Early-Onset Cataracts, Spastic Paraparesis, and Ataxia Caused by a Novel Mitochondrial tRNA\textsubscript{Glu} (\textit{MT-TE}) Gene Mutation Causing Severe Complex I Deficiency: A Clinical, Molecular, and Neuropathologic Study

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Abstract
Mitochondrial respiratory chain disease is associated with a spectrum of clinical presentations and considerable genetic heterogeneity. Here we report molecular genetic and neuropathologic findings from an adult with an unusual manifestation of mitochondrial DNA disease. Clinical features included early-onset cataracts, ataxia, and progressive paraparesis, with sequencing revealing the presence of a novel de novo m.14685G→A mitochondrial tRNA\textsubscript{Glu} (\textit{MT-TE}) gene mutation. Muscle biopsy showed that 13% and 34% of muscle fibers lacked cytochrome c oxidase activity and complex I subunit expression, respectively. Biochemical studies confirmed a marked decrease in complex I activity. Neuropathologic investigation revealed a large cystic lesion affecting the left putamen, caudate nucleus, and internal capsule, with evidence of marked microvacuolation, neuron loss, perivascular lacunae, and blood vessel mineralization. The internal capsule showed focal axonal loss, whereas brainstem and spinal cord showed descending anterograde degeneration in medullary pyramids and corticospinal tracts. In agreement with muscle biopsy findings, reduced complex I immunoreactivity was detected in the remaining neuronal populations, particularly in the basal ganglia and cerebellum, correlating with the neurologic dysfunction exhibited by the patient. This study emphasizes the importance of molecular genetic and postmortem neuropathologic analyses for furthering our understanding of underlying mechanisms of mitochondrial disorders.

Key Words: Mitochondrial disease, Neurodegeneration, Neurologic impairments, Neuronal complex I deficiency, tRNA mutations.

INTRODUCTION
Mitochondrial DNA (mtDNA) diseases encompass a wide variety of disorders characterized by multisystem involvement and pronounced neurologic defects. Mitochondrial DNA diseases can be caused by mutations in either the nuclear DNA or mtDNA (in the form of point mutations or large-scale mtDNA rearrangements) (1). A surprisingly large proportion of pathologic mtDNA point mutations occur within the mitochondrial-tRNA (mt-tRNA) genes, although these only account for less than 10% of the coding mtDNA sequence (2, 3). Mitochondrial DNA genetics differ from nuclear genetics because the mitochondrial genome is maternally inherited and is present as multiple copies within a single cell, leading to a situation whereby mutated and wild-type mtDNA molecules can coexist within a cell, resulting in a phenomenon termed heteroplasmy. When the heteroplasmy level of mutated mtDNA reaches a critical threshold, the cell may develop a biochemical defect that can be detected using a histochemical assay for cytochrome c oxidase ([COX] complex IV) and succinate dehydrogenase ([SDH] complex II) activities. This histochemical stain typically reveals a mosaic pattern of COX...
activity in affected tissues. More recently, the development of immunohistochemical techniques using monoclonal antibodies has permitted the evaluation of individual subunits comprising the mitochondrial respiratory chain, thereby providing insights into the molecular pathogenesis of mtDNA disease (4).

There are a myriad of clinical features associated with mt-tRNA mutations that vary according to the nature of the mt-tRNA defect and tissue segregation of the mutation. Indeed, clinical manifestations of patients harboring the same mutation vary considerably and the m.3243A→G mutation is a good example of this diversity. This mutation within the MT-TE gene is the most common mt-tRNA mutation affecting approximately 1 in 400 people (5, 6) and has been shown to cause a number of different syndromes, including MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) (7), chronic progressive external ophthalmoplegia (8), and MIDD (maternally inherited diabetes and deafness) (9).

Although mt-tRNA mutations lead to considerable variation in clinical phenotype, progressive neurologic deficits are prominent and often are the most disabling feature of disease. Attempts to understand how neurologic impairments such as recurrent headaches, cerebellar ataxia, seizures, and cognitive decline develop have taken the form of neuroradiologic and postmortem studies. Typically, neuroradiologic investigation demonstrates atrophy, bilateral basal ganglia calcification, and gray matter T2-weighted hyperintensities (10, 11). Pathologic analysis of CNS tissues reflects the genetic and phenotypic heterogeneity associated with mt-tRNA mutations. The findings reveal diverse changes according to the mt-tRNA defect and variability within patient populations harboring the same mutated mt-tRNA. In patients with m.3243A→G, there typically are multiple ischemic-like lesions in posterior cerebral regions, including the occipital, temporal, and parietal cortices, and the cerebellum and abnormalities of the cerebral vasculature (12, 13). Patients with the m.8344A→G mutation associated with myoclonic epilepsy and ragged red fibers (MERRF) show severe degenerative CNS changes with neuronal cell loss and astroglialosis in the cerebellum, inferior olives, red nucleus, and substantia nigra (14, 15). Recently, we have shown that quantitative evidence of neuronal cell loss in the cerebellum is linked with severe respiratory chain defects for complexes I and IV, particularly in patients harboring the m.8344A→G, m.3243A→G, and m.14709T→C point mutations (14). Molecular analysis does not show a relationship between the heteroplasmic level of mutated mtDNA and pathology (14, 16); thus, the molecular mechanisms involved in determining cellular fate are not clear.

