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Loss of Myelin-Associated Glycoprotein in Kearns-Sayre Syndrome

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Abstract

Objective—To explore myelin components and mitochondrial changes within the central nervous system in patients with well-characterized mitochondrial disorders due to nuclear DNA or mitochondrial DNA (mtDNA) mutations.

Design—Immunohistochemical analysis, histochemical analysis, mtDNA sequencing, and real-time and long-range polymerase chain reaction were used to determine the pathogenicity of mtDNA deletions.

Setting—Department of Clinical Pathology, Columbia University Medical Center, and Newcastle Brain Tissue Resource.

Patients—Seventeen patients with mitochondrial disorders and 7 controls were studied from August 1, 2009, to August 1, 2010.

Main Outcome Measure—Regions of myelin-associated glycoprotein (MAG) loss.

Results—Myelin-associated glycoprotein loss in Kearns-Sayre syndrome was associated with oligodendrocyte loss and nuclear translocation of apoptosis-inducing factor, whereas inflammation, neuronal loss, and axonal injury were minimal. In a Kearns-Sayre syndrome MAG loss region, high levels of mtDNA deletions together with cytochrome- *c* oxidase–deficient cells

and loss of mitochondrial respiratory chain subunits (more prominent in the white than gray matter and glia than axons) confirmed the pathogenicity of mtDNA deletions.

Conclusion—Primary mitochondrial respiratory chain defects affecting the white matter, and unrelated to inflammation, are associated with MAG loss and central nervous system demyelination.

Demyelination resulting from oligodendrocyte dysfunction or a distal "dying-back" oligodendrogliopathy has been distinguished from direct injury to myelin based on preferential loss of myelin-associated glycoprotein (MAG).[1-6] MAG is expressed in oligodendrocyte processes at the axoglial junction, the most distal part of the cell.[7] Preferential loss of MAG relative to myelin basic protein (MBP) and damage to oligodendrocyte processes are neuropathologic features described in acute lesions of multiple sclerosis (MS), white matter stroke (WMS), and progressive multifocal leukoencephalopathy (PML).[1,3- 5,8- 9] Cellular energy failure has been implicated in the pathogenesis of demyelination under these inflammatory and hypoxic conditions, where modes of tissue damage are multiple. Whether preferential loss of MAG is a feature of primary mitochondrial disorders,[10] due to either mutations in nuclear genes encoding mitochondrial proteins or mitochondrial DNA (mtDNA) and where inflammation is minimal, is not known.

Mitochondria are responsible for the generation of adenosine triphosphate through oxidative phosphorylation.[11] However, they are also known to play a pivotal role in apoptosis and calcium handling. Uniquely, mitochondria contain their own circular genome (mtDNA), which encodes for 13 subunits of the mitochondrial respiratory chain complexes, all excluding complex II.[11] The central nervous system (CNS) is frequently affected in primary mitochondrial disorders, and white matter disease (myelinopathy) is described as the neuropathologic hallmark of Kearns-Sayre syndrome (KSS).[10- 13] Whether there is evidence of preferential MAG loss in KSS, which is caused by either a clonally expanded, single, large-scale deletion or a complex rearrangement of mtDNA or indeed other primary mitochondrial disorders where the gray matter is predominantly affected, is not known.

In this study, we explored the expression of CNS myelin components, including MAG and MBP, in autopsy tissue from patients with well-characterized primary mitochondrial disorders, including KSS, myoclonic epilepsy with ragged red fibers, mitochondrial encephalopathy lactic acidosis and strokelike episodes, mitochondrial neurogastrointestinal encephalomyopathy, Leber hereditary optic neuropathy, and mitochondrial disease due to *POLG* (mitochondrial DNA polymerase gamma) mutations (Table).[10,17] We show preferential loss of MAG in patients with KSS. In patients with mitochondrial disorders other than KSS, preferential MAG loss was not present, and all myelin components were equally lost within affected regions. High levels of mtDNA deletions, cytochrome-*c* oxidase (COX)–deficient cells, and a loss of mitochondrial respiratory chain complex subunits in a MAG loss region in KSS confirmed the pathogenicity of mtDNA mutations. Our findings in KSS indicate an association between mitochondrial respiratory deficiency in white matter and the development of distal dying-back oligodendrogliopathy.

