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A newborn with severe liver failure, cardiomyopathy and transaldolase deficiency

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Summary: This paper describes the second patient found to be affected with a deficiency of transaldolase (TALDO1; EC 2.2.1.2). Clinically, this patient presented in the neonatal period with several signs of severe liver failure: severe coagulopathy, low serum protein, elevated blood ammonia, and hypoglycaemia. She had generalized oedema, moderate muscular hypotonia, and dysmorphic signs. Liver size was decreased, and the spleen was moderately enlarged. There was severe cardiomegaly. The clinical course was characterized by intractable liver failure and progressive myocardial hypertrophy. The child died at the age of 18 days from respiratory failure. In urine, elevations of erythritol, arabitol and ribitol were found, suggesting a deficiency of transaldolase. Enzyme studies in cultured fibroblasts showed undetectable transaldolase activity. DNA sequence analysis of the TALDO1 gene showed a homozygous missense mutation (575G>A), resulting in an amino acid alteration at position 192 (arginine to histidine, R192H). This amino acid is part of the catalytic site of the transaldolase protein. Discovery of this second patient affected with transaldolase deficiency and liver failure suggests that this disorder has a heterogenous clinical presentation with highly variable severity.

Transaldolase (EC 2.2.1.2) is the second enzyme of the nonoxidative part of the pentose phosphate pathway. Together with transketolase, it creates a reversible link between the oxidative branch of the pentose phosphate pathway and glycolysis. Transaldolase transfers a three-carbon unit by converting glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate into erythrose 4-phosphate and fructose 6-phosphate.

In 2001, we described a patient affected with a genetic deficiency of transaldolase (Verhoeven et al 2001) (McKusick 606003). The patient, a girl who is now 13 years of age, presented in the neonatal period with low birth weight, aortic coarctation, mild bleeding problems and enlarged clitoris. Within several months, this girl developed hepatosplenomegaly. At the age of 2 years, a liver biopsy showed micronodular liver cirrhosis with no specific characteristics. At the age of 10 years, she had thrombocytopenia, mildly increased coagulation times and intermittently elevated ammonia. Psychomotor development has always been normal.

In the neonatal period, gas chromatographic analysis of sugars and polyols in urine showed elevated concentrations of ribitol, D-arabitol and erythritol. Repeated analysis at the age of 3 and 10 years revealed the same profile of abnormalities. In plasma and CSF, arabitol and ribitol were also elevated, though to a lesser extent. The metabolite profile suggested a defect in the pentose phosphate pathway at the level of transaldolase. An enzyme assay performed in erythrocytes and lymphoblasts showed absence of transaldolase activity. The defect was proved by mutation studies of the TALDO gene, showing a homozygous deletion of three base pairs. The father of the patient was heterozygous for the mutation.

These findings prompted us to investigate urinary sugars and polyols in patients with liver problems of unknown origin. This resulted in the diagnosis of the second patient with a deficiency of transaldolase. This patient presented with a more severe clinical course.

METHODS

Analysis of monosaccharides and polyols in urine and plasma was performed as described before (Jansen et al 1986). Plasma samples (0.5 ml) were deproteinized using methanol. A minimum of 1 ml of urine was used. After evaporation to dryness, samples were derivatized to their trimethyl derivatives using a mixture of *N*-trimethylsilylimidazole, *N,O*-bis(trimethylsilyl)acetamide and trimethylchlorosilane (3:3:2 v/v) (Tri-sil TBT, Pierce Chemicals, Rockford, USA) for 30 min at 100°C. The sugar and polyol derivatives were extracted into hexane, after which the organic layer was washed with a 0.1 mol/L HCl solution. The samples were analysed by gas chromatography (CP SIL 5 CB column, 25 m × 0.25 mm ID) (Chrompack, The Netherlands) and detected by flame ionization detection.

The transaldolase enzyme assay and DNA studies were performed as described earlier (Verhoeven et al 2001). The enzyme assay was performed in fibroblasts, instead of lymphoblasts, without changing the experimental procedure. However, results obtained in these cell types are comparable.