Here, we report on the case of a 45-year-old woman with an unusual clinical course of mtDNA disease, featuring cataracts and progressive spastic paraparesis, for whom a molecular genetic diagnosis was not forthcoming until after death despite a lifelong illness. Laboratory investigation of a muscle biopsy revealed clear mitochondrial abnormalities, leading to the identification of the novel mt-tRNA mutation within the MT-TE gene. The pathogenicity of this mutation was confirmed by single-fiber segregation studies. We also report the CNS neuropathology that included a large cystic lesion within the basal ganglia, degeneration of the pyramids, optic nerve microvacuolation, and loss of myelinated axons in conjunction with severe neuronal respiratory chain deficiency involving complex I. We correlate the neuropathologic features of this patient with her neurologic deficits and attempt to understand the mechanisms underpinning neural dysfunction and degeneration in relation to the presence of the pathogenic m.14685G→A mutation.

**MATERIALS AND METHODS**

All human studies were approved and performed under the ethical guidelines issued by Newcastle University, with written informed consent obtained from the patient’s family.

**Case Report**

The patient developed bilateral cataracts at age 7 years that were surgically treated. Ophthalmic examination at that time also revealed pigmentary retinopathy and evidence of severe peripheral retinal degeneration on electroretinogram. She developed bilateral sensorineural hearing impairment in her teenaged years, which, combined with the visual problems, led to a working diagnosis of either post–rubella syndrome, although there had been no positive history of maternal rubella during pregnancy, or Usher syndrome. No new neurologic features occurred until she was aged 40 years when she developed severe pain in both legs associated with walking difficulties and incontinence. Magnetic resonance imaging (MRI) scan at the time showed bilateral symmetric calcification in the basal ganglia and posterior thalami, with no evidence of a spinal cord lesion to account for her symptoms and no other obvious changes (Fig. 1A). Her walking...
deteriorated further during the next 5 years, with increasing unsteadiness and spasticity in the lower limbs resulting in a stiff broad-based gait. Computed tomographic and MRI scans at this time revealed severe cortical atrophy, calcification of the basal ganglia and posterior thalamus, and mild high-signal changes in periventricular white matter (Fig. 1B, C). The ventricles and cisterns were normal, as were the cerebellum and brainstem. At the age of 44 years, 5 months before her death, she developed sudden-onset right-sided weakness, aphasia, and dysphagia that lasted a few weeks. She became completely bed bound and displayed behavioral disturbances, with pressure of speech and signs of aggression. She also developed disturbed sleep patterns, followed by agitation and acute confusion requiring hospitalization. Physical examination at this time revealed sensorineural deafness, poor speech, persistent head tremor, and spasticity of all 4 limbs, with brisk reflexes and extensor plantars. Laboratory investigations revealed no evidence of infection or metabolic derangement. There was, however, an elevated plasma lactate level at 3.32 mmol/L (reference range, 0.7–2.2 mmol/L) and mildly elevated urinary pyruvate levels that prompted referral and a muscle biopsy. There were no clinical signs of hypertension or vascular disease. Her family history included a brother with spina bifida and a maternal grandmother diagnosed as having multiple sclerosis. She has 1 teenaged daughter who remains well.

The patient died at age 45 years from bronchopneumonia. A general autopsy did not show any significant abnormalities of the cardiovascular system. Specifically, gross examination revealed a heart weight of 240 g (reference range, 0.7–166 g). The coronary arteries at this site.

Muscle Histology, Histochemistry, and Respiratory Chain Analysis

Standard histologic and histochemical analyses of a left quadriceps muscle biopsy were performed on fresh-frozen 10-μm cryosections. Standard methods included hematoxylin and eosin (H&E) stain, oil Red O, Gomori trichrome stain, and sequential COX/SDH histochemistry to assay both complex IV (COX) and complex II (SDH) activities (17). Immunohistochemistry (IHC) was performed on patient and age-matched control muscle sections using the following antibodies: anti-complex I 20 kd (1:600; Mitosciences, Abcam, Cambridge, MA), anti–complex II 70 kd (1:800; Mitosciences), and anti-complex IV subunit I (1:1000; Mitosciences) to determine complex I, complex II, and complex IV expressions, respectively, according to previously described methods (18). The activities of the individual respiratory chain complexes were measured in a post–600 × gav muscle supernatant and expressed relative to the activity of the matrix marker enzyme citrate synthase (19).