METHODS

Autopsy Samples

Samples from patients 2 through 5, 10, 11, and 14 were obtained from the Department of Clinical Pathology, Columbia University Medical Center, and the remaining samples were from the Newcastle Brain Tissue Resource. All cases of mitochondrial disease were diagnosed before death, and these patients underwent standard diagnostic tests, including a

muscle biopsy. Patient 1 with KSS developed a left-sided, divergent strabismus at the age of 3 years. She developed bilateral ptosis and progressive external ophthalmoplegia at 15 years of age and impaired vision and trifascicular block at 27 years of age. In addition, neurologic examination revealed impaired cognition and hearing, retinal pigmentation, proximal myopathy, and marked cerebellar ataxia. Magnetic resonance imaging of the brain revealed white matter hyperintensities and cerebellar atrophy. A muscle biopsy revealed COX-deficient, ragged red fibers (5%), and a single, large-scale mtDNA deletion was detected in routine diagnostic testing. She died at 40 years of age after a respiratory infection. Details of patients 2 and 3, who had KSS due to 12.2- and 10.3-kb mtDNA deletions, respectively, were previously reported in a study of respiratory chain subunit expression.[14] A number of additional patients with mitochondrial disease due to mtDNA or nuclear DNA mutations and age-matched controls without neurologic disease were evaluated (Table). The diseases included Leber hereditary optic neuropathy, mitochondrial encephalopathy lactic acidosis and strokelike episodes, myoclonic epilepsy with ragged red fibers, mitochondrial neurogastrointestinal encephalomyopathy, and *POLG* mutations. All cases received a molecular diagnosis after death.

In total, 54 fixed tissue blocks from patients with primary mitochondrial disorders were studied. Inflammatory components were compared with cases (eTable 1) in which MAG loss has already been reported (pattern III MS,[9] WMS,[1] and PML[8]).

Tissue Preparation And Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded (FFPE) and snap-frozen tissues from the cerebral and cerebellar hemispheres and the spinal cord were processed as previously described.[17] Institutional ethical approval was obtained for all tissues used in this study. To evaluate the extent and nature of neuropathologic changes, we applied routine histopathologic stains on FFPE tissue sections, including cresyl fast violet stain for the neuronal population density, Loyez stain for myelin, and Bielschowsky silver stain for axons. Immunohistochemical analysis was performed on 5 μm of FFPE tissue from the cerebellum, occipital lobe, spinal cord, and frontal and parietal lobes using a number of antibodies against myelin, axons, macrophages, and mitochondrial proteins (eTable 2). Detection of primary antibodies was performed using a polymer detection system (Menapath kit; Menarini Diagnostics), visualized with 3,3′-diaminobenzidine. Loss of MAG when remaining myelin proteins were relatively spared was termed preferential MAG or MAG loss.

Quantification Of Olig2- And Cd68-Positive Cells And Densitometric Analysis Of Myelin Immunoreactivity

The number of CD68- and Olig2-positive cells stained using the corresponding antibodies was determined in regions of white matter with an area of approximately 10 000 μm^2 using StereoInvestigator software (MBF Bioscience). Immunoreactivity of myelin components and 2′,3′-cyclic nucleotide phosphodiesterase (CNPase) was densitometrically assessed using Axiovision (Carl Zeiss Microimaging GmbH).

Cox And Succinate Dehydrogenase Histochemical Analysis

For histochemical studies, sequential COX (complex IV) and succinate dehydrogenase (SDH or complex II) analysis was performed, as previously described,[18] on 10-μm, snap-frozen sections. Images were captured with a BX54 microscope (Olympus America, Inc). For molecular genetic studies, 20-μm frozen sections were mounted on polyethylenenaphthalate-membrane slides, subjected to sequential COX and SDH histochemical analysis, and air-dried after ethanol dehydration. A variety of different regions were excised using a Leica Laser Microdissection System (Leica Microsystems) and lysed overnight according to previous methods.[19]

Neuronal Density

We subjected 20-μm FFPE cerebellar sections from KSS patients and 7 age-matched controls to cresyl fast violet stain and subsequently analyzed the sections using StereoInvestigator software (MBF Bioscience). The boundaries of the gray matter were outlined, and the total number of neurons, defined by possessing a nucleolus and clear cell body profile, were counted. A neuronal cell density was then derived.

