Identification and functional characterization of a novel *MTFMT* mutation associated with selective vulnerability of the visual pathway and a mild neurological phenotype

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ABSTRACT

Mitochondrial protein synthesis is initiated by formylated tRNA-methionine, which requires the activity of MTFMT, a methionyl-tRNA formyltransferase. Mutations in *MTFMT* have been associated with Leigh syndrome, early-onset mitochondrial leukoencephalopathy, microcephaly, ataxia, and cardiomyopathy. We identified compound heterozygous *MTFMT* mutations in a patient with a mild neurological phenotype and late-onset progressive visual impairment. MRI studies documented a progressive and selective involvement of the retrochiasmatic visual pathway. MTFMT was undetectable by immunoblot analysis of patient fibroblasts, resulting in specific defects in mitochondrial protein synthesis and assembly of the oxidative phosphorylation complexes. This report expands the clinical and MRI phenotypes associated with *MTFMT* mutations, illustrating the complexity of genotype-phenotype relationships in mitochondrial translation disorders.

INTRODUCTION

Mitochondria maintain an independent translation system to synthesize the 13 essential structural subunits of the oxidative phosphorylation (OXPHOS) system encoded on the mitochondrial genome (mtDNA). Defective mitochondrial translation, first documented in patients with mutations in the translation elongation factor GFM1 [1], has been reported in an increasing number of neurologic conditions [2-7]. The mitochondrial protein synthesis machinery resembles that found in prokaryotes, and in both systems translation is initiated by formylmethionine. *MTFMT* encodes the mitochondrial methionyl-tRNA formyltransferase (MTFMT) that is responsible for formylating Met-tRNA^{Met} [8]. Unlike the system in prokaryotes, there is only a single Met-tRNA in mitochondria, so the ratio of fMet-tRNA^{Met} (for

initiation) to Met-tRNA^{Met} (for translation elongation) must be tightly regulated to ensure efficient mitochondrial translation.

Mutations in *MTFMT* were first identified by Mito exome sequencing (nuclear-encoded mitochondrial genes plus mtDNA) in two children with Leigh syndrome and a combined OXPHOS deficiency [9]. Subsequent studies reported several new *MTFMT* mutations and expanded the clinical phenotypes to include early-onset, severe encephalopathy with white matter and basal ganglia involvement, microcephaly, ataxia, and cardiomyopathy [10-13], and recently a relapsing-remitting form of demyelinating disorder [14].

Patients with *MTFMT* mutations presented either combined OXPHOS defects or isolated complex I deficiency [13,10,12,11,9] resulting from impaired mitochondrial translation. Strikingly, MTFMT was virtually undetectable in fibroblasts from all patients investigated, independent of the severity of the clinical phenotype [12,13,11]. The initial study on fibroblasts from two patients with Leigh syndrome reported a global translation defect [9]; however, two other studies [13,15] demonstrated that the protein synthesis defect is more selective, affecting most significantly 3 complex I subunits (ND5, ND4, ND1) and one complex IV subunit (COX II), consistent with the pattern of assembly defects in complexes I and IV observed by BN-PAGE analysis. The defects in OXPHOS assembly appear to be disproportionately severe relative to nature and extent of the translation defects, suggesting that translation can be initiated with unformylated methionine, but that the N-terminal formyl-methionine residue is somehow important for the subsequent assembly into a functional OXPHOS complex [13].

Whole exome sequencing (WES) has revolutionized the diagnostic workup of patients with unknown rare genetic conditions, uncovering novel causal genes even when very few or single cases from non-consanguineous families are available for analysis, and has contributed

substantially to the broadening of clinical and radiological phenotypes of known diseases. The current report significantly expands the clinical and radiological phenotype associated with *MTFMT* mutations.

METHODS

Patient

We investigated the first child of healthy, non-consanguineous parents of French Canadian ancestry, with a mitochondrial disorder of undetermined origin. The Institutional Review Board of our Institution approved the research study and the subject here reported gave her consent to participate in the study.