Molecular Genetic Studies

Total DNA was extracted from several tissues provided by the patient, her mother, and her daughter by standard procedures and from individual muscle fibers using laser capture microdissection (Leica Microsystems AS-LMD 6000, Wetzlar, Germany). These tissues included samples of the index patient’s antemortem urine and skeletal muscle and postmortem cardiac muscle and of both mother and daughter’s urine and blood. Total DNA was extracted from formalin-fixed paraffin-embedded tissues (spinal cord, dorsal root ganglia [DRG], optic nerve, basal ganglia, cerebellum, medulla oblongata, and pons) using a NucleoSpin Tissue XS kit (Macherey-Nagel, Düren, Germany) and following the manufacturer’s instructions.

The entire sequence of the mitochondrial genome was determined using 36 pairs of M13-tagged oligodeoxynucleotide primers, as previously described (20). Amplified polymerase chain reaction (PCR) products were sequenced using BigDye Terminator v3.1 chemistries (Applied Biosystems, Cheshire, UK) on an ABI3130xL Genetic Analyser and directly compared with the revised Cambridge reference sequence (GenBank Accession No. NC_012920.1) using SeqScape software (Applied Biosystems). PyroMark Assay Design Software v.2.0 (Qiagen, Crawley, UK) was used to design locus-specific PCR and pyrosequencing primers, which amplified a 169-bp PCR product spanning the m.14685 nucleotide using a forward primer (nt 14584-14607) and a biotinylated reverse primer (nt 14730-14753). Pyrosequencing was achieved on the PyroMark Q24 platform according to the manufacturer’s protocol using a mutation-specific pyrosequencing primer (nt 14686-14702). PyroMark Q24 software was used to quantify the m.14685G→A heteroplasmy levels by directly comparing the relevant peak heights of both wild-type and mutant mtDNA at this site.

Neuropathology

Because of a lack of suitable age-matched control tissue, 5 control samples from patients between 68 and 78 years were obtained from the Newcastle Brain Tissue Resource to allow interpretation of the pathologic changes occurring in our patient (Table, Supplemental Digital Content 1, http://links.lww.com/NEN/A427). The processing of CNS tissues was performed as previously described (12).

After the removal and snap-freezing of patient cerebral and frontal cortex samples, the brain and spinal cord were formalin fixed for 12 days before routine neuropathologic examination. Sequential COX/SDH histochemistry was performed on frozen tissue sections as previously described (12). Sections were mounted on glass slides for quantitation of percentage COX-deficiency.

Sampling of the formalin-fixed brain for microscopic/immunohistochemical analysis on paraffin-embedded sections was restricted to the left basal ganglia, cerebellum, brainstem, spinal cord, DRG, and optic nerve. Histologic stains included H&E, cresyl fast violet, and Loyez for the assessment of cellular morphology, neuronal population density, and myelin, respectively, as previously described (12). Immunohistochemistry was performed on 5-μm-thick formalin-fixed paraffin-embedded sections using a panel of primary antibodies (Table, Supplemental Digital Content 2, http://links.lww.com/NEN/A428), as previously described (21). These included markers for components of the mitochondrial respiratory chain (complex I: anti–complex I 19 kd [CI-19], anti–complex I 20 kd [CI-20],...

**Quantification of Neuronal Respiratory Chain Deficiency**

Cells were identified as neurons depending on their clear cell body profile and size and a visible nucleolus. Respiratory-deficient neurons were identified either by IHC on formalin-fixed sections or by histochemistry on frozen sections that reveal protein expression levels or enzyme activity, respectively. A neuron was judged to be respiratory chain deficient by an absence of 3,3’diaminobenzidine staining in the neuronal cell cytoplasm after IHC or by blue staining of the neuronal cytoplasm after histochemical staining for COX/SDH (Figure, Supplemental Digital Content 3, http://links.lww.com/NEN/A429).

Gray matter regions were identified using a motorized Olympus microscope equipped with StereoInvestigator software (MBF Bioscience, Magdeburg, Germany). All neurons within the Purkinje cell layer, dentate nucleus, inferior olivary nucleus, and arcuate nucleus were counted. In the remaining gray matter nuclei, 5-mm² regions were outlined and all neurons within this area were assessed. The number of respiratory-deficient neurons and total number of neurons were then counted, and the proportion of respiratory chain–deficient neurons was given as a percentage. For control tissue, those respiratory chain markers where a high level of deficiency was observed in the patient were also quantified (Table, Supplemental Digital Content 4, http://links.lww.com/NEN/A430).

**Statistical Analysis**

Statistical analysis was performed using an unpaired Student t-test where a significant difference was determined when p < 0.05.

**RESULTS**

**Morphologic, Histochemical, and Biochemical Analyses**

Muscle histology showed fiber size variation, numerous “pyknotic nuclear bags,” and subsarcolemmal or diffuse basophilic granularity. Some fibers were vacuolated (Fig. 2A) and contained neutral lipid (Fig. 2B). A normal spatial distribution of type I and II fibers was detected, but type II fibers revealed atrophy (Fig. 2C). Sequential COX/SDH histochemistry documented 13% COX-deficient fibers (Fig. 2D); many of these showed mitochondrial accumulation typical of “ragged-red” changes (Fig. 2D insert). Analysis of the mitochondrial respiratory chain complexes suggested evidence of isolated complex I deficiency in muscle homogenate, most notably after comparing the activity of complex I with that of complex II (which is entirely nuclear-encoded); patient complex I activity showed a dramatic reduction.

