are often, but not always, affected with clinical disease. The history and clinical and laboratory evidence of liver disease in the patient and his sister are consistent, thus far, with the fairly benign course observed by Talamo and Feingold (1973) in two ZZ sibs.

Most individuals with the ZZ alpha₁-antitrypsin genotype do not develop liver disease. Alper and Johnson (1970) have pointed out that the frequency of neonatal hepatitis is of the order of 1 in 25 000 live births whereas the ZZ genotype has an incidence of 1 in 2500. If the incidence of liver disease is indeed higher among ZZ sibs of probands with liver disease than among unrelated ZZ individuals (Talamo, 1971), then some other familial genetic or environmental factor may also be important in the causation of the liver injury. We expect to report elsewhere on the question of whether the Duarte gene for galactose-1-phosphate uridyl transferase has a high incidence among those individuals with the ZZ genotype for alpha₁-antitrypsin who develop liver disease.

We thank Ms. E. Johnston and J. Van Ess for technical assistance. This research was supported in part by Research Career Development Award 5-K04-HD-50248 (L.R.W.), Grant 1-RO1-GM19962 and Contracts N01-H0-1-2221 and N01-HB-1-2404.

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