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Neonatal mitochondrial hepatoencephalopathy caused by novel *GFM1* mutations

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ABSTRACT

Disorders caused by defects in the mitochondrial translation system are clinically and genetically heterogeneous. The elongation phase of mitochondrial protein synthesis requires, among many other components, three nuclear-encoded elongation factors: EFTu (*TUFM*; 602389), EFTs (*TSFM*; 604723), and EFG1 (*GFM1*; 606639). Mutations have been identified in the genes encoding all three elongation factors, and they result in combined respiratory chain deficiencies and severe phenotypes with an early fatal outcome. So far, only eleven patients have been reported with mutations in *GFM1*. Here we describe an additional three patients with novel *GFM1* mutations. Our results confirm the tissue-specific effect of *GFM1* mutations, since we found only slightly decreased respiratory chain enzyme activities in muscle and fibroblasts, but a severe deficiency in the liver. Hence, a thorough biochemical evaluation is important to guide genetic investigation in patients suspected for a mitochondrial disorder.

1. Introduction

Eukaryotes contain two translational systems, one in the cytosol and one in the mitochondria. The mitochondrial translation machinery comprises mitochondrial DNA (mtDNA) encoded rRNA and tRNAs as well as numerous nuclear-encoded proteins, including mitochondrial ribosomal proteins, and initiation, elongation and termination factors [1]. All these components are essential for maintaining the primary function of the mitochondria, the biosynthesis of energy (ATP) via the oxidative phosphorylation (OXPHOS) pathway. Disorders caused by defects in the mitochondrial translation system are clinically and genetically heterogeneous. The elongation phase of mitochondrial protein synthesis requires, among many other components, three nuclear-encoded elongation factors: EFTu (*TUFM*; 602389), EFTs (*TSFM*; 604723), and EFG1 (*GFM1*; 606639). Mutations have been identified in the genes encoding all three elongation factors, and they result in severe phenotypes with an early fatal outcome [2,3]. Usually, a combined respiratory chain (RC) deficiency is found, i.e. decreased activity of two or more RC complexes, excluding complex II, which is the only complex where the subunits are encoded entirely by the nuclear genome. So far, only eleven patients have been reported with mutations in *GFM1* [3–9]. Here we describe an additional three patients with novel *GFM1* mutations.

2. Materials and methods

2.1. Patients

2.1.1. Patient 1

Patient 1 was a boy, the first of three children from healthy Danish parents. The parents were consanguineous since they had common second great grandparents. He was born at term after a normal pregnancy and delivery. All birth parameters were below average, weight was 2200 g (<3 percentile), birth length 45 cm (<3 percentile) and head circumference 32 cm (<3 percentile). At birth, only a single umbilical artery was noted, and he had dysmorphic features with retrognathia, epicanthus, simply shaped external auricles, a unilateral simian crease, hypospadia, maldescensus testis and a left-sided paralysis of the facial nerve. Abnormal posturing was also noted.

Within the first 10 h he was admitted to the neonatal department due to lactic acidosis with symptoms consisting of twitching of the extremities, abnormal head movements, abnormal breathing

Abbreviations: OXPHOS, oxidative phosphorylation; RC, respiratory chain; CS, citrate synthase

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Table 1
Results of respiratory chain enzyme analysis.

	CI/CS	CI/CS	CIII/CS	CIV/CS	CS
Muscle					
Patient 1	**58** (65–135)	81 (59–141)	106 (44–156)	**28** (43–157)	104 (40–161)
Patient 2	89 (65–135)	94 (59–141)	138 (44–156)	**42** (43–157)	65 (40–161)
Patient 3	66 (65–135)	127 (68–133)	128 (56–144)	**62** (65–135)	102 (25–175)
Liver					
Patient 3	**4** (43–176)	43 (28–195)	**24** (45–208)	**3** (42–181)	**410** (51–176)
Fibroblasts					
Patient 3	N.p.[a]	102 (67–141)	95 (75–130)	**36** (67–155)	112 (44–156)

	CI	CII	CIII	CIV
Fibroblasts				
Patient 1	17 (11–26)	23 (7–21)	65 (25–65)	85 (73–197)
Patient 2	26 (11–26)	14 (7–21)	59 (25–65)	**56** (73–197)

Enzyme activities of citrate synthase (CS) and respiratory chain complexes I–IV corrected for CS activity. Values are expressed as percentage of control mean (reference ranges in parentheses). The enzyme analyses were carried out at different times, and the reference values have been changed slightly. The activities of complexes I through IV in fibroblasts from patients 1 and 2 are expressed as mU/mg protein (reference ranges in parentheses). Abnormal values are shown in bold.
[a] Not performed.

and vomiting. There was hypoglycemia with a blood glucose of 0.6 mmol/l (normal value [NV] >2.5 mmol/l), standard base-excess −25.1 (NV +/−3) and serum lactate of 23.4 mmol/l (NV <2.0). He had a normal ECG. Initially he was treated with intravenous glucose, which was discontinued after four days. The following days his respiration normalized and he became clinically stable.