**FIGURE 2.** Skeletal muscle biopsy histology, histochemistry, and immunohistochemistry (IHC). (A) Muscle histology shows numerous degenerative changes with fiber size variation, pyknotic nuclear bags, internal nuclei (arrowheads), and vacuolated (lipidized) fibers (hematoxylin and eosin). (B) Evidence of lipid accumulation (oil Red O). (C) Atrophy of type II fibers despite normal distribution of type I and II fibers on the ATPase (pH 9.9) reaction. (D) There is a mosaic pattern of cytochrome c oxidase (COX) deficiency (COX/succinate dehydrogenase [SDH] histochemistry); deficient fibers are blue. There is a granular and subsarcolemmal accumulation of mitochondria (insert, Gomori trichrome). (E, F) Control muscle fibers show high complex I expression (complex I 20 kd IHC) (E), whereas the patient muscle biopsy reveals evidence of multiple complex I deficient fibers ([F] complex I 20 kd IHC). (G, H) Complex II is intact in both control (G) and patient (H) (complex II 70 kd). (I, J) Control muscle fibers show high complex IV expression (II complex IV subunit I IHC); few complex IV–deficient fibers are present in the patient muscle ([J] complex IV subunit I IHC). Scale bar = 100 μm.
(0.323) relative to control values (0.817 ± 0.153). The activities of other respiratory chain enzymes, including complex IV, were all within reference ranges (Table 1). In agreement with these observations, further analysis of respiratory chain protein expression using IHC revealed that 34% of the muscle fibers lacked immunoreactivity for complex I 20 kd (Fig. 2F), whereas only 9% exhibited an absence of immunoreactivity for complex IV subunit I (Fig. 2H), in contrast to age-matched control tissues (Fig. 2E, G), whereas complex II 70 kd immunostaining was high in control and patient samples (Fig. 2I, K).

**Molecular Diagnosis of a Novel mtDNA Mutation**

Sequencing of DNA isolated from skeletal muscle revealed a novel heteroplasmic mtDNA mutation at position m.14685G>A in the MT-TE gene (Fig. 3A, B). This mutation affects an evolutionarily conserved Watson-Crick base pair within the T₃C stem loop of the mt-tRNAGlu molecule (Fig. 3C). Heteroplasmic levels of the m.14685G>A mutation were

<table>
<thead>
<tr>
<th>Complex</th>
<th>Patient</th>
<th>Controls (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>0.085</td>
<td>0.104 ± 0.036</td>
</tr>
<tr>
<td>Complex II</td>
<td>0.265</td>
<td>0.145 ± 0.047</td>
</tr>
<tr>
<td>Complex III</td>
<td>1.131</td>
<td>0.554 ± 0.345</td>
</tr>
<tr>
<td>Complex IV</td>
<td>1.242</td>
<td>1.124 ± 0.511</td>
</tr>
<tr>
<td>Complex I: Complex II</td>
<td>0.323</td>
<td>0.817 ± 0.153</td>
</tr>
</tbody>
</table>

All activities are expressed as ratios to the activity of citrate synthase ± SD. Complex I is expressed as nmol nicotinamide adenine dinucleotide dehydrogenase (NADH) oxidized.min⁻¹unit citrate synthase⁻¹. Complex II is expressed as nmol dichlorophenolindopheno (DCPIP) reduced.min⁻¹unit citrate synthase⁻¹. Complex III and Complex IV activities are expressed as first-order rate constants (K.sec⁻¹unit citrate synthase⁻¹).
determined in skeletal muscle, urine, and heart by pyrosequencing at 44%, 24%, and 7%, respectively. We performed single muscle fiber analysis to determine whether the amount of mutated mtDNA correlated with the observed biochemical phenotype in individual fibers. Substantially higher levels of the m.14685G>A mutation were detected in COX-deficient fibers (95.5% ± 3.5%, n = 14) than in the COX-positive fibers (29.9% ± 31.5%, n = 15) (p < 0.001, 2-tailed Student t-test), confirming segregation of the m.14685G>A genotype with respiratory chain dysfunction (Fig. 3D). To investigate the inheritance of the novel m.14685G>A variant, we screened blood and urine samples from the patient’s asymptomatic mother and daughter and did not find any evidence of the mutation, suggesting that the m.14685G>A mutation was de novo and had arisen sporadically. Screening of available patient CNS tissues confirmed the presence of the m.14685G>A mutation at high levels in the medulla oblongata (82%) and basal ganglia (68%), whereas moderately high levels were detected in the cerebellum (58%), spinal cord (52%), pontine nuclei (51%), DRG (48%), and optic nerve (44%).