CASE HISTORY

The patient was the first of dizygotic female twins delivered by emergency Caesarean section at 36 weeks' gestation because of HELLP syndrome (haemolysis, elevated liver enzymes, low platelets) of the mother. The parents were of Turkish origin and full cousins. A 2-year-old sister had one dysplastic kidney but was otherwise

healthy. The second twin was perfectly healthy. Birth weight was 2550 g (50th centile for dichorionic twins); her head circumference was normal. She displayed generalized oedema, moderate hypotonia, and discrete dysmorphic signs with down-slanting palpebral fissures, low-set ears and increased intermamillary distance. There was a striking bleeding tendency and severe coagulopathy unreactive to intravenous vitamin K (Table 1). Additional signs of liver failure included low serum protein, elevated ammonia and low glucose. Transaminases were remarkably low (Table 1). Because of a rapidly increasing unconjugated hyperbilirubinemia in the presence of anaemia and elevated lactate dehydrogenase (Table 1), haemolysis was considered. The mother had blood group A positive, the infant B positive. However, the Coombs test was negative, and haemoglobin electrophoresis was normal. Fresh frozen plasma and packed red cells were transfused, and diuretic therapy was started. The infant required a glucose intake of 72 μmol glucose/kg per min to keep the blood glucose level above 2.8 mmol/L, which can be considered normal for a neonate with severe liver failure. Ultrasound of the abdomen demonstrated a decreased liver size with inhomogenous echogeneity of the parenchyma, and a normal gallbladder. On ultrasound Doppler examination the hepatic blood vessels appeared normal, except for a persistent large venous duct. The spleen was moderately enlarged. There were no collaterals. The kidneys appeared normal. On magnetic resonance tomography of the abdomen and heart, there was no evidence of iron storage. Ultrasound studies of the brain and quadriceps muscle were unremarkable. A cardiac ultrasound demonstrated a severe cardiomegaly with marked biventricular myocardial thickening and mild regurgitation at most valves. There were no structural defects. Shortening fraction was normal. There was no pulmonary hypertension. Renal function tests revealed a glomerular proteinuria. Cerebrospinal fluid (CSF) cell content and glucose concentration were normal. An ophthalmological examination showed no abnormalities. Karyotype analysis demonstrated XX/XO mosaicism.

A diagnostic work-up for neonatal liver failure was performed. No evidence was found for a bacterial infection, syphilis, viral infections including hepatitis A, B and C, herpesviruses, parvovirus B19, adenovirus and toxoplasmosis. Enterovirus RNA polymerase chain reactions were performed on pharyngeal aspirate, stool, serum and CSF, all of which were found negative. In addition, tests for Coxsackie A9 and B and Echo 9 were negative. No evidence was found for neonatal lupus, α_1-antitrypsin deficiency, neonatal haemochromatosis, galactosaemia, tyrosinaemia type I, disorders of bile acid metabolism, disorders of carnitine metabolism, peroxisomal disorders, defects in organic and amino acid metabolism, urea cycle defects, and glycogen storage disorders (types Ia, III, IV, VIa). Fatty acid oxidation defects were excluded (total and free carnitine in plasma were within reference ranges (total 18 μmol/L, reference range 17–41 μmol/L, free carnitine 12 μmol/L, reference range 10–21 μmol/L; the acylcarnitine profile in plasma was normal and *in vitro* probing of fibroblasts of the patient (Dr C. R. Roe and D. S. Roe, Dallas, Texas, USA) showed no abnormalities). The urinary bile acids profile showed no abnormalities. A bone marrow aspirate showed no evidence of storage disease, malignancy, or haemophagocytic lymphohistiocytosis. In plasma, elevated concentrations of glycine, glutamine and methionine were observed. Urinary amino acids

Table1 Clinical chemistry parameters in the transaldolase-deficient patient as determined directly *post partum*, and at days 3 and 17 of life