Long-Range Polymerase Chain Reaction

Long-range polymerase chain reaction (PCR) was performed using the Expand Long Template PCR system and DNA was extracted from homogenates of specific brain sections prepared using a QIAamp DNA Micro Kit (QIAGEN, Inc) per the manufacturer's guidelines. The following cycle conditions were used: 3 minutes at 93°C; 10 cycles of 93°C for 30 seconds, 58°C for 30 seconds, and 68°C for 12 minutes; 20 cycles of 93°C for 30 seconds, 58°C for 30 seconds, and 68°C for 12 minutes plus 5 seconds per additional cycle; and a final extension of 11 minutes at 68°C. The PCR products were diluted 1:30, and 1 μL was used as a template for a second round of amplification using the same conditions as previously mentioned. Primers were designed to cover the major arc of the mitochondrial genome, as previously described.[20] DNA from a normal blood sample was used as a positive control.

Gel Extraction And Sequencing

To confirm the presence of mtDNA deletions, amplified products were extracted using a gel extraction kit (QIAGEN, Inc) and cycle sequenced on an ABI3100 Genetic Analyzer (Applied Biosystems), as previously described.[20] Sequences were compared with the revised Cambridge reference sequence using SeqScape software (Applied Biosystems).

Real-Time Pcr

A multiplex real-time PCR assay using specific fluorogenic Taqman probes (Applied Biosystems) was used to determine the relative amplification of mtDNA within the *MTND1* and *MTND4* genes, as previously described.[19] This quantitative assay allows for calculation of the percentage level of deleted mtDNA and was performed in a control (white and gray matter), KSS patient 1 (white and gray matter), KSS patient 15 (*POLG* mutation; white matter), and KSS patient 17 (*POLG* mutation; white matter).

Statistical Analysis

Two-way analysis of variance was used to assess differences between more than 2 groups. The Bonferroni test was used to compare differences between 2 selected groups. Minitab statistical software, version 15 (Minitab, Inc), was used and $P < .05$ was considered significant.

RESULTS

We determined the pattern of MAG, MBP, myelin oligodendrocyte glycoprotein (MOG), and CNPase loss in a number of primary mitochondrial disorders due to mutations of mtDNA (deletions or point mutations) or nuclear genes encoding mitochondrial proteins (Table).[10,17] We then performed a detailed analysis of mtDNA and mitochondrial respiratory chain subunits and activity within a preferential MAG loss region and adjacent gray matter (dentate nucleus) from KSS patient 1, for whom snap-frozen mirror image tissue blocks were available.

Preferential Loss Of Mag In KSS

Preferential MAG loss and CNPase loss, which mirrors MAG loss, were seen in KSS (Figure 1A-D). The MAG and CNPase loss was particularly apparent at high magnification in cerebellar white matter in KSS patient 1 when compared with controls (Figure 1A and B). Within the same region, MBP and MOG staining in serial sections was relatively spared (Figure 1C and D). In all other affected regions in KSS patients (n = 3), preferential MAG loss was seen in 5 additional regions (eFigure 1A), with the remaining regions showing equal loss of MBP, MOG, MAG, and CNPase in serial sections. Evaluation of other primary mitochondrial cases did not show preferential loss of MAG. In mitochondrial disorders other than KSS, myelin proteins were either intact in regions without neuroaxonal degeneration or equally absent in regions often with severe neuroaxonal degeneration (eFigure 1B and C and (eFigure 2).

The assessment of Olig2-positive nuclei showed a significant reduction in oligodendrocyte lineage cells in the MAG loss regions of all 3 KSS patients compared with controls (Figure 2A, B, and D; P = .006). In contrast, Olig2 nucleus counts did not significantly differ in nondemyelinated regions of KSS cerebrum compared with corresponding white matter from control cases or nondemyelinated cerebellum from other mitochondrial cases. There was evidence of nuclear translocation of the apoptosis-inducing factor, which is stored in the intermembrane space of mitochondria,[21] in MAG loss regions of KSS patients (Figure 2C). In contrast, only cytoplasmic apoptosis-inducing factor staining was seen within adjacent neurons in KSS dentate nucleus, consistent with its physiologic localization within mitochondria. Neuronal density within the dentate nucleus of KSS patient 1, in whom preferential MAG loss was seen, was comparable ($43.20/mm^2$) to controls ($55.86 \pm 7.61/mm^2$, n = 8), and standard neuropathologic methods revealed relative sparing of axons and neurons in the dentate nucleus (Figure 2E-G). Given that inflammation was a prominent feature of all the patients with distal dying-back oligodendrogliopathy, we determined the density of CD68-positive cells in primary mitochondrial disorders and compared this with the pattern III MS, WMS, and PML lesions. As expected, the density of CD68-positive cells in KSS and other primary mitochondrial disorders was significantly lower than in acute pattern III MS, WMS, or PML lesions (Figure 2H-J, P = .01) and not significantly different from controls.