Neuropathology

The fixed brain weight was 1,072 g (brainstem and cerebellum 131 g [12.2%]). Externally, the hemispheres were symmetric and demonstrated only mild widening of sulci posteriorly. There was also some variation in color and thickness of the cortical ribbon. Cut surfaces revealed bilateral palpably calcified globi pallidi. There was a cavitated calcified lesion in the left putamen crossing the top of the internal capsule and encroaching on the caudate nucleus (Fig. 4A); a smaller calcified region in the right putamen abutted the internal capsule. The hippocampi were normal, the lateral ventricles were mildly dilated, and the cerebral peduncles were narrow. The cerebellum showed a thin dentate nuclear ribbon but normal cortex and white matter. Lobar white matter was generally normal, and infarcts were not seen. Thus, neuropathologic assessment confirmed the distribution of atrophy, basal ganglia calcification, and white matter pathology observed on MRI and computed tomographic scans.

Microscopic examination of the left basal ganglia revealed a large cystic lesion involving the dorsal caudate nucleus, lateral putamen, and the interconnecting upper internal capsule, thus corresponding with a large elongated lacunar infarct (Fig. 4A). This lesion showed prominent neuropil microvacuolation, neuronal cell loss, perivascular lacunae, and concentric medial mineralization in arterioles, whereas capillaries showed droplet micromineralization (Fig. 4B, C). Foamy macrophages and hypertrophic astrocytes were prominent in the vicinity of the infarct (Fig. 4D, E). Transversely and obliquely cut segments of lenticulostriate arteries showed obvious atherosclerotic changes, including severe thickening of the intima, an atherosclerotic core filled with foamy macrophages, and focal fibrosis of the media (Fig. 4H). The lumen was significantly occluded. Immunohistochemistry confirmed the presence of vascular smooth muscle cell invasion of the intima and separation of the smooth muscle layer consistent with atherosclerotic changes (Fig. 4I, 1 and 2). Analysis of the endothelium showed decreased immunoreactivity for the endothelial-expressed protein GLUT1; this layer was often absent from arterioles (Fig. 4J, 3 and 4). Axonal spheroids labeled with nonphosphorylated neurofilament antibodies (indicative of axonal degeneration) were present in the neuropil adjacent to the cystic lesion. In the part of the internal capsule affected by the cystic lesion, there was prominent degeneration and loss of myelin and axons (Fig. 4K–N). There was mild microvacuolation and mild neuronal cell loss accompanied by astrogliosis and proliferation of macrophages in the caudate nucleus. In the internal globus pallidus and the preserved medial part of the putamen, there was extensive mineralization with the walls of small arterioles formed by concentric mineral aggregates, whereas capillaries showed droplet mineralization (Fig. 4F, G).

There was marked vessel wall mineralization in the deep white matter of the cerebellum (Fig. 4O) that was associated with loss of vascular smooth muscle and endothelial immunoreactivity. The cerebellar cortex demonstrated normal Purkinje cell complement and cytology, but there were numerous axonal swellings/torpedoes within the granular cell layer (Fig. 4P). The dentate nucleus showed some loss of the large neurons with the population of small neurons relatively preserved (Fig. 5E). Associated with this, there was a high density of CD68-positive activated microglia/macrophages and a high number of abnormally beaded astrocytic processes (Fig. 4Q, R). White matter myelin in this region was intact.

The pons demonstrated mild neuronal loss from the locus coeruleus with an increase in astroglia and some macrophage infiltration in the left corticospinal tracts. In the medulla oblongata, there was a moderate bilateral degree of myelin loss in the pyramids and prominent unilateral axonal degeneration in the left pyramid. There was loss of phosphorylated neurofilaments (as judged using SMI-31R; data not shown) that were replaced with nonphosphorylated neurofilaments (as judged by SMi-32P), myelin degeneration was evident with a Loyez stain; there was a high density of macrophages/activated microglial infiltration (as judged by anti-CD-68 IHC). In the spinal cord, neuronal density was preserved at all levels examined, but there was axon and myelin degeneration and loss in the medial portion of the right anterior funiculus; this most likely represented the distal portions of the corticospinal fibers lesioned in the left internal capsule. There was also moderate chronic bilateral loss of myelin and axons elsewhere in anterior and lateral funiculi of the spinal cord (data not shown) and prominent chronic bilateral loss of myelin and axons in the posterior funiculi, particularly in the gracile fasciculus and in the spinal roots (assessed with Loyez stain and SMi31R IHC), indicative of bilateral loss of neurons in the DRG. A few nodules of Nageotte were seen in a DRG.