Mutation detection and functional analysis

Variants identified by WES performed on genomic DNA from the patient were annotated and filtered as previously described [13]. Functional analyses were performed as in [13].

RESULTS

Case report

The patient was born at term after an uneventful pregnancy. The psychomotor development was always considered mildly delayed for her age. Her weight and height growth were at the 3rd centile of the growth charts. At the age of seven years she presented with right fourth cranial nerve palsy. A brain MRI performed at that time revealed the presence of multiple areas of abnormal signal in the cerebral white matter, corpus callosum, basal ganglia, and brainstem, some of which with cystic degeneration. The involvement of the striata nuclei raised the possibility of a mitochondrial disease and the analysis of the respiratory chain performed on muscle biopsy tissue confirmed an assembly defect in complexes I and IV. Molecular testing for

common MELAS and MERRF mutations was negative. The patient was regularly followed up by neuro-ophthalmology and the fourth cranial nerve palsy improved spontaneously over time. A follow-up MRI performed at the age of 17 showed no major changes since the previous exam (Fig 1A-C). At the age of 18 years, bilateral vision loss developed over a period of two months, prompting the need for help in daily life activities involving vision. The ophthalmological evaluation performed at that time revealed vision of 20/200 in each eye, almost normal color vision (8/10 HRR plates, each eye), normally reactive pupils, and normal looking optic nerves and retinas. The electroretinogram was normal and flash and pattern visual evoked potentials were decreased. Subsequent examinations showed unchanged appearance of the optic nerves for six months until mild bilateral temporal optic atrophy appeared (Supplementary Fig 1A). At that moment vision had decreased to 20/250 OD and 20/400 OS, and color vision to 0/10 in both eyes. The follow up brain MRI showed selective bilateral involvement of the entire visual pathway, particularly severe at the level of the optic radiations (Fig 1D-F), and the presence of signal changes in the central portion of the brainstem. These specific findings were not present in the exam performed one year before (Fig 1A-C).

At the last neurological and ophthalmological examination, performed at the age of 24 years, the patient was oriented, cooperative and has mild cognitive deficit. She is autonomous for the majority of the daily life activities, requiring only minimal supervision for the visual impairment; she has a part-time employment for individuals with disabilities. The motor exam documented paratonia, hyperreflexia to the upper and lower limbs, and Babinsky sign bilaterally. Her vision was 20/800 OD and 20/500 OS, and both optic nerves became severely atrophic (Supplementary Fig 1B). On confrontational visual field, she also presented a left homonymous visual field deficit, in keeping with the mildly asymmetric postchiasmatic white matter changes.

Her medical history is negative for metabolic crises, diabetes, epilepsy and deafness. She never had cognitive or motor regression.

The last MRI exam was performed on a 3T Siemens TRIO machine with a specific protocol for white matter disorders (Fig 1G-I). The supratentorial white matter and corpus callosum abnormalities were unchanged since previous. The specific involvement of the optic pathway was again documented and appeared stable. The head of the putamina nuclei became atrophic. The MR spectroscopy did not show any lactate peak.

Genetic results

We identified two heterozygous variants in the *MTFMT* gene: the first variant (c.626C>T; p.Arg181Serfs5*) is a common, previously described, mutation [12,10,13,14,9], known to cause skipping of exon 4 and producing a premature stop codon. The second mutation (c.176C>T; p.Ala59Val) has never been reported before. It is predicted as damaging by SIFT [16] and probably pathogenic by PolyPhen-2 [17]. Clinical analysis of the gene confirmed the heterozygous status of the mother for the variant c.626C>T.