Association Of Mag Loss With Pathogenic Levels Of mtDNA Deletion And Respiratory Deficiency In Oligodendrocytes And Astrocytes

A single mtDNA deletion was detected, using long-range PCR, in tissue homogenates from dentate nucleus gray and white matter in KSS patient 1, in whom preferential MAG loss was evident and snap-frozen tissue available (Figure 3A). Sequencing of deleted mtDNA extracted from long-range PCR identified the deletion breakpoints at positions 11657 and 15636, giving rise to a deletion of 3978 base pairs (bp) associated with an imperfect 12-bp repeat (Figure 3C). Real-time PCR revealed a significantly higher heteroplasmy level of mtDNA deletions (the percentage of mutant to total mtDNA copies) in the dentate nucleus white matter (77.1%) compared with gray matter (44.3%, P = .02). This finding was in contrast to the patients who harbored multiple mtDNA deletions (3 *POLG* patients) and lacked evidence of preferential MAG, where the levels were low (Figure 3B). The pathogenicity of the single mtDNA deletion in the white matter of the dentate nucleus in this case (KSS patient 1) was supported by a disproportionate decrease in the immunoreactivity of the mitochondrial respiratory chain complex I 30-kDa subunit and the COX subunit I (COX-I, Figure 4A-D) compared with the complex II 70-kDa subunit and porin in the white matter. This pathogenicity was also supported by cells lacking COX activity after COX and SDH histochemical analysis (those cells deficient in COX activity but with intact SDH activity stain blue) (Figure 4H and eFigure 3D). Oligodendrocytes (Figure 4F, red) and

astrocytes (Figure 4G, red) in this case contained mitochondria (green) but were devoid of COX-I (blue) relative to controls (eFigure 3A and B), whereas most axonal mitochondria contained COX-I (Figure 4E). In contrast to the dentate nucleus white matter, COX-deficient cells were not present in the dentate nucleus gray matter in this case (eFigure 3C), and COX and complex II subunits were intact in neurons (Figure 4J-L). Furthermore, COX-deficient axons were not detected in the white matter. Neurons in the dentate nucleus of this case were not completely spared by the mtDNA deletion because several cells with faint complex I 30-kDa staining were observed in the dentate nucleus gray matter neurons (Figure 4K) compared with controls (Figure 4K).

COMMENT

Oligodendrocyte dysfunction and a distal dying-back oligodendrogliopathy are considered important in the pathogenesis of a number of CNS disorders,[22-23] including MS.[9] For the first time, we show preferential MAG loss, a sign of distal dying-back oligodendrogliopathy, in a primary mitochondrial disease, KSS.

Several findings in KSS point toward a pathogenic role for mtDNA deletions in the white matter, including the following: (1) presence of cells devoid of COX activity in white but not gray matter, (2) more prominent decrease in mitochondrial respiratory chain complex subunits in white than gray matter, (3) much greater mtDNA deletion levels in dentate nucleus white matter than in gray matter, and (4) loss of oligodendrocytes and nuclear translocation of apoptosis-inducing factor in the MAG loss regions, which is similar to findings after experimental demyelination.[24] Compared with the KSS white matter, the effect of mtDNA deletions on neurons in dentate nucleus was subtle, and axonal injury was rarely detected where MAG loss was apparent. It is possible that dysfunction of neurons due to complex I defects contributed to the MAG loss in KSS. In this regard, reports of preferential MAG loss after wallerian degeneration in spinal cord injury patients and experimental spinal cord trauma are worthy of note.[25-26] In such situations, preferential MAG loss occurred in conjunction with loss of axonal proteins and extensive degradation of the distal portions of axons by calpains. Activated calpains were suggested to also degrade myelin closest to the axons (MAG) after spinal cord injury.[25] In the white matter of KSS patients, however, the low rate of chronic axonal degeneration is unlikely to have extensively and diffusely activated calpains, or an unidentified protease,[4] to cause the MAG loss. Furthermore, absence of preferential MAG loss in primary mitochondrial cases other than KSS, where neurodegeneration is well recognized, suggests that oligodendrocyte dysfunction in KSS was not secondary to neuronal and axonal degeneration or dysfunction.

