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Mutation of the human mitochondrial phenylalanine-tRNA synthetase causes infantile-onset epilepsy and cytochrome *c* oxidase deficiency☆

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ABSTRACT

Mitochondrial aminoacyl-tRNA synthetases (aaRSs) are essential enzymes in protein synthesis since they charge tRNAs with their cognate amino acids. Mutations in the genes encoding mitochondrial aaRSs have been associated with a wide spectrum of human mitochondrial diseases. Here we report the identification of pathogenic mutations (a partial genomic deletion and a highly conserved p. Asp325Tyr missense variant) in *FARS2*, the gene encoding mitochondrial phenylalanyl-tRNA synthetase, in a patient with early-onset epilepsy and isolated complex IV deficiency in muscle. The biochemical defect was expressed in myoblasts but not in fibroblasts and associated with decreased steady state levels of COXI and COXII protein and reduced steady state levels of the mt-tRNA^{Phe} transcript. Functional analysis of the recombinant mutant p. Asp325Tyr FARS2 protein showed an inability to bind ATP and consequently undetectable aminoacylation activity using either bacterial tRNA or human mt-tRNA^{Phe} as substrates. Lentiviral transduction of cells with wildtype FARS2 restored complex IV protein levels, confirming that the p.Asp325Tyr mutation is pathogenic, causing respiratory chain deficiency and neurological deficits on account of defective aminoacylation of mt-tRNA^{Phe}.

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1. Introduction

Human mitochondria possess their own translation machinery in order to produce 13 mitochondrially-encoded polypeptides that are subunits of the oxidative phosphorylation (OXPHOS) complexes. The translation machinery has a dual origin and comprises mitochondrially (mt-) encoded transfer RNAs (mt-tRNAs) and ribosomal RNAs (mt-rRNAs) as well as numerous, nuclear-encoded proteins including mitochondrial ribosomal proteins, initiation, elongation and termination factors, a methionyl-tRNA transformylase and mitochondrial aminoacyl-tRNA synthetases. Hence, mutations in either the mitochondrial genome (mtDNA) [1,2] or the nuclear DNA [3] can cause defects in mitochondrial protein synthesis resulting in a variety of mitochondrial disease phenotypes affecting both children and adults.

Aminoacyl-tRNA synthetases (aaRSs) play a key role in the faithful translation of the genetic code since they catalyse the attachment of each amino acid to its cognate tRNA. The human proteome includes two sets of aaRSs that are encoded by nuclear genes and are involved in either cytosolic or mitochondrial protein synthesis [4], with the exception of *GARS* and *KARS* that function in both domains. Based on the

Abbreviations: OXPHOS, oxidative phosphorylation; aaRS, aminoacyl-tRNA synthetase; mt-, mitochondrial; mtDNA, mitochondrial DNA; MRI, magnetic resonance imaging; LBSL, leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation; PCH6, pontocerebellar hypoplasia type 6; MLASA, myopathy, lactic acidosis and sideroblastic anaemia

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tRNA recognition mode and the domain organisation, aaRSs are divided into two classes: Class I aaRSs (mainly active as monomers) and Class II enzymes (mainly active as dimers or tetramers) [5]. In recent years, recessively-inherited mutations in a growing number of mitochondrial aminoacyl-tRNA synthetases of both classes have been associated with a diverse spectrum of early-onset mitochondrial clinical presentations [6]. These include mutations in the genes for *DARS2* causing leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) [7,8], *RARS2* causing pontocerebellar hypoplasia type 6 (PCH6) [9], *YARS2* causing myopathy, lactic acidosis and sideroblastic anaemia (MLASA) syndrome [10,11], *SARS2* causing hyperuricemia, pulmonary hypertension, renal failure in infancy and alkalosis (HUPRA) syndrome [12], *HARS2* associated with ovarian dysgenesis and sensorineural hearing loss [13], *AARS2* causing infantile cardiomyopathy [14], *EARS2* associated with leukoencephalopathy [15], *MARS2* causing neurodegenerative phenotype in flies and autosomal recessive spastic ataxia frequently associated with leucoencephalopathy (ARSAL) in humans [16], *LARS2* associated with premature ovarian failure and hearing loss in Perrault syndrome [17] and *KARS*, which encodes both the cytosolic and mitochondrial lysl-tRNA synthetases, and is associated with non-syndromic hearing impairment [18]. Recently, mutations have been reported in the *FARS2* (mitochondrial phenylalanyl-tRNA synthetase) gene in two families with infantile mitochondrial encephalopathy reminiscent of Alpers' syndrome [19,20]. In the present study, we report novel *FARS2* mutations including a large scale genomic deletion, in a child with muscle-restricted OXPHOS deficiency associated with intractable infantile epilepsy and abnormal brain MRI findings.

