Postnatal and antenatal laboratory diagnosis of glutaric aciduria II in a South African family


Summary
Glutaric aciduria type II (GA II) is a rare inherited disorder of organic acid metabolism, first reported in 1976.1 Mild and severe forms have been diagnosed, with the latter presenting in the neonatal period. The inheritance is autosomal recessive and biochemical studies on cultured cells have shown a markedly impaired ability to oxidise fatty acids of varying chain lengths.2 In the severe form the initial presentation is one of respiratory distress and lethargy. Blood glucose levels fall sharply and metabolic acidosis accompanies, often accompanied by an odour of sweaty feet. Plasma and urine levels of glutaric acid are usually strikingly elevated; this is accompanied by lesser elevations in other organic acids. Treatment is difficult and most neonates progress rapidly to coma and death. The diversity in the chain lengths of the fatty acids encountered has led to the alternate listing of this disorder as multiple acyl-CoA dehydrogenase deficiency.3

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A case of GA II, presenting in the neonatal period, and the prenatal diagnosis of this disorder during the following pregnancy is reported. To the best of our knowledge, this is the first such case detected in South Africa.

Index case
The index case (baby W) was a white male infant, normal at birth and born to healthy, non-related parents. The first-born child in the family, a boy, had died shortly after birth with respiratory distress but no details are available. Two hours after birth signs of respiratory distress were noted. On day 2 tube feeding with milk formula was started. Vomiting ensued after each feed and blood gas analysis showed metabolic acidosis. On day 3 he was transferred to the intensive care unit (ICU) at Tygerberg Hospital and found to be hypotonic, 10% dehydrated and clinically mildly jaundiced. His pulse rate was 170/min, and he was tachypnoeic (70/min). Central nervous system examination revealed a bulging fontanelle and hypotonia. Septicaemia was suspected and treatment with penicillin and gentamicin was started.

Laboratory analysis showed a metabolic acidosis and hypoglycaemia without ketosis. Results of all other investigations were normal except for a moderate uraemia (10 mmol/l; normal 2.5 - 6.6 mmol/l) and a raised anion gap (21.7 mmol/l; normal 4 - 14 mmol/l). Liver function tests showed an elevated total serum bilirubin level (133 µmol/l; normal 2 - 17 µmol/l), with a normal conjugated fraction. The levels of the following enzymes were elevated: aspartate aminotransferase 97 U/l (normal < 18 U/l) and lactate dehydrogenase 684 U/l (normal 215 - 370 U/l). The plasma ammonia level was slightly raised on two occasions (104, 112 µmol/l; normal 55 - 90 µmol/l). A urinary odour of sweaty feet was noticed on the day after admission to the ICU, leading to a provisional diagnosis of isovaleric acidemia. Tube feeding with a commercial formula low in branch chain amino acids (maple syrup urine disease feeds) was started but vomiting persisted and the acid-base status remained unchanged.

Organic acid analysis of urine revealed a marked excretion of several acids, particularly glutaric acid, in a profile compatible with GA II and not isovaleric acidemia. Protein intake was restricted (1.5 g/kg/d) but vomiting and hypotonia remained a consistent feature. At 7 weeks of age, computed tomography of the brain showed diffuse low-density areas and at 2 months the patient died after a sudden deterioration in his clinical condition.

Prenatal diagnosis of fetus W. The parents were counselled about the 25% risk of recurrence. Approximately 6 months thereafter the mother presented for antenatal diagnosis at 17 weeks' gestation. Amniocentesis showed a chromosomally normal female fetus and marker studies excluded maternal contamination of the specimen. Organic acid analysis of amniotic fluid and in vitro oxidation studies on the cultured cells revealed an abnormal biochemical profile consistent with GA II. The pregnancy was terminated at 23 weeks' gestation, no morphological abnormality being detected in the fetus.

Cell cultures. Skin fibroblasts and amniotic fluid cells were cultured in standard growth medium and all assays were performed on cultures between passages 2 and 10.

Substrate oxidation by cultured cells. The oxidation of (1-14C) butyric acid, (1-14C) oleic acid, (U-14C) lysine and (1-4,14C) succinic acid was tested on intact cells by measurement of the release of 14C-C02 as described by Saudubray et al.5 Assays were carried out in the wells of microtitre plates and all isotopes were obtained from Amersham (UK).
Organic acids. Urine and amniotic fluid organic acids were extracted into organic solvents and converted into their trimethylsilyl derivatives. Positive identification and quantitation were effected by combined gas/liquid chromatography-mass spectrometry (Hewlen Packard 5988 A).

Results

Organic acid analysis. The gas chromatogram of urine revealed raised levels of a number of organic acids, with glutaric acid the most striking (600-fold). The other acids detected in increased amounts were 2-hydroxyglutaric, ethylmalonic, adipic and lactic acids (Table I). The chromatogram of the at-risk amniotic fluid is shown in Fig. 1 and clearly demonstrates an elevated glutaric acid level (8.0 μmol/l; i.e. 10 times normal).