Optic Nerve

Severe chronic degenerative changes with microvacuolation were observed in the optic nerve. There was a lack of myelin staining (H&E and Loyez) and lack of labeling with all neurofilament markers. Sparse rounded cells, most likely representing macrophages digesting cell debris, were detected at the periphery of the nerve using CD68, SMi-31R, and S100B IHC. Blood vessels showed fibrous hyalinization (data not shown).
FIGURE 4. Neuropathologic changes are prominent in the basal ganglia and cerebellum. (A) A coronal section of the cerebral hemisphere reveals atrophy and a cystic lesion on the left side involving the dorsal caudate nucleus, internal capsule, and lateral putamen (arrowhead). (B, C) The putamen shows neuropil microvacuolation (B cresyl fast violet), perivascular lacunae, and vessel wall mineralization (C hematoxylin and eosin), involving arterioles and capillaries with droplet mineralization. (D, E) Infiltration of foamy macrophages (D anti–CD-68) and hypertrophic astrocytes (E anti–glial fibrillary acidic protein [GFAP]) in the vicinity of the cystic lesion. (F, G) There is prominent droplet mineralization within capillaries in the internal globus pallidus (hematoxylin and eosin). (H) Atherosclerotic changes are present in the lenticulostriate arterioles with abnormal thickening of the vessel wall (hematoxylin and eosin), vascular smooth muscle cell invasion of intimal wall (I anti–α-smooth muscle actin), and separation of vascular smooth muscle cell layer (I anti–α-smooth muscle actin) and loss of the endothelium (I anti-GLUT1). (K–N) The internal capsule shows microvacuolation (K hematoxylin and eosin), myelin loss (L Loyez), axon loss (M anti-SMI31R), and degenerating axons and axon loss (N anti-SMI32P). (O) There is prominent mineralization of blood vessels in the cerebellar white matter (hematoxylin and eosin). (P) The cerebellar cortex shows numerous Purkinje cell axon torpedoes in the granular cell layer (anti-SMI31R). (Q, R) Activated microglia/macrophages (Q anti–CD-68) and abnormally beaded astrocytic processes (R anti–GFAP) are present within the dentate nucleus. Scale bar = 100 μm.
Mitochondrial Respiratory Chain Complex I Deficiency Is Prominent in Neuronal Populations

Further IHC evaluation of patient and control CNS tissues was performed using markers of the mitochondrial respiratory chain complexes to investigate possible abnormalities in their expression. This included analysis of subunits comprising complex I (Cl-19, CI-20, CI-30, and CI-39), complex II (CII-70), and complex IV (COX-I and COX-IV) (Fig. 5) (14). Neurons were deemed respiratory chain deficient when the neuronal cell body showed an absence of immunoreactivity. This permitted quantification of the percentage level of neurons demonstrating respiratory chain deficiency for the complete panel of mitochondrial antibodies (Table 2; Table, Supplemental Digital Content 4, http://links.lww.com/NEN/A430, for patient and control CNS regions, respectively). Of the 3 mitochondrial respiratory chain components investigated, expression of subunits comprising complex I were markedly affected throughout all CNS tissues investigated (Fig. 5). The most marked respiratory chain deficiencies were observed in the basal ganglia, particularly in the putamen, caudate nucleus, and insular cortex, where 94%, 92%, and 87% of the neuronal populations displayed loss of CI-20 immunoreactivity (Table 2; Fig. 5A, B). This observed respiratory deficiency was much higher than that seen in the same neuronal populations in control tissues (Table, Supplemental Digital Content 4, http://links.lww.com/NEN/A430). No similar reduction in the labeling was seen for complex II, whereas only mild loss of immunoreactivity was observed for complex IV, with only 6% and 1% of the neurons within the putamen displaying deficiencies for COX-I and COX-IV, respectively. Neurons and foamy macrophages adjacent to the putaminal lesion lacked complex I expression but retained weak expression of complex II and complex IV subunits. The choroid plexus demonstrated profound loss of complex I staining (Fig. 5C i) and only mild reduction of COX-I staining (Fig. 5C iii); CII-70 (Fig. 5C ii) and COX-IV (Fig. 5C iv) subunits were strongly expressed.

Abnormalities in the expression of complex I were prominent in the gray matter and white matter neurolpid in the cerebellum (Fig. 5D, E), affecting both Purkinje cells and the dentate nucleus neurons. This was in contrast to the immunoreactivity observed against CII-70, COX-I, and COX-IV subunits, where strong staining could be determined and only a small proportion of the neuronal population were affected (Table 2).

In agreement with the neurohistopathologic observations, the optic nerve tissues demonstrated an absolute loss of immunoreactivity for complexes I, II, and IV. This is likely a consequence of severe chronic degenerative changes associated with visual abnormalities of the patient. The inferior olivary nucleus neurons demonstrated moderately high levels of complex I deficiency, with 49% of neurons exhibiting CI-30 deficiency, and also showed an unusual distribution of mitochondria (as judged by expression of nuclear-encoded complex II), whereby only the periphery of the neuronal cytoplasm retained immunoreactivity. This is probably related to the premature accumulation of lipofuscin pigment in the neuronal cell body. A similar pattern was observed using antibodies to complex IV, including COX-I and COX-IV.

