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

Fumarate hydratase (FH) deficiency (OMIM 606812) is a rare autosomal recessive defect of the Krebs Tricarboxylic Acid (TCA) cycle caused by mutations in fumarate hydratase (*FH*) gene (OMIM 136850). The *FH* gene on chromosome 1q42 encodes two isoenzymes, one in the cytosol and the other in the mitochondrial matrix (Bourgeron et al., 1994).

Patients with fumarase deficiency can present with poor feeding, microcephaly, fumaric aciduria, and limb dystonia. Neurological symptoms can include severe developmental delay, infantile spasms, seizures, and regression (Allegri et al., 2010; Kerrigan et al., 2000). All patients described so far appear to have had a defect of both cytosolic and mitochondrial fumarase. Infantile lethality is commonplace in this rare inborn error of metabolism. Carriers of a single *FH* gene mutation are at risk of developing the hereditary leiomyomatosis and renal cell cancer (Hereditary leiomyomatosis and renal cell cancer [HLRCC], OMIM 136850) phenotype.

We identified FH deficiency in a dichorionic diamniotic (DCDA) twin pregnancy. Our patients demonstrated hep-atic disease as part of their clinical phenotype, and a novel mutation was identified in the *FH* gene.

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

TABLE 1

Biochemical Results for Twin 1

Date	Substrate	Reference range	Result	Outcome
Urine results				
02.10.11	Fumarate	<25 mmol/mol creatinine	493.2	20 × ULR
02.10.11	Alpha-ketoglutarate	<350 mmol/mol creatinine	1,172.1	3 × ULR
02.10.11	Succinyladenosine	<5 mmol/mol creatinine	14.6	3 × ULR
02.10.11	SAICAR	<1 mmol/mol creatinine	<0.3	Within normal range
Urine test repeated 5 days later				
07.10.11	Fumarate	<25 mmol/mol creatinine	313.4	12 × ULR
07.10.11	Alpha-ketoglutarate	<350 mmol/mol creatinine	438	Slightly raised
07.10.11	Succinyladenosine	<5 mmol/mol creatinine	22	4 × ULR
07.10.11	SAICAR	<1 mmol/mol creatinine	<0.3	Within normal range
CSF results				
14.10.11	CSF lactate	0–2.8 mmol/L	5	2 × ULR
14.10.11	CSF pyruvate	30–130 μmol/L	345	3 × ULR

Note: 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.
ULR = Upper limits of reference range.

TABLE 2

Biochemical Results for Twin 2

Date	Substrate	Reference range	Result	Outcome
Urine results				
02.10.11	Fumarate	<25 mmol/mol creatinine	402.6	16 × ULR
02.10.11	Alpha-ketoglutarate	<350 mmol/mol creatinine	385.2	Slightly raised
02.10.11	Succinyladenosine	<5 mmol/mol creatinine	14.2	3 × ULR
02.10.11	SAICAR	<1 mmol/mol creatinine	<0.3	Within normal range
Urine test repeated 5 days later				
07.10.11	Fumarate	<25 mmol/mol creatinine	482.3	19 × ULR
07.10.11	Alpha-Ketoglutarate	<350 mmol/mol creatinine	1367.3	4 × ULR
07.10.11	Succinyladenosine	<5 mmol/mol creatinine	24.4	5 × ULR
07.10.11	SAICAR	<1 mmol/mol creatinine	<0.3	Within normal range
CSF results				
14.10.11	CSF lactate	0–2.8 mmol/L	3.9	1.5 × ULR
14.10.11	CSF pyruvate	30–130 μmol/L	253	2 × ULR

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.
ULR = Upper limits of reference range.

presented for pediatric assessments in view of global developmental delay, poor feeding, and extreme irritability.

Abnormal neurological examination findings included postnatal microcephaly, hypotonia, dystonic limb posturing, cortical visual impairment, convergent strabismus, no developmental milestone attainment, tongue thrusting and an unsafe/uncoordinated oral motor swallow mechanism, and features of the brain stem dysfunction. An initial diagnosis of cerebral palsy was postulated, but surmised to be unlikely as there was no obstetric or postnatal setting to explain hypoxic ischemic encephalopathy.

Brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) identified global cerebral atrophy, corpus callosum thinning, hypoplastic brainstem, and hyperintense lesions in the basal ganglia, especially in the caudate and thalamic nuclei, with an elevated lactate peak on MRS.

Cerebrospinal fluid had elevated lactate and pyruvate, further suggesting a mitochondrial disease process. Urine organic acids were suggestive of FH deficiency as repeated samples indicated high fumarate, alpha-ketoglutarate, and succinyladenosine (see Tables 1 and 2).

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 has been described previously (Loeffen et al., 2005) and was inherited from their mother. The p.Gly346Asp mutation, inherited from their father, is a novel mutation hitherto unreported in the medical literature; however, silico analysis (http://genetics.bwh.harvard.edu/pph2/, http://blocks.fhcrc.org/sift/SIFT.html) 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 hepatomegaly with a hard cirrhotic liver edge on clinical

TABLE 3

Fumarase Enzymology Results

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

Note: The fumarase enzymology resulted in undetectable fumarase levels in both patients, with deficient control values as expected for the assay.
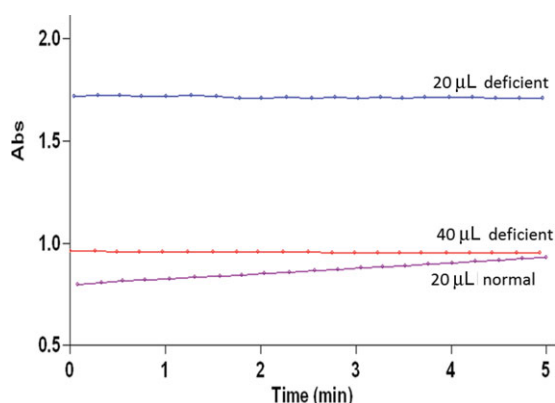
FIGURE 1

(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.

palpation, and pitting peripheral edema suggestive of liver cirrhosis and hepatic synthetic dysfunction. However, this clinical component of their phenotype was not actively investigated further, as a non-invasive palliative care approach to their management had been adopted to maximize quality of life. Both boys succumbed to their disease three months apart in their second year of life.

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 FH deficiency include elevated urine excretion of fumarate, alpha-ketoglutarate, and succinyladenosine. Our cases highlight the importance of considering a neurometabolic 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 (Loeffen et al., 2005) and has been reportedly found nine times on the 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.

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