Fumarase Deficiency in Dichorionic Diamniotic Twins

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Fumarase deficiency is a rare autosomal recessive inborn error of metabolism of the Krebs Tricarboxylic Acid cycle. A heavy neurological disease burden is imparted by fumarase deficiency, commonly manifesting as microcephaly, dystonia, global developmental delay, seizures, and lethality in the infantile period. Heterozygous carriers also carry an increased risk of developing hereditary leiomyomatosis and renal cell carcinoma. We describe a non-consanguineous family in whom a dichorionic diamniotic twin pregnancy resulted in twin boys with fumarase deficiency proven at the biochemical, enzymatic, and molecular levels. Their clinical phenotype included hepatic involvement. A novel mutation in the fumarate hydratase gene was identified in this family.

Keywords: fumarase deficiency, Krebs tricarboxylic acid cycle, dichorionic diamniotic, leiomyomatosis, renal cell carcinoma, hepatomegaly

Case Report

Our patients were the products of a non-consanguineous Caucasian family with no family history of twinning. The antenatal period was unremarkable, with ultrasound scans performed at 11, 19, and 30 weeks gestation indicating a DCDA twin pregnancy of morphologically normal males. They were delivered via emergency lower segment cesarean section at 32 weeks gestation for premature onset of labor. Both neonates developed hyaline membrane disease requiring assisted ventilation. At 6 months of age they...
Organic acids were suggestive of FH deficiency as repeated urine peak on MRS. Brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) identified global cerebral atrophy, corpus callosum thinning, hypoplastic brainstem, and hypointense lesions in the basal ganglia, especially in the caudate and thalamic nuclei, with an elevated lactate and hyperintense lesions in the basal ganglia, especially in the putamen, globus pallidus and substantia nigra. Abnormal neurological examination findings included an unsafe/uncoordinated oral motor swallow mechanism, developmental milestone attainment, tongue thrusting and delayed speech, visually-guided movement, cortical visual impairment, convergent strabismus, no head control, poor feeding, and extreme irritability. Abnormal postural tone was present with hyperreflexia and a stiff, jerky gait. Cerebrospinal fluid had elevated lactate and pyruvate, with a ratio of 47.5:1 within normal limits of 0.3-15.2.

Notes: The biochemical results found for twin 1 indicate persistent high levels of fumarate, indicative of fumarase deficiency. Raised alpha-ketoglutarate can be increased in this condition and raised levels of succinyladenosine can be secondary to increased fumarate levels. Fumarase enzyme activity was assayed on cultured fibroblasts for both twins and was undetectable in both patients’ cell lines (see Table 3 and Figure 1).

Sequence analysis of the FH gene showed the siblings were compound heterozygous for c.1037G>A (p.Gly346Asp) in exon 7, and c.1431_1433dup (p.Lys477dup) in exon 10. The p.Lys477dup mutation, inherited from their father, is a novel mutation hitherto unreported in the medical literature; however, siliho analysis (http://genetics.bwh.harvard.edu/pph2/) predicted this to be a pathogenic mutation. A prenatal diagnostic test was performed via chorionic villous sampling at 12 weeks gestation for a subsequent unaffected pregnancy.

Over the subsequent months both boys developed hepaticomegaly with a hard cirrhotic liver edge on clinical examination and persistent jaundice. Biochemical tests confirmed a hematological disorder and investigations were performed via chorionic villous sampling at 12 weeks gestation for a subsequent unaffected pregnancy.

Note: The biochemical results found for twin 2 are similar to those for twin 1 and there are persistent high levels of fumarate, indicative of fumarase deficiency. Raised alpha-ketoglutarate can be increased in this condition and raised levels of succinyladenosine can be secondary to increased fumarate levels.
TABLE 3
Fumarase Enzymology Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fumarase level (nmol/min/mg)</th>
<th>Citrate synthase level (nmol/min/mg)</th>
<th>% citrate synthase ratio (relative to mean controls)</th>
<th>% fumarase level (relative to mean controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>146.2</td>
<td>43.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Normal control</td>
<td>73.2</td>
<td>29.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Deficient control</td>
<td>12.8</td>
<td>28.9</td>
<td>15.3%</td>
<td>11.6%</td>
</tr>
<tr>
<td>Deficient control</td>
<td>5.1</td>
<td>16.9</td>
<td>10.4%</td>
<td>4%</td>
</tr>
<tr>
<td>Twin 1</td>
<td>0</td>
<td>27.5</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Twin 2</td>
<td>0</td>
<td>28.6</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Note: The fumarase enzymology resulted in undetectable fumarase levels in both patients, with deficient control values as expected for the assay.