	Post partum		*Day 3*		*Day 17*	
Parameter	*Patient*	*Reference*	*Patient*	*Reference*	*Patient*	*Reference*
White cells (10^9/L)	17	8–30	8	9–34	4	5–20
Hb (mmol/L)	6.3	9.0–13.7	7.6	8.4–12.5	6.1	7.8–12.5
Platelets (10^9/L)	75	150–400	87	150–400	58	150–400
Reticulocytes (%)	4.2	1.5–6	5.5	1–5	2.8	0.3–1.3
Prothrombin time (%)	<5	65–100	17	65–100	25	65–100
Fibrinogen (g/L)	<0.65	1.67–3.99	0.54	1.62–4.62	1.81	1.62–3.78
Factor II (%)	ND		24	33–93	41	34–102
Factor V (%)	ND		16	45–145	34	62–134
Factor VII (%)	ND		8	35–143	8	42–138
Bilirubin [conjugated] (μmol/L)	55 [ND]	<205	198 [31]	<257	446 [258]	<17 [<3.4]
ASAT/ALAT (U/L)	62/31	<39/<34	40/19	<39/<34	25/19	<39/<34
γGT (U/L)	27	<250	13	<250	21	<150
Alkaline phosphatase (U/L)	ND		596	<650	1356	<650
LDH (U/L)	2519	<800	1441	<800	357	<800
Total protein (g/L)	29	46–68	37	46–68	65	46–68
Albumin (g/L)	ND		28	30–45	ND	
Ammonia (μmol/L)	110	<107	166	<92	130	<92

Except for the postpartum tests, all blood tests were drawn during, or after a short break of fresh frozen plasma transfusion. Platelets and red packed cells were transfused intermittently

ND not determined

Reference values are for full term neonates, and have been compiled from several sources including Avery et al (1994) and Roos et al (2000)

were unremarkable, except for a mild elevation of glycine. Low serum transferrin and haptoglobin were attributable to liver failure. Serum ferritin was elevated to 612 ng/ml (<200). Creatine kinase, lactate, pyruvate and the lactate/pyruvate ratio (L/P) were normal in blood and CSF until the terminal phase, when blood L/P rose rapidly. No mutations of the mitochondrial DNA were found. Analysis of the complexes of the respiratory chain in frozen muscle and liver from the patient showed reduced activities for all complexes (I–IV) (Dr A. Slama, Paris, France). Values were between 40% and 98% of the lower limit of the reference range (mean ± 2SD), with complex I being most affected. These findings rule out a primary defect of the respiratory chain but suggest mitochondrial dysfunction, probably secondary to the transaldolase deficiency.

CSF protein concentration was elevated to 4200 mg/L (550–1200), with increased concentrations of several amino acids, but notably glutamine and tyrosine.

The clinical course was characterized by an intractable liver failure and progressive myocardial hypertrophy. Liver failure with a small liver size, persistence of a large venous duct—probably secondary to portal hypertension—and hypersplenism associated with low transaminases suggested chronic liver disease. Liver transplantation was not considered an option in view of the multisystem disease of possible metabolic origin. The infant developed a respiratory failure and severe lactic acidosis. She died at day 18 on the respirator due to bradycardic heart failure.

(A)