The pontine nucleus demonstrated milder complex I deficiencies, whereas subunits comprising complexes II and IV were highly expressed (Table 2; Fig. 5F). Throughout all spinal cord levels, evidence of mild complex I deficiency was observed in the motor neurons, which was highest in the lumbar levels, whereas complex II and complex IV expression was preserved (Table 2). Evaluation of the DRG situated adjacent to the spinal column showed only low levels of mitochondrial respiratory deficiencies (Table 2).

Despite the pronounced complex I deficiencies observed, biochemical COX deficiencies were present at relatively low levels in the neurons studied, affecting approximately 14.5% and 4.2% of neuronal populations in the frontal cortex and cerebellum, respectively. Intriguingly, blood vessels and glial cells within the white matter of the frontal lobe and cerebellum also demonstrated COX-deficiency (data not shown).

**DISCUSSION**

This study has allowed us to address clinicopathologic correlations for a previously unknown mutation and enabled us to explore the relationship between genotype and phenotype. We describe a 45-year-old woman with an atypical clinical presentation of mtDNA disease with early-onset development of cataracts followed by pigmentary retinopathy and hearing loss in her teenage years. Many years later, she developed progressive spastic paraparesis, ataxia, slurred speech, and incontinence. Subsequent molecular genetic testing identified a novel and sporadic mt-tRNA Glu m.14685G>A transition in the mitochondrially encoded MT-TE gene. Evaluation of affected tissues, including muscle and brain, revealed profound respiratory deficiency and degenerative changes confirming the pathogenicity of this novel mutation.

We have identified a novel heteroplasmic variant, m.14685G>A, within the mt-tRNA Glu molecule. Evaluation of affected muscle biopsy showed high levels of the m.14685G>A mutation and strong expression of the T-Q-C stem of the T-Q-C stem of the mt-tRNA Glu molecule. Eight other mutations are reported in the MT-TE gene, and these score as “definitely pathogenic” (http://www.mitomap.org/MITOMAP/MutationsRNA); 4 occur in the T-Q-C stem of the mt-tRNA. It is suggested that alterations in base composition of the T-Q-C stem might compromise the structural integrity of the mt-tRNA molecule (23). Further evidence from sequence homology analysis for this mutation showed that G-C base pairing is highly conserved in other species. Analysis of the affected skeletal muscle biopsy showed high levels of the m.14685G>A changes segregating with COX-deficiency in individual muscle fibers and therefore corroborates the pathogenicity of this mutation (22). Biochemical assessment of respiratory chain function in the patient’s muscle homogenate revealed mild complex I deficiency, whereas complex IV activity was within the reference range. Because biochemical measurements performed on homogenate muscle samples can often mask some of the subtle focal changes in enzyme activity, histochemistry and IHC was performed on muscle sections. This confirmed a mosaic pattern of COX-deficiency, with
TABLE 2. Percentage of Mitochondrial Respiratory-Deficient Neurons Throughout CNS Tissues in the Patient With the Pathogenic m.14685G>A Mutation, Aged 45 Years

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CI-19</th>
<th>CI-20</th>
<th>CI-30</th>
<th>CI-39</th>
<th>CII-70</th>
<th>COX-I</th>
<th>COX-IV</th>
<th>COX/SDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>14.5</td>
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<tr>
<td>Insular cortex</td>
<td>75</td>
<td>87</td>
<td>79</td>
<td>90</td>
<td>0</td>
<td>14</td>
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All respiratory deficiencies are expressed as percentage levels.
*Neurons for which overall immunoreactivity was low.
†Neurons for which there was variability in immunoreactivity without evidence of deficiency.

FIGURE 5. Selective complex I deficiency in multiple brain regions. (A-G) Immunohistochemical analysis of the mitochondrial respiratory chain proteins shows prominent loss of complex I expression throughout the CNS. The cystic lesion within the putamen shows a near global loss of complex I subunit expression ([A] i, complex I 19 kd [CI-19]), whereas complex II 70 kd (CII-70) ([A] ii) and IV remain relatively intact ([A] iii and iv, cytochrome c oxidase I [COX-I] and COX-IV). The insular cortex shows a dramatic loss of complex I subunits ([B] i, CI-19), preserved complex II expression ([B] ii, CII-70) and mild loss of complex IV subunits ([B] iii and iv, COX-I and COX-IV). A similar pattern of respiratory deficiency is observed in the choroid plexus, with dramatic loss of complex I subunits ([C] i; CI-19), relatively intact expression of complexes II ([C] ii, CII-70) and IV ([C] iii and iv, COX-I and COX-IV). The cerebellar Purkinje cells show moderate deficiency for complex I ([D] i, CI-19), with some variation in complex II expression ([D] ii, CII-70) and minimal complex IV deficiency with COX-I ([D] iii, COX-I), whereas nuclear-encoded COX-IV is unaffected ([D] iv, COX-IV). The dentate nucleus reveals numerous complex I-deficient neurons ([E] i, CI-19), altered complex II expression ([E] ii, CII-70) and mild complex IV deficiencies ([E] iii and iv, COX-I and COX-IV). The neurons within the basilar pontine nuclei demonstrate a mild complex I deficiency ([F] i, CI-19) and preserved expression of complexes I ([F] ii, CI-19) and complex IV subunits ([F] iii and iv, COX-I and COX-IV). Inferior olivary neurons show moderately high number of complex I-deficient cells ([G] i, CI-19) in conjunction with altered CI-70 expression indicative of a lack of cytoplasmic staining ([G] ii, CII-70). In this nucleus, mild neuronal deficiency of COX-I is present ([G] iii, COX-I), whereas nuclear-encoded COX-IV is unaffected ([G] iv, COX-IV). Scale bar = 100 μm.
As a consequence, portions of the descending projections from the internal capsule to the left pyramid and the medial portion of the right anterior corticospinal tract in the cervical spinal cord were affected by axonal and myelin degeneration.