FIGURE 1
(Colour online) Example of kinetic spectrophotometric trace. Note: Fumarase enzyme levels are tested spectrophotometrically on whole cell homogenates in an assay that measures the rate of dehydration of L-malate to fumarate at a maximum absorption of 250 nm over 5 min, therefore obtaining an estimate of the level of fumarase enzyme activity in cell extracts.

Discussion
Fumarate hydratase deficiency is a rare inborn error of metabolism, with less than 50 cases reported to date (Allegri et al., 2010). Most affected infants have demonstrated severe global developmental delay, encephalopathy, microcephaly, seizures, and hypotonia (Allegri et al., 2010; Kerrigan et al., 2000; Ottolenghi et al., 2011). The biochemical clues suggestive of FH deficiency include elevated urine excretion of fumarate, alpha-ketoglutarate, and succinyladenosine. Our cases highlight the importance of considering a neuro-metabolic etiology in infants with an encephalopathy, especially where there is no clear history to indicate hypoxic ischemic encephalopathy. An accurate diagnosis of neuro-metabolic diseases in infants is usually of most importance for predicting prognosis and for determining reproductive options. However, carriers of the FH gene are at risk of HLRCC, and thus accurate diagnosis opens the issue of cascade testing and tumor surveillance.

Mitochondrial disorders present with a range of hepatic clinical phenotypes, including acute fulminant hepatic failure, hepatic synthetic failure, cholestatic jaundice, steatosis, hepatomegaly, and cirrhosis (Lee & Sokol, 2007). Hepatic involvement has only been reported in four infants with FH deficiency to date, manifesting with cholestasis and hepatomegaly (Allegri et al., 2010; Walker et al., 1989; Zeman et al., 2000). Our siblings demonstrated hepatomegaly and hard cirrhotic liver edge on palpation, while tissue samples were not obtained in view of the palliative nature of their clinical management at the time.

Of the two compound heterozygous mutations identified, the c.1431_1433 dup (p.Lys477dup) mutation in exon 10 has been previously described to be deleterious (Loffen et al., 2005) and has been reportedly found nine times on the FH gene mutation database (Bayley et al., 2008). The second mutation, c.1037G>A (p.Gly346Asp) in exon 7, has not been previously reported but is predicted (via in silico analysis) to be a pathogenic change. Pathogenic mutations in the FH gene have included 57% missense, 27% frameshift and nonsense mutations, and diverse deletions, insertions, and duplications (Bayley et al., 2008). To date, genotype–phenotype correlations in FH deficiency are lacking (Bayley et al., 2008); however, our siblings demonstrated a very severe clinical–biochemical phenotype.

Approximately one-third of monozygotic twins will be DCDA (Weber & Sebire, 2010), which is the most likely explanation as to why such a rare inborn error of metabolism has manifested in this twin pregnancy. Rare autosomal recessive inborn errors of metabolism have been infrequently described in the literature secondary to monozygous twinning, including mitochondrial neurogastrointestinal encephalomyopathy (MNGIE; Schupbach et al., 2007), sialic acid storage (Martin et al., 2003), and aspartylglucosaminuria (Opladen et al., 2012). Our patients represent the first reported case of FH deficiency in twins.
The FH gene has also been shown to act as a tumor suppressor gene, predisposing carriers to autosomal dominant benign fibroid tumors of skin, uterus, hereditary leiomyomatosis, and renal cell carcinoma (HLRCC), as well as multiple cutaneous and uterine leiomyosarcoma (MCUL) later in life (Deschauer et al., 2006; Tomlinson et al., 2002). Tumor surveillance programs are well described for select genetic cancer predisposition syndromes, for example, juvenile polyposis coli. The tumor surveillance required for FH mutation carriers is not clear at this stage. We elected to perform 6-monthly abdominal ultrasounds in the parents.

Our cases highlight the importance of considering a neuro-metabolic diagnosis in patients with a ‘cerebral palsy phenotype’, especially when there has been no clear indication of a hypoxic event. The diagnosis of FH deficiency can not only provide some certainty for the parents in terms of natural history progression, but is also a diagnosis with relevance of their own health status, given the cancer predisposition associated with being an FH carrier.

References