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<th>TABLE I. URINE ORGANIC ACID LEVELS (μmol/g CREATININE)</th>
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<tr>
<td>Ethylmalonic</td>
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<td>2-hydroxyglutaric</td>
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<td>Methylsuccinic</td>
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In vitro oxidation studies. The ability of cultured skin fibroblasts from baby W to oxidise each of the substrates butyric acid, oleic acid and lysine was impaired (Table II); oxidation of butyric acid was the most reduced at 14% of mean activity of the control cultures. Oleic acid and lysine gave values of 20% and 24% respectively. In keeping with obligate heterozygote status, the fibroblast cultures from the parents showed a reduction of approximately 50%. The amniotic fluid cell cultures and a fibroblast culture from fetus W were only assayed for their ability to oxidise butyric acid. Here, control amniotic fluids gave results similar to the fibroblast controls while cells from the pregnancy at risk (fetus W) showed very little activity, almost identical to that of cells from baby W. The oxidative defect in the fetus was thus confirmed by the low activity in fibroblasts cultured from the abortus. Normal mitochondrial function in cells with the fatty acid oxidative defect was demonstrated by the efficient oxidation of succinic acid.

14C-butyrate/1H-leucine incorporation ratio. A second measure of the efficiency of butyrate oxidation was obtained by analysis of the amount of 14C label incorporated into trichloroacetic acid precipitable macromolecules. The incorporation of tH leucine, a metabolite not in the same pathway, was taken as a general indicator of metabolism in the cell population. The 14C/tH incorporation ratios are shown in Fig. 2. The ratio for cells from baby W is clearly decreased (3-fold).

Discussion

GA II is typical of the inherited disorders of organic acid metabolism presenting with a fulminant or catastrophic picture in the neonatal period. Many of these disorders have been diagnosed in the South African population and must be considered in the critically ill neonate. Early detection and appropriate dietary and/or megavitamin therapy can minimise asso-
cated mental and physical sequelae. Metabolic clues to early recognition are acidosis with a raised anion gap, hypoglycaemia with or without ketosis, hyperammonaemia and a urinary odour. Ancillary findings of leukopenia and thrombocytopenia are also suggestive. For definitive diagnosis urine and plasma organic acids must be analysed by gas/liquid chromatography — mass spectrometry. This investigation is now offered by the Department of Biochemistry at the University of Potchefstroom.

Patients with GA II have a defect in the process of fatty acid β-oxidation through a deficiency in the activity of several classes of the acyl-CoA dehydrogenases which initiate the oxidative cycle. In contrast, for GA I there is a selective deficiency of glutaryl-CoA dehydrogenase activity leading to tissue accumulation of glutaric acid only. Although the number of cases of GA II described to date is small (less than 20) it is clearly a heterogeneous disorder. Most patients have presented shortly after birth, a minority having suffered delirious intra-uterine effects resulting in dysmorphic features (large head, odd facies, polyctytic kidneys and genital abnormalities). Others have shown symptoms of hypoglycaemia, acidosis and lethargy only later in life, usually after infection and/or pregnancy. The majority of cases have yielded a characteristic abnormal serum and urine organic acid profile with glutaric acid predominating. Deviations include one patient showing predominantly ethylmalonic-adipic acid excretion and another C6 - C10 dicarboxylic acids. The heterogeneity underlying GA II has not been fully characterised at the molecular level, but a recent study has demonstrated that the disorder can be caused by deficiencies of either of the two linked flavoproteins which transfer electrons from the acyl-CoA dehydrogenases to co-enzyme Q of the mitochondrial electron transfer chain.

Success in the treatment of neonatal GA II has been limited. The most encouraging case study employed a regimen of a high-energy diet, restricted in protein and fat, together with DL-carnitine HCl and riboflavin. This regimen was not available here at the time and baby W only received a low-protein diet which failed to alter the clinical course.

A few reports have appeared on the prenatal diagnosis of GA II. In affected pregnancies, amniotic fluid organic acid analysis at 15 - 18 weeks' gestation revealed a 10 - 30-fold increase in glutaric acid levels. Some authors list accompanying, but less marked, increases in levels of 2-hydroxyglutaric, adipic and suberic acids. In the South African pregnancy similar results were obtained, with glutaric acid levels increased 10-fold above normal. Also 2-hydroxyglutaric acid was increased but to a lesser extent. The consistency in the elevation of amniotic fluid glutaric acid levels in affected pregnancies attanges considerable reliability to the chemical method in the prenatal diagnosis of this condition.

The diagnosis of GA II should be considered in a patient with respiratory distress, metabolic acidosis, non-ketotic hypoglycaemia and muscular hypotonia occurring in the neonatal period or later in life. A diet high in carbohydrates but low in protein and fat should be implemented immediately, together with riboflavin and carnitine supplementation. Additional treatment with insulin has been reported to improve glucose utilisation and dramatically improve the metabolic condition of these patients.

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REFERENCES