An immunoblot analysis of whole cell extracts from patient fibroblasts, showed that MTFMT was undetectable compared to control. This result is similar to that previously observed in an MTFMT patient with the phenotypically more severe Leigh syndrome (c.626C >T; p.Arg181Serfs5* and c.994C>T; p.Arg332*) which we used as a positive control [13], and indeed all previously investigated patients, suggesting that the missense substitution in MTFMT destabilizes the protein. In addition, the steady-state levels of ND1 and COX1, the core structural mtDNA-encoded subunits of complexes I and IV were decreased (Fig 2A). A mitochondrial pulse translation assay [18] showed a decrease in the rate of synthesis of ND5 (55%), ND4 (63%), and COX II (25%). The rates of synthesis of other mtDNA-encoded polypeptides were

similar or higher to those observed in controls. The global reduction in mitochondrial protein synthesis was smaller as compared to the previously published MTFMT patient (patient 1 in (Hinttala, et al., 2015)) (8% vs. 30%), which was consistent with the milder phenotype in the patient studied here (Fig 2A, C). An investigation of the assembly of OXPHOS enzyme complex by blue-native polyacrylamide gel electrophoresis (BN-PAGE) of patient fibroblasts showed a decrease in fully assembled complexes I and IV in fibroblasts and accumulation of a small amount of a subcomplex of complex V (F1), despite the fact the both mtDNA subunits were synthesized at greater than control rates, and the level of fully assembled complex V does not appear to be reduced (Fig 2D). Complex III was assembled normally and complex II was unaffected as it is encoded entirely by nuclear DNA.

To confirm the pathogenicity of the *MTFMT* mutation, we used a retroviral vector (pLSXH) to express a wild-type *MTFMT* cDNA in immortalized patient fibroblasts. Expression of *MTFMT* from the retroviral vector resulted in significantly higher steady-state levels of MTFMT than control levels in bulk culture, resulting in a dominant negative phenotype, with decreased translation of all mitochondrial polypeptides and decreased assembly of all OXPHOS complexes (Fig 2B, D) as we have previously observed [13]. We therefore isolated clones from the transduced patient fibroblasts to identify those expressing MTFMT at close to control levels. Analysis of two such clones showed rescue of the steady-state levels of ND1 levels, the protein synthesis (Fig 2B, C), and the OXPHOS assembly defect (Fig 2D). These results confirm that *MTFMT* is the gene responsible for translation and OXPHOS assembly defect in the patient.

DISCUSSION

Clinical and MRI heterogeneity is not unexpected in diseases caused by dysfunction of the mitochondrial translation machinery, but it remains largely unexplained. The biochemical defects we observed in our patient are similar to those previously reported in patients with more severe phenotypes and a fatal course [12,13,11]. Biochemical studies of the *in vitro* activity of two pathogenic *MTFMT* variants showed substantially decreased activities (>36-fold compared to wild-type) [19]. This, coupled with the fact that the steady-state levels of MTFMT are below immunodetectable levels in all reported studies of patient fibroblasts, implies that the residual activity of MTFMT must be exceedingly low. It is not, however, known what level of formylation could be supported under these conditions, or whether there are tissue-specific differences in the expression or activity of the pathogenic variants. Even small differences associated with different mutations, which would be very difficult to measure accurately, could make a substantial difference in the severity of the biochemical and clinical phenotype. Neither the global translation defect nor the OXPHOS assembly defect in our patient was as severe as in the Leigh syndrome MTFMT patient we used as a positive control (patient 1 in [13]), consistent with the milder phenotype we observed.

The initial clinical history of our patient corresponds to the spectrum of late onset Leigh disease caused by mutations in a nuclear gene [20-23]. However, the subsequent evolution, characterized by a sudden-onset, rapidly evolving visual deficit is unusual and unique.

