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Short communication

Mild clinical presentation and prolonged survival of a patient with fumarase deficiency due to the combination of a known and a novel mutation in *FH* gene

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ABSTRACT

Mutations in the *FH* gene cause the deficiency of the enzyme fumarase (fumarate hydratase, EC 4.2.1.2) which result in autosomal recessive fumaric aciduria in early childhood with failure to thrive, seizures, developmental delay, mental retardation, hypotonia and sometimes with polycythemia, leukopenia, and neutropenia. Many children with fumarate hydratase deficiency do not survive infancy or childhood; those surviving beyond childhood have severe psychomotor retardation. Recently, FH gene was also identified as a "non-classical" tumor suppressor gene and heterozygous mutations were shown to cause multiple cutaneous and uterine leiomyomas as well as hereditary leiomyomatosis and renal cell cancer. A male patient who was referred to investigate the etiology of psychomotor retardation was later diagnosed to have fumaric aciduria due to the combination of a previously known (c.1431_1433dupAAA) and a novel (c.782G>T) mutation. The patient had an unusually mild clinical course without acidotic attacks. Interestingly his father who was heterozygous for the c.1431_1433dupAAA mutation in the *FH* gene had cutaneous leiomyoma.

1. Introduction

Fumarase (fumarate hydratase, EC 4.2.1.2) (FH) is a component of the tricarboxylic acid cycle and catalyzes the conversion of fumarate to malate (Pithukpakorn, 2005). The *FH* gene is located on chromosome 1q42.1 and codes for two isoforms that are produced by alternative initiation and vary at the amino terminus and for electrophoretic mobility. Mutations in the *FH* gene cause autosomal recessive fumaric aciduria that presents in the neonatal period or early infancy with failure to thrive, seizures, developmental delay, mental retardation, hypotonia and sometimes with polycythemia, leukopenia, and neutropenia. Many children with *FH* deficiency do not survive infancy or childhood; those surviving beyond childhood have severe psychomotor retardation (Coughlin et al., 1998; Loeffen et al., 2005).

In 2002, the Multiple Leiomyoma Consortium identified *FH* as a "non-classical" tumor suppressor gene responsible for multiple cutaneous and uterine leiomyomas (MCUL) as well as hereditary leiomyomatosis and renal cell cancer (HLRCC) (Alam et al., 2005; O'Flaherty et al., 2010; Toro et al., 2003).

We report the clinical and molecular data of a child with early onset disease and prolonged survival. His father has cutaneous leiomyoma.

2. Case report

A 19-month-old male infant was referred to the Metabolic Clinic for developmental delay and encephalopathy. The infant was the second child of a gravida 2, para 2, 37 year old mother and a 34 year old father, born after an uneventful pregnancy. He was born term at 39 weeks of gestation via cesarean section due to fetal distress. The birth weight and length as well as the head circumference were in the normal range. There were no postnatal complications, or feeding difficulties.

The infant was the second child of his parents. He had a three-year-old sister who was in good health. There was no history of consanguinity or birth defects in the family. Examination of the father showed subcutaneous leiomyomas on his arms. Renal ultrasounds of the parents were normal.

Despite good feeding his weight was below 3rd centile by 4 months of age. By 9 months, he was not sitting and was referred to a neurologist. The neurological examination revealed mild truncal hypotonia with normal muscle strength. The deep tendon reflexes were 2+ in all extremities. There was no ankle clonus and no abnormal movements.

Abbreviations: *FH*, Fumarate hydratase gene; FH, Fumarate hydratase protein; HLRCC, hereditary leiomyomatosis and renal cell cancer; HIF, hypoxia-inducable factor.

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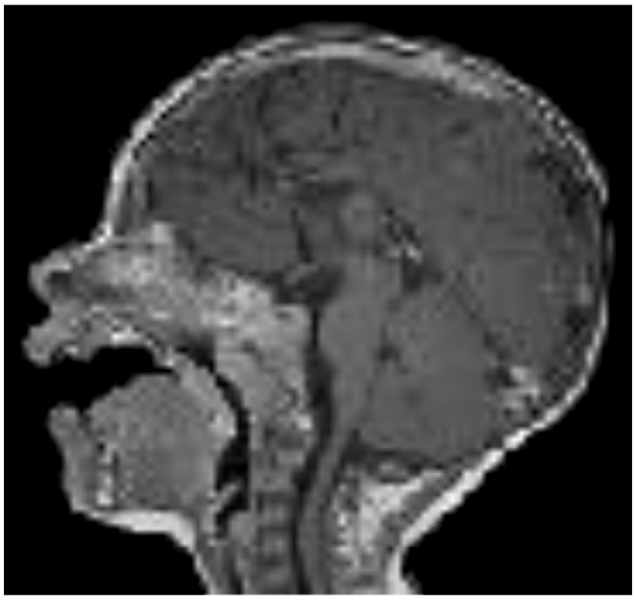

Fig. 1. MRI of the brain at 24 months. There is hypomyelination of white matter and marked thinning of the corpus callosum.

A karyotype and subtelomeric FISH studies were normal.

MRI of the brain at 24 months revealed hypomyelination of white matter and marked thinning of the corpus callosum (Fig. 1). The EEG was normal.