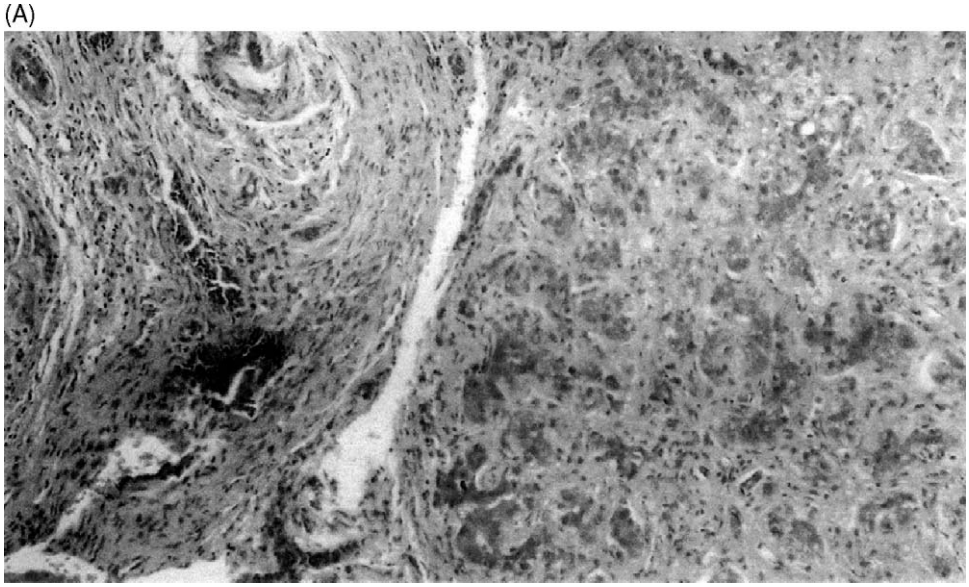

Figure 1 (A) Liver showing evident hepatocellular and canalicular cholestasis, signs of massive hepatocellular damage and degeneration of hepatocytes and fibrosis. Magnification × 120, haematoxylin–eosin staining. (B) Electron microscopy of liver, showing mitochondrial abnormalities as described in the text

(B)

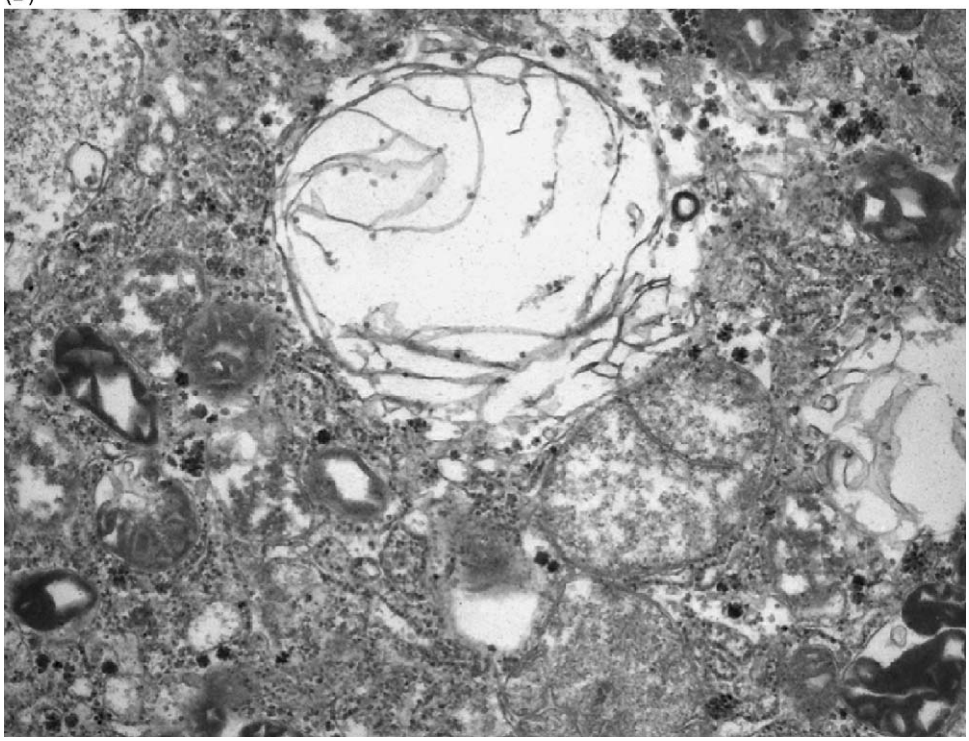

Figure 1 Continued

Postmortem samples of several tissues were obtained. The liver showed massive fibrosis with large areas of cell death and necrotic hepatocytes (Figure 1A). Intact hepatocytes appeared ballooned. There were broad fibrous tracts, and marked hepatocellular and canalicular cholestasis. Mitochondria were coarse, the cristae were irregular and their number was decreased (Figure 1B). On light microscopy the myocardium appeared unremarkable. However, on electron microscopy, mitochondria showed similar abnormal features as seen in the liver. Skeletal muscle mitochondria displayed the same findings, although less pronounced.