2. Material and methods

2.1. Patient case report

This young boy is the first child of healthy non-consanguineous, white British parents. He was born at term following an uneventful pregnancy weighing 3132 g (9th–25th centile). Early development was thought to be normal. At approximately 6 months of age he developed tonic upward eye deviation associated with flexion of his arms and neck consistent with infantile spasms. An electroencephalograph (EEG) at this time was grossly abnormal (hypsarrhythmia) and strongly supported a diagnosis of West Syndrome. Cranial MRI was reported as normal. Prednisolone was prescribed and treated the seizures effectively. Steroids were weaned over 6 weeks and he remained seizure free for a further 6 months. By the age of 1 year, it was apparent that his early developmental progress was not being maintained and that he was functioning at the 6–8 month developmental stage. Seizures returned shortly after his first birthday and were prolonged, frequent and on occasion focal, involving his right arm, leg and right side of face. Clonazepam briefly improved seizure frequency, but subsequently his epilepsy has proved refractory to various combinations of anticonvulsant therapy. Prolonged seizures of more than 60 min have been associated with a stepwise regression in his neurodevelopment. Seizure semiology is now predominantly one of epilepsia partialis continua involving the right side of his face, right arm and right leg. The development of focal seizures and the progressive nature of the condition prompted a second cranial MRI at the age of 2 years 6 months. By contrast with the previous scan, this MRI revealed symmetrical subcortical white matter lesions (Fig. 1A) with thinning of the anterior and genu of the corpus callosum (Fig. 1B).

On examination, the patient had small, round, anteriorly rotated ears and a broad nasal root. He demonstrated no visual awareness but startled to loud noise. Tone was increased in all 4 limbs with internal rotation of both legs at the hips. Reflexes were pathologically brisk. Brief myoclonic jerks were evident throughout the examination.

2.2. Structural investigation of the nuclear genome using genome-wide array

Early genetic screening included an Affymetrix Genome-wide Human SNP 6.0 array, which was used to detect DNA copy number changes in our patient. Samples were prepared and processed according to the manufacturer's specifications and analysed using locally-established methods [21]. Array CEL intensity files were loaded into Genotyping Console (Affymetrix UK Ltd.) for analysis. An approximately 88 kb deletion was identified within the short arm of chromosome 6, band p25.1 but no similar deletions were identified in the controls. This reported deletion was interrogated against ~5000 control samples using Nexus Copy Number v6.1™ (BioDiscovery Inc.). Quantitative polymerase chain reaction (qPCR) using the SYBR-Green method was used to confirm the reported deletion and determine the inheritance pattern [21]. Identification of the genes within the reported deletion locus was achieved using the UCSC genome browser build GRCh36/hg18 at the time.

2.3. LYRM4 and FARS2 gene sequencing

Total genomic DNA was obtained using standard methods and the coding region plus intron–exon boundaries of the *LYRM4* and *FARS2*

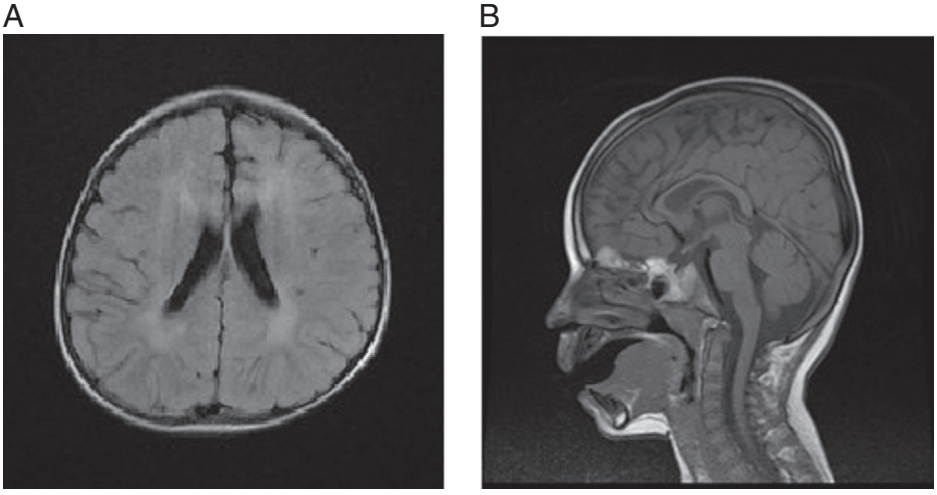

Fig. 1. Cranial MRI performed at age 2.5 years. A. Transverse T1 FLAIR image illustrating symmetrical, anterior predominant, white matter signal changes. B. Sagittal T1 weighted image demonstrating thinning of the anterior and mid portions of the corpus callosum.

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