The single mtDNA deletion in KSS removed several functionally important protein-coding and *tRNA*genes. Complex I subunits are directly affected, and the removal of specific *tRNA* genes would directly lead to a defect in overall mitochondrial protein synthesis, including COX-I. For a biochemical defect to manifest, the heteroplasmy level of a mtDNA deletion needs to exceed a certain threshold, generally 60%, because there are multiple copies of mtDNA in a single cell.[27-28] In KSS patients, the white matter showing MAG loss contained above-threshold heteroplasmy levels of mtDNA deletion, whereas the gray matter and the corresponding white matter from control cases and those with other primary mitochondrial disorders harboring mtDNA deletions and without MAG loss (*POLG* patients) showed below-threshold levels. The pathogenic levels of mtDNA deletions in the KSS white matter had rendered both oligodendrocytes and astrocytes respiratory deficient. On the basis of the findings of this study, it is not possible to establish whether the energy defect in oligodendrocytes and/or astrocytes was the driving force of oligodendrocyte dysfunction. Metabolites and ions are exchanged between astrocytes and oligodendrocytes through gap junctions, disruption of which causes white matter disease, including loss of

oligodendrocytes.[29]Whether distal dying-back oligodendrogliopathy due to energy failure is a direct consequence of mitochondrial defects within oligodendrocytes or an indirect process due to lack of metabolic support from compromised astrocytes may be investigated using relevant animal models.

Although preferential MAG loss was detected in 6 of the 54 blocks of rare autopsy tissue from primary mitochondrial cases, in principle, this study indicates an association between respiratory deficiency in the white matter and oligodendrocyte dysfunction. The opportunities to detect preferential MAG loss in postmortem tissue derived from chronic neurodegenerative disorders are limited. Preferential MAG loss is thought to be an early neuropathologic sign of oligodendrocyte dysfunction. Most CNS disorders and animal models of MS with preferential MAG loss have been reported using postmortem tissue after acute illness, brain biopsies, or tissue from the acute stage of experimental demyelination.[1,9,30] Muscle rather than brain biopsy is the investigation of choice in patients with suspected primary mitochondrial disorders, and to our knowledge, brain biopsy tissue from mitochondrial cases is not available. Because postmortem tissues often represent the late stages of chronic neurodegeneration, it is entirely possible that we might not see changes directly related to early disease stages. Nevertheless, MAG loss in KSS is an important finding and shows that genetically determined mitochondrial respiratory chain defects may cause distal dying-back oligodendrogliopathy in relatively noninflammatory environments.

Inflammation, hypoxia, and viral infections have been shown to perturb cellular function in multiple ways, including inhibition of mitochondrial respiratory chain activity.[31] Our findings suggest that mitochondrial defects reported in MS lesions involving oligodendrocytes and astrocytes may play an important role in CNS demyelination. As shown in this study, inflammation is not a prominent feature in primary mitochondrial disorders, and the relative sparing of axons also argues against a hypoxic injury in KSS patients. Taken together, these observations provide evidence for the proposal that distal dying-back oligodendrogliopathy in KSS is a primary event due to the high heteroplasmy levels of mtDNA deletion.[13]