RESULTS

Metabolite analyses: Table 2 shows the concentrations of monosaccharides and polyols in a urine and a plasma sample from the patient at the age of 2 weeks. The values are compared with those found in the first transaldolase-deficient patient at almost the same age (at 5 weeks and at 1 day).

The chromatogram of the analysis of sugars and polyols showed a large, unidentified peak (Figure 2). The same compound was observed in the urine of the first

Table 2 Concentrations of pentitols and erythritol in urine and plasma of the patient, compared to the values of the first transaldolase-deficient patient (Verhoeven et al 2001) and the reference values

	Patient	*First TALDO-deficient patient*	*Reference range*
Urine (mmol/mol creatinine)	*2 weeks*	*5 weeks*	
Arabitol	463	404	27–99
Ribitol	722	247	7–24
Erythritol	976	914	58–192
Plasma (μmol/L)	*2 weeks*	*1 day*	
Arabitol	26	37	<5
Ribitol	33	20	<5
Erythritol	28	72	<5

transaldolase-deficient patient. This compound has a mass:charge ratio of 660 as determined by mass spectrometry, which corresponds to the expected signal of the trimethylsilyl derivative of sedoheptulose.

In addition to arabitol, ribitol and erythritol, an elevated concentration of galactose was observed (1105 mmol/mol creatinine; normal <382), which was ascribed to the liver failure.

Enzyme Assay: Analysis of transketolase and transaldolase in fibroblasts from the patient showed the presence of transketolase activity (formation of glyceraldehyde 3-phosphate and sedoheptulose 7-phosphate from ribose 5-phosphate). Transaldolase activity, however, was below the limit of detection.

Mutation analysis: In DNA from fibroblasts from the patient, a homozygous missense mutation in the TALDO1 gene was detected by sequence analysis. This mutation is located in exon 5 and was detected at both the mRNA and genomic DNA levels. The mutation, a G-to-A transition at position c575 of the coding sequence results in replacement of arginine 192 by histidine (575G>A, R192H).

DISCUSSION

This paper describes the second patient affected with a genetic deficiency of transaldolase. The clinical symptoms in this second patient are more severe than in the first patient. Both patients were born with a low birth weight. Both patients had related parents of Turkish origin. The first patient presented in the neonatal period with an aortic coarctation, which was surgically corrected. Both patients presented with signs of liver disease. The first patient gradually developed hepatosplenomegaly within the first months of life, whereas the second patient showed liver failure directly after birth. Remarkably, in both patients, transaminases and γ-glutamyltransferase remained within the reference range. Furthermore, both patients presented with bleeding tendency, which was mild in the first patient and severe and vitamin K-resistant in the second. The liver problems observed in the second patient cannot be ascribed to the Turner syndrome mosaicism, as severe liver