The MRI findings documented in our case, in which the entire visual pathway is selectively involved with a symmetric and homogeneous pattern has not, to our knowledge, been reported before. The sensitivity of the visual pathway to mitochondrial stress is well known. Optic atrophy was previously reported in patients with *MTFMT* mutations [10,9], and is commonly associated with Leigh syndrome [24]. However, the involvement of the entire visual

pathway is novel. We cannot determine if the whole pathway was affected simultaneously; nonetheless, the delayed appearance of optic atrophy more than six months after the visual symptoms started suggests that the posterior visual pathway involvement dominated the initial clinical presentation. Furthermore, the most striking MRI finding in our case is the bilateral, symmetric and severe optic radiation damage. In Leber's hereditary optic neuropathy (LHON), another mitochondrial disorder, the microstructural involvement of the optic radiations- invisible to conventional MRI sequences- was documented with advanced imaging techniques, but it is still unclear whether it results from direct postgeniculate damage or anterograde degeneration [25,26]. In contrast to LHON, in our case the visual pathway changes were severe enough to be detected with conventional MRI sequences and they dominated the entire MRI picture. Infectious events or silent metabolic decompensation contributing to visual loss cannot be fully excluded in our case, but are unlikely given the clinical, ophthalmological and imaging presentation.

In conclusion, *MTFMT* mutations are associated with a wide variety of phenotypes that encompass very early onset and fatal disease to milder phenotypes with specific sensory involvement as illustrated here. Despite this dramatic variability, the underlying biochemical defect appears to be only subtly different across patients suggesting that clinical expression is influenced by small differences in residual MTFMT activity and genetic background.

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Figure legends

Figure 1. Brain MRI of the patient performed in 2009 (A-C), 2010 (D-F) and 2014 (G-I). A-C, axial T2-weighted images showing signal changes in the striata nuclei, with cavitations in the putamina (B, C), involvement of the splenium of the corpus callosum (C), and multifocal white matter abnormalities in the frontal regions. D-E, axial T2- and F, axial T1-weighted images documenting the symmetric involvement of the retrochiasmatic visual pathway, specifically of the optic radiations. Focal mesencephalic abnormalities are also seen (D). G-H, coronal and I, axial FLAIR T2-weighted images showing the selective damage of the optic radiations (arrows) (G-H), unchanged since previous, and the progressive atrophy of the striata nuclei (I).

Figure 2. Characterization and rescue of the biochemical defect in control and *MTFMT* patient fibroblasts (A) Pulse labeling of newly synthesized mitochondrial polypeptides in patient, a previously studied *MTFMT* patient with Leigh syndrome as a positive control (c.626C>T, c.994C>T), and control fibroblasts. The seven subunits of complex I (ND), one subunit of complex III (cyt b), three subunits of complex IV (COX), and two subunits of complex V (ATP) are indicated on the left (upper panel). Immunoblot analysis of MTFMT, COX I and ND1 (lower panel). The complex II 70-kDa subunit was used as loading control. (B) Rescue of the translation defect in two clones expressing MTFMT near control levels, and dominant negative effects associated with overexpression of MTFMT (upper panel). Immunoblot analysis of MTFMT and ND1 in the rescued clones (lower panel). The complex II 70-kDa subunit was used as loading controls (C) Quantification of the rate of protein synthesis in current patient compared with previously analyzed *MTFMT* patient with Leigh Syndrome. Rates of synthesis of individual polypeptides were normalized to the steady state level of the complex II 70-kDa subunit and are shown as the % of the average of four controls (top panel). Quantification of the rates of

synthesis of the individual polypeptides in the two rescued clones analyzed in (C). Rates of synthesis of individual polypeptides were normalized with steady state level of the complex II 70-kDa subunit and shown as the % of the average of two controls. (D) BN-PAGE analysis of the assembly of individual OXPHOS complexes in control and two rescued patient fibroblasts clones expressing wild-type *MTFMT*. Antibodies against an individual subunit of OXPHOS complex I-V were used for immunoblotting and complex II was used as the loading control.

Supplementary Figure 1. Fundus photographs showing the progressive atrophy of the left optic nerve (A) in 2010 and (B) at the last clinical follow up in 2014.