A large cystic lesion was observed in the basal ganglia, with evidence of microvacuolation, neuronal cell loss, foamy macrophages, and astroglial cell proliferation. This lesion is similar to those frequently described in late-stage ischemic-like lesions documented in mtDNA disease (10–12, 28). In conjunction, microvascular abnormalities consisting of atherosclerotic changes and blood vessel mineralization were prominent. Although atherosclerotic changes and blood vessel mineralization can occur in basal ganglia of aged individuals, they are highly unusual in a 45-year-old person. The precise mechanisms leading to vascular mineralization remain unknown, but some studies suggest that smooth muscle cell or pericyte dysfunction may lead to osteogenic differentiation of these cells into osteoclast and osteoblast cells (29, 30). We have previously speculated that mitochondrial dysfunction within the smooth muscle layer could initiate smooth muscle cell transdifferentiation as a protective mechanism (31). These abnormalities are particularly interesting given the location of the lesion, which corresponds well with the watershed territory supplied by the lenticulostrate arteries and distal arteries of the middle cerebral artery (32). This allows us to speculate that mitochondrial defects in the microvasculature led to functional and pathologic changes in the vessels, that combined with a lack of functional redundancy in the lenticulostrate arteries, were directly responsible for the formation of this lesion (33). It is noteworthy that this patient did not show clinical signs of diabetes or hypertension, which are known risks of development of infaracts. It is intriguing that putaminal lesions are frequently seen on MRI in patients harboring MT-TLI mutations (34) and patients with Leigh syndrome (35). We hypothesize that the vascular trees of the short perforating lenticulostrate arteries might be particularly vulnerable to defects in mitochondrial metabolism; this presents an interesting area for further examination.

Ataxia is a common clinical manifestation in patients with mtDNA disease, and neuropathologic changes and respiratory chain deficiencies have been documented within the olivo-cerebellum (14, 36–38). Although our patient exhibited ataxia, the cerebellum showed only mild neuron loss from the dentate nucleus, some changes in cerebellar white matter microvasculature, and Purkinje cell torpedoes. It is feasible to suggest that these changes, together with the loss of sensory neurons and their peripheral and central projections, contributed to the genesis of her ataxia.

Our understanding of mtDNA disease pathogenesis has been facilitated by the use of IHC techniques to evaluate protein expression of mitochondrial respiratory chain complexes (4). Our analysis, using monoclonal antibodies raised against mitochondrial respiratory complexes I, II, and IV, suggested that the basal ganglia, inferior olives, and cerebellum had the most striking deficiencies for subunits of complex I, with near total loss of subunits expression in neuronal populations within the basal ganglia. This correlates with our finding of high heteroplasmy of the m.14685G>A defect in these brain regions. In addition, subtle complex IV deficiency was also observed in a small proportion of neurons. In other CNS regions where minimal degenerative changes occurred, milder complex I deficiency could be seen, including the pontine nuclei and spinal cord posterior neurons. This, in conjunction with our muscle biochemical data, confirms that complex I subunits are most sensitive to the novel m.14685G>A mutation in mt-tRNA\textsubscript{Gln}. A propensity toward development of neuronal complex I deficiency has been described previously in the olivo-cerebellum of a large cohort of patients with mtDNA disease (14). Understanding the molecular mechanisms underpinning neuronal complex I deficiency is therefore an important area for future investigation.

This study builds on the complex clinical picture seen in patients with mtDNA disease and highlights the importance of elucidation of the precise molecular defect involved in disease pathogenesis. The identification of mtDNA defects is particularly crucial when providing families with appropriate genetic counseling. Although we were not able to investigate postmitotic tissues from our patient’s daughter, the available evidence suggests that the mutation arose sporadically and has not been transmitted and, as such, the daughter is not likely at risk for developing disease as a consequence of the m.14685G>A mutation. However, situations involving mt-tRNA mutations typically require careful consideration for genetic counseling when the mutation may be passed on to the offspring. Because mtDNA diseases caused by pathogenic mt-tRNA mutations are notoriously difficult to treat, understanding the molecular mechanisms underpinning neurologic impairments in these patients is integral to the development of appropriate treatment strategies.

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