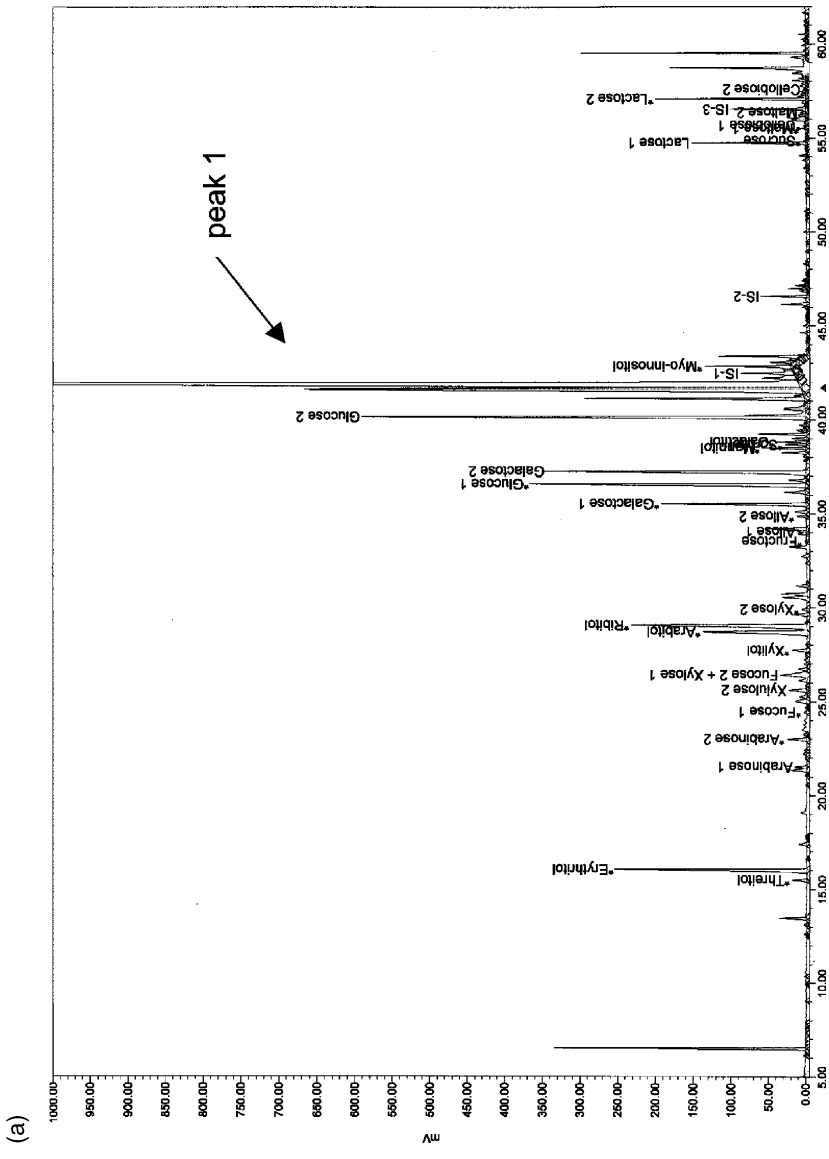

Figure 2 Gas chromatograms of the analysis of monosaccharides and polyols in urine of the transaldolase-deficient patient and a control. Indicated is peak 1, of which the exact nature is unknown. Mass spectrometric analysis has shown a mass to charge ratio of 660, corresponding with a trimethylsilyl ester of sedoheptulose