In summary, we report preferential loss of MAG in KSS patients, in whom the tissue injury is due to pathogenic levels of mtDNA deletions, causing mitochondrial respiratory chain dysfunction rather than inflammation or hypoxia. MAG loss in KSS patients, although a rare finding in postmortem tissue of primary mitochondrial disorders, suggests an important role for mitochondria in demyelinating disorders of the CNS, such as MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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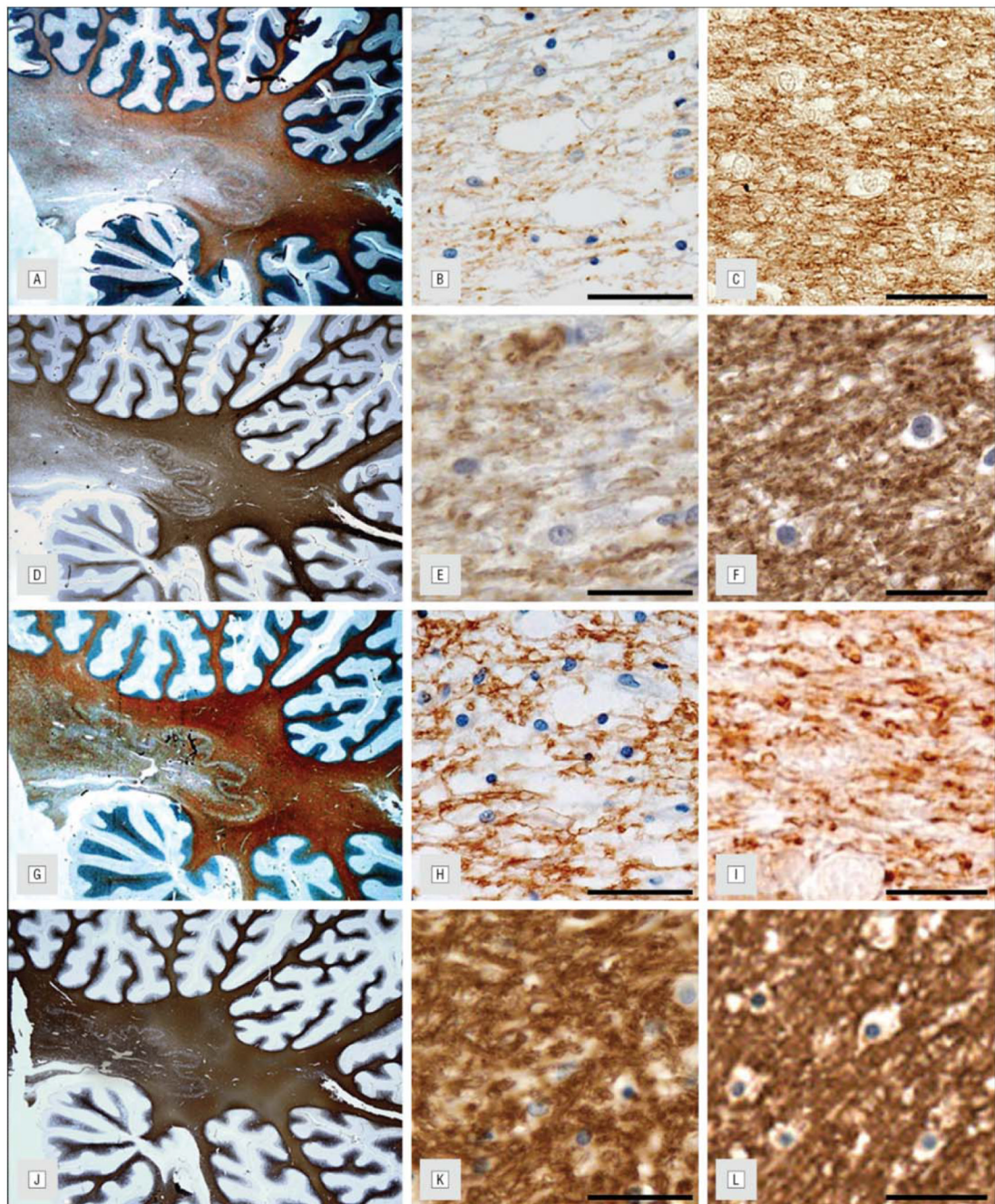
Figure 1.
Pattern of myelin loss within the dentate nucleus in Kearns-Sayre syndrome (KSS). There was an apparent loss of myelin-associated glycoprotein (MAG) (A-C) and 2′,3′-cyclic nucleotide phosphodiesterase (CNPase) (D-F) compared with myelin oligodendrocyte glycoprotein (MOG) (G-I) and myelin basic protein (MBP) (J-L) immunoreactivity within the dentate nucleus white matter (KSS patient 1). G-I, The loss of MOG was much less striking than MAG loss in serial sections. At high magnification (original magnification ×63), the loss of MAG (A-C) and CNPase (D-F) in KSS was notable compared with corresponding control tissue. In contrast, the difference in MOG (G-I) and MBP (J-L)

