Hereditary fructose intolerance and α_1 antitrypsin deficiency

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Abstract

A patient with coexisting hereditary fructose intolerance (HFI) and α_1 antitrypsin deficiency (α_1 ATD) is described. Protease inhibitor typing was not conclusive, presumably because of impaired N-glycosylation secondary to HFI. The case underlines the diagnostic role of molecular genetic techniques in inborn errors of metabolism.

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Both α_1 antitrypsin deficiency (α_1 ATD) and hereditary fructose intolerance (HFI) are important differential diagnoses in infants with cholestatic liver disease and failure to thrive. Because of their extremely rare occurrence, the potential problems in diagnosis resulting from an overlap of clinical symptoms, and the fact that the conventional diagnostic variable of one disease, electrophoretic mobility of the protease inhibitor (PI), is affected by the presence of a second disorder, we describe a patient in whom coexistence of α_1 ATD and HFI was ultimately confirmed by molecular genetic methods.

Case report

A term male neonate (2840 g, 49 cm) presented at age 4 weeks with vomiting, pale loose stools, and failure to thrive (3150 g) while being fed an infant formula (PreHumana, Humana, Herford, Germany) containing lactose as the only carbohydrate. His liver was slightly enlarged and he had cholestasis with a total and conjugated plasma bilirubin of 78.7 and 49.6 $\mu\text{mol/l}$ respectively. As α_1 AT in plasma was decreased to values between 22 and 70 mg/dl, α_1 ATD was suspected and the patient was changed to a medium chain triglyceride-containing formula (Portagen; Mead Johnson, Dietzenbach, Germany; 2.0 g sucrose/100 ml). As stool consistency and vomiting worsened, an acute enteric infection was assumed, and the formula was again changed to a carbohydrate and fat reduced brand (Humana Heilnahrung; 0.6 g fructose and 0.8 g sucrose/100 ml). While the child was on a third, fructose-free, infant formula (Aptamil; Milupa, Friedrichsdorf, Germany), vomiting subsided temporarily, but when small amounts of vegetable were introduced into the diet at age 3 months, vomiting exacerbated and the patient was referred to us.

On physical examination, he was observed to have severe dystrophy (3700 g, 53 cm). Both liver and spleen were enlarged (6 cm and 2 cm

respectively). Aspartate aminotransferase (80 U/ml (normal range 7-28)), alanine aminotransferase (61 U/ml (normal range 6-31)), and γ -glutamyltransferase (206 U/ml (normal range 4-91)) were increased, total protein was decreased (44.0 g/l (normal range 45.7-73.3)), blood glucose levels were slightly decreased (minimum 2.7 mmol/l (normal range 3.3-5.5)), and prothrombin time was in the low normal range. Plasma α_1 AT was again low (87 mg/dl (normal range 152-329)), and both parent's α_1 AT levels were in the low normal range (178 and 156 mg/dl). On isoelectric focusing, the patient's PI type was equivocal, with one broad band between the S and Z position, not allowing a definitive classification. Both parents unequivocally had the PI type MZ. Histology of the liver disclosed mild periportal fibrosis, macrovesicular steatosis, and mild pseudoacinar arrangement of hepatocytes in addition to small amounts of diastase resistant, periodic acid Schiff positive material within the hepatocytes.

From the patient's history, laboratory results, and liver histology, an additional diagnosis, HFI, was suspected. An intravenous fructose tolerance test (200 mg/kg) resulted in hypoglycaemia (0.83 mmol/l) and a decreased plasma phosphate level (1.17 mmol/l (normal range 1.38-2.05)). Fructose-1,6-bisphosphate aldolase activity in liver was 0.43 $\mu\text{mol/min/g}$ (normal range 1.0-12.7) and fructose-1-phosphate aldolase activity was not detectable (normal range 1.0-5.6), resulting in a ratio of "infinity" (normal value < 2.5).

In order to exclude a secondary decrease in fructaldolases, enzyme determinations in liver and jejunal biopsy specimens were repeated at age 16 months when the patient had completely recovered clinically on a fructose restricted diet. Fructose-1,6-bisphosphate aldolase activity in liver was now 0.45 $\mu\text{mol/min/g}$, and fructose-1-phosphate aldolase activity was 0.12 $\mu\text{mol/min/g}$, resulting in a ratio of 3.75 (< 2.5). Fructose-1,6-bisphosphate aldolase in small intestine was 2.0 $\mu\text{mol/min/g}$ (normal range 0.7-5.8), and fructose-1-phosphate aldolase was not detectable (normal range 0.7-2.6), resulting in a ratio of infinity.

Recently, at the patient's age of 14 years, we were able to confirm both diagnoses using molecular genetic methods (fig 1): the patient was found to be homozygous for both the common Ala150Pro mutation of HFI and the Glu342Lys mutation of α_1 ATD(ZZ). His asymptomatic brother was homozygous for α_1 ATD(ZZ) both by isoelectric focusing and molecular genetic testing.

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Figure 1 Analysis of polymerase chain reaction (PCR) amplified DNA segments by polyacrylamide gel electrophoresis. The mutation *Ala150Pro* was detected by amplification of a DNA segment containing exon 5 of the *ALDB* gene and subsequent restriction enzyme digestion with *BsaHI*, an isoschizomer of *AhaII*.¹ The protease inhibitor (PI) mutation *Glu342Lys* was detected by amplification of a DNA segment containing exon 5 by a mismatch primer pair and subsequent restriction enzyme digestion with *TaqI*.² Bold black arrows indicate homozygosity in the patient and his brother. Note that the PI mutation results in the loss of a restriction site, and the presence of the *ALDB* mutation creates a new restriction site. *Bpl*, Base pair ladder.

Discussion

About 4% of the northern European population are carriers of, and consequently about 1 in 2500 is homozygous for, α_1 ATD(ZZ).³ For HFI, it has been estimated that up to 1 person in 20 000 is born with this metabolic disorder.⁴ Thus, on the basis of the known location of the genes involved on two different chromosomes, coexistence of the two diseases in a patient has to be expected in about 1 in 40 million. With this rare occurrence, a false positive diagnosis of one of the two diseases had to be considered in this case. An incorrect diagnosis of HFI seemed unlikely, as determination of enzyme activity in the liver was based on ratios, as usually performed with fructose 1,6-bisphosphate

and fructose 1-phosphate as substrate, which should eliminate decreased activities secondary to parenchymal injury. Furthermore, enzyme activity in small intestine, a tissue not affected by α_1 ATD, was investigated, and this confirmed the diagnosis of HFI. However, hypoglycosylation of proteins is known in patients with HFI. Hypoglycosylation is normally identified by a mobility shift of serum sialotransferrins on isoelectric focusing, but it also affects other glycoproteins. In retrospect, we interpret the potentially misleading result of PI typing as a consequence of coexisting HFI. It has only recently been reported that fructose 1-phosphate, which accumulates in HFI, is an inhibitor of phosphomannose isomerase, the first enzyme of the N-glycosylation pathway, thus explaining N-glycosylation disturbances in HFI.⁵

In our case, coexistence of HFI and α_1 ATD was finally confirmed by molecular genetic techniques. Thus molecular genetic diagnosis offers the advantage of not being influenced by secondary effects of coexisting diseases, and also circumvents invasive approaches—for example, liver biopsy—often necessary for conventional diagnostic methods.

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