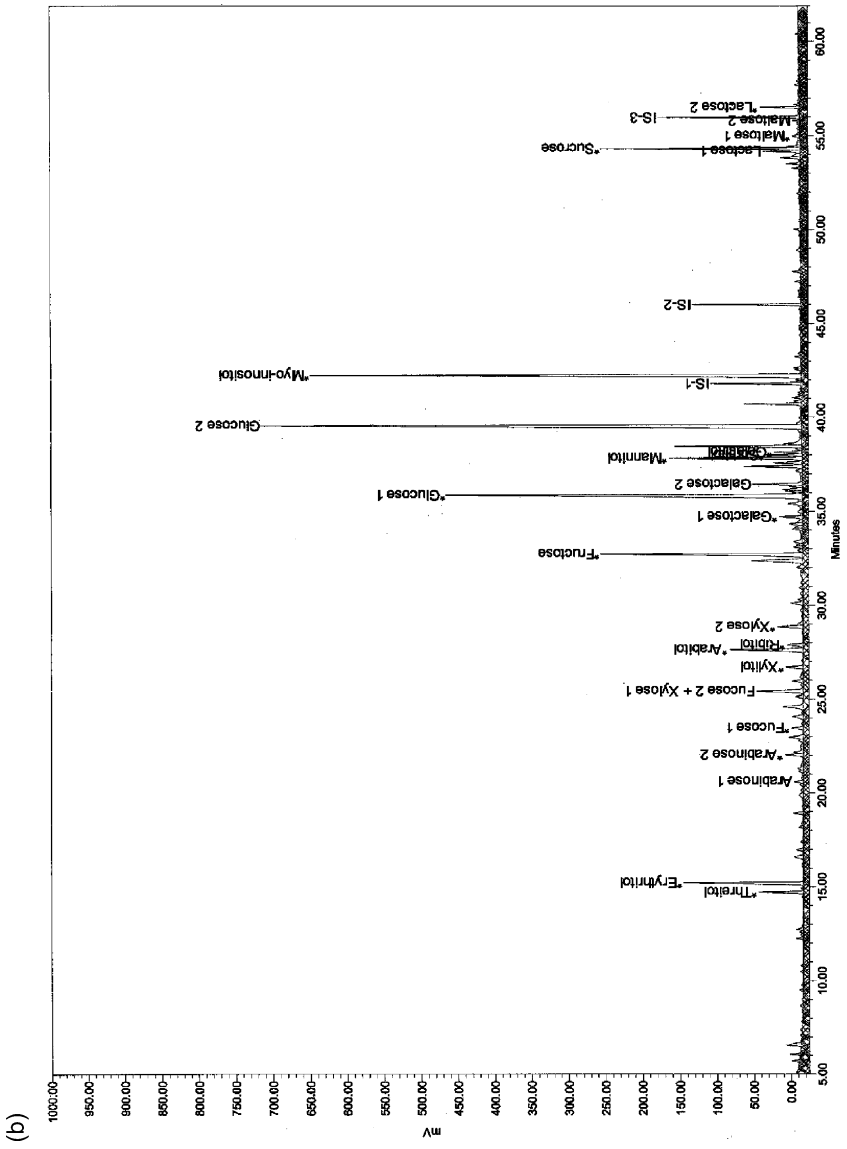

Figure 2 Continued

disease has not been described in infants with Turner syndrome. However, the generalized oedema, moderate muscular hypotonia and dysmorphic signs, as observed in this patient, can be part of the Turner syndrome (mosaicism) and were absent in the first patient.

A remarkable finding was the progressive cardiomegaly in the patient described here. Postmortem analysis of cardiac tissue showed coarse mitochondria with irregular cristae and a decrease in number. No signs of cardiac abnormalities have been observed in the first transaldolase-deficient patient, who is now 13 years of age.

The first patient is neurologically normal. The second patient showed hypotonia, which may have been caused by the severe illness.

The biochemical findings in both patients were similar. In urine from the first weeks of life, there were highly elevated levels of ribitol, D-arabitol and erythritol. Furthermore, in both patients we found an abnormal compound in the monosaccharide and polyol chromatogram, which is presumed to be sedoheptulose. This unidentified compound is seen in control urines as well, but is highly elevated in both transaldolase-deficient patients.

Analysis of transketolase and transaldolase in both patients showed the presence of transketolase and complete absence of transaldolase activities in lymphoblasts of the first patient and in fibroblasts of both patients (no lymphoblasts were available from the second patient).

On the molecular level, two different mutations have been identified. Both patients are homozygous. The first patient showed a homozygous deletion of three base pairs (nucleotides 561–563) in the TALDO gene, resulting in loss of serine 171 of the transaldolase protein. This amino acid is part of a highly conserved region. The patient described in the present paper showed a homozygous missense mutation of one base pair, resulting in an amino acid change (arginine 192 to histidine). Arginine 192 has been proposed to be part of the phosphate-binding site, which is part of the catalytic site of the enzyme (Thorell et al 2000). It is likely that the observed mutations cause the transaldolase deficiencies as found in fibroblasts from both patients.

No explanation for the difference in clinical presentations of the two transaldolase-deficient patients has been found. Although they were affected by different mutations, no residual enzyme activity was found in either of them. Furthermore, at the metabolite level, the patients were remarkably similar. Similarities between the two transaldolase-deficient patients reported are the neonatal liver problems with relatively low transaminases, normal γGT and initially low bilirubin levels.

Currently, there is no therapy available for transaldolase deficiency. As long as it is unknown whether the polyols or sugar phoshates are the toxic agents in this disorder, it is impossible to design a logical treatment stategy. Liver transplantation may, in the first patient, be necessary to prolong life expectancy. As only the liver seems to be affected in this patient, liver transplantation may be beneficial. Prenatal diagnosis at the enzyme and molecular level may be offered to families with an affected patient.

The observations described in this paper suggest that screening for transaldolase deficiency should be performed in neonates and children affected with liver problems in whom no other diagnosis can be found. Analysis of urinary sugars and polyols seems a valuable tool for screening for transaldolase deficiency.

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N.M.V. and M.W. contributed equally to this paper.

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