Slow progression in the development continued without any regression. He could sit unsupported at 11 months, was able to pull to stand at 13 months and started crawling at 14 months. No babbling was noticed. At the age of 33 months he was able to pull up to stand, to grasp and put things on one another and started to make some words. The ophthalmologic examination did not show any other abnormality, aside from strabismus.

At age of 46 months he was able to walk some distance with assistance and showed slow progress in development and growth without seizures and deterioration in skills.

At the age of 6 years and three months he had no speech but only some signs and did not have any additional neurologic signs except episodes of shivers and loss of eye control lasting for 3–5 min.

At age of 7 years, the patient was still developmentally delayed and had short stature. He also had possible seizures with normal EEG and walked with an ataxic gait. He did not speak any words but was very social.

The blood lactate showed minimal elevation at 16.9 mg/dl. The pyruvate was 0.96 mg/dl, within the normal range. Ammonia was normal at 13 μmol/l. Blood acylcarnitine and amino acid investigation with tandem mass spectrometry were in the normal range. Quantitative plasma amino acids were normal, but urine organic acids revealed a highly elevated fumarate at 304 mmol/mol of creatinine (normal <8).

With these results, a preliminary diagnosis of fumarate hydratase deficiency was considered and the *FH* gene was sequenced.

The patient was put on oral carnitine 500 mg daily and on multivitamins. He also had occupational, speech and physical therapy. For the risk of renal carcinoma, the parents were recommended to have urinalysis and renal ultrasound at least once a year.

3. Molecular studies

Sequence analysis was carried out for the *FH* gene (GenBank NG_012338.1), using both genomic and cDNA samples of the index case and the parents. Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA) and RNA was extracted from the lymphoblasts by using the RNeasy Midi Kit (Qiagen) according to the manufacturer's instructions. Poly-dT-primed cDNA was synthesized from 3 μg of lymphoblast RNA using the Omniscript RT Kit (Qiagen). The genomic primers covered the intron–exon boundaries. All primers for cDNA were designed to prevent amplification of genomic DNA and verified by PCR containing genomic DNA as a template (Tables 1 and 2).

PCR reactions were carried out using TaqDNA polymerase (Applied Biosystems, Foster City, CA). The identity of PCR products was confirmed by direct sequencing on an automatic sequencer (3730 DNA Analyzer, Applied Biosystems) using the BIG DYE Terminator Cycle Sequencing Kit (Applied Biosystems). The PCR amplification of *FH* exons 1–10 was performed in a 50 μl reaction mixture which contained 100 ng of genomic or cDNA, 25 mM MgCl2, 10 mM of each deoxynucleotide triphosphate, 2.5 U of DNA polymerase (Gold Taq, Applied Biosystems), and 20 pmol of each primer. PCR conditions included an initial denaturation at 95 °C for 10 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 45 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 7 min. PCR products were resolved by electrophoresis on a 1% agarose gel. For the description of alterations, nucleotide numbering using the A of the ATG translation initiation start site of the coding DNA reference sequence was denoted as nucleotide +1. The alteration nomenclature was arranged according to the current guidelines at the HGVS website (http://www.hgvs.org/mutnomen/).

4. Result

Sequencing of the *FH* gene of the infant identified two mutations c.1431_1433dupAAA creating an additional lysine at amino acid position 477 (p.Lys477dup) (Fig. 2) and a novel c. 782G>T (p.R261I) (Fig. 3) mutation. The mother was heterozygous for the novel p.R261I change and the father was heterozygous for c.1431_1433dupAAA mutation, which has also been reported in patients with tumors (Ylisaukko-oja et al., 2006).

Table 1
Primers for *FH* genomic DNA.

Exon	Forward	Reverse	Product size (base pair)
1	5′-CCCAGAAATTCTACCCAAGC-3′	5′-AGGGCTGAAGGTCACTGC-3′	214
2	5′-TGATCCTGGGTTTCTTTTCAAC-3′	5′-ATGAATACAGCCTACTTCATCC-3′	240
3	5′-CCAAAATAATAAACTTCCATGC-3′	5′-ATGGGTCTGAGGTTATTAAG-3′	221
4	5′-CTGTATTCAAACTCTGTGGC-3′	5′-TTATAACCAAAAAACAGCAAAGC-3′	288
5	5′-GTTTTTGTTGCCTCTGATTTAAC-3′	5′-TGGCCATTTGTACCAAGCTC-3′	290
6	5′-GAGTAACTTGTAAGCTATTAGG-3′	5′-AATGTACAGACCACGTA-3′	285
7	5′-TAACTTGTTCACCCATCTAGG-3′	5′-CTAGTCAAGTTTTAGCTCCAAC-3′	287
8	5′-TTAGTCTTTACTCTGTCATTGG-3′	5′-TAATAAGCCTTTGGTCAAAAAAC-3′	212
9	5′-ATTGTATATTTACTGTCAACCAG-3′	5′-AAACACTGATCCACTTGTCTCT-3′	356
10	5′-CTGCTAACCCATATGTCGTC-3′	5′-CGTTTTTAAGAAATGGGAGTCTG-3′	252