At three months, a brain CT showed bilateral temporal cortical atrophy and widespread hypodense areas in the white matter. He had poor eye contact and his mental development was markedly delayed. He developed liver failure with coagulopathy and encephalopathy and died at four months of age. A muscle biopsy showed hypotrophic muscle fibers and abnormal lipid vacuoles in the sarcoplasm. An autopsy showed severely disordered cortical migration with polymicrogyria, glandular hypospadia, micronodular cirrhosis and germ cell hypoplasia.

2.1.2. Patient 2

Patient 2 was the younger sister of patient 1, born two and a half years later. She was born after a normal pregnancy and delivery at 38 weeks. The birth parameters were all <3 SD. She developed hypoglycemia with lactic acidosis in the first day of life, and within days, signs of liver dysfunction. She had failure to thrive, with poor weight gain. She died 14 days old. Autopsy was not performed, but a postmortem muscle biopsy showed normal muscle histology.

2.1.3. Patient 3

Patient 3 was a girl, the second child of healthy non-consanguineous parents. She was born after a normal pregnancy and delivery at gestational age 41 + 5. Her birth weight and length were normal (2970 g and 51 cm, respectively), but her head circumference was

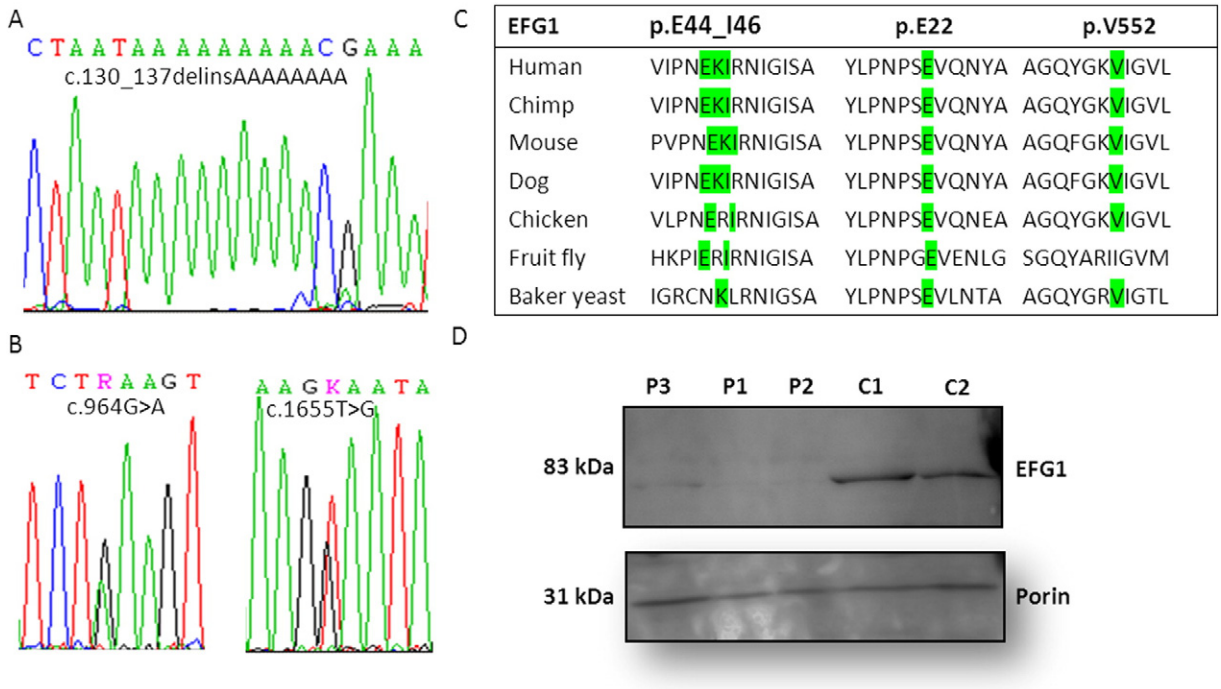

Fig. 1. Detection and characterization of the *GFM1* mutations. (A) Sequence traces of c.130_137delinsAAAAAAAA, (B) c.964G>A and c.1655T>G. (C) EFG1 sequence alignment between different species. All the affected amino acids are located in highly conserved regions across species. (D) Western blot analysis of EFG1, lanes P1–3: patients' fibroblasts, lanes C1–C2: controls' fibroblasts. The band of ~83 kDa corresponds to the expected size for EFG1. An antibody against porin was used as a loading control.