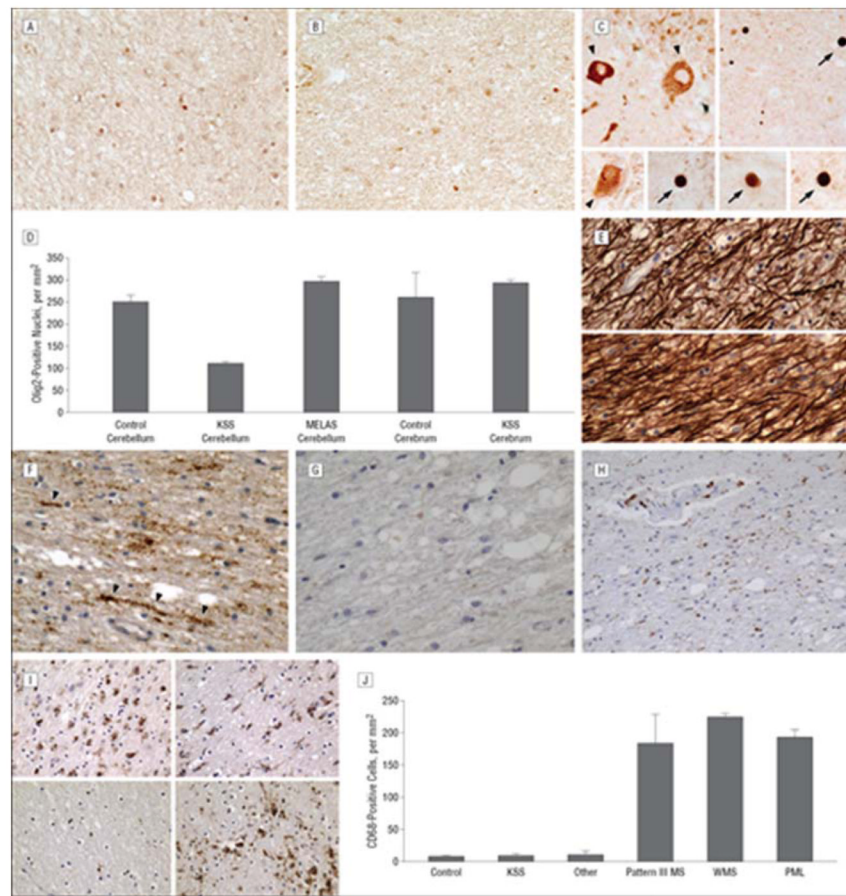immunoreactivity between KSS and control tissue was subtle, if at all. Scale bar represents 12 µm.

Figure 2.
Injury to oligodendrocytes, relative sparing of axons, and lack of inflammation within dentate in Kearns-Sayre syndrome (KSS). A-D, Loss of Olig2-positive nuclei showed a notable decrease in oligodendrocyte lineage cells within the white matter of the dentate nucleus in KSS patients (B) compared with controls (A and D). Quantitation of Olig2-positive cells in KSS dentate nucleus white matter demonstrated the loss of oligodendrocytes to be significant ($P = .008$, analysis of variance) compared with controls and patients with mitochondrial encephalopathy lactic acidosis and strokelike episodes (MELAS) in which myelin-associated glycoprotein (MAG) loss was not detected in the dentate (D). In nondemyelinated regions in KSS cerebrum, we did not detect a significant loss of Olig2-positive cells compared with corresponding white matter. Apoptosis-inducing factor (AIF) was located in the cytoplasm of neurons (C, arrowheads), whereas neuronal nuclei did not show AIF immunoreactivity. In contrast, numerous nuclei with intense AIF staining were detected in the KSS dentate white matter where preferential MAG loss was evident (C, arrows). E-G, Axons in the dentate nucleus white matter, where MAG loss was apparent, and control tissue were morphologically intact as judged by Bielschowsky silver stain. Axons in the MAG loss region rarely showed accumulation of synaptophysin, a marker of fast axonal transport block (G). In contrast, synaptophysin-positive axons were detected within affected regions with equal loss of myelin components in primary mitochondrial disorders, reflecting ongoing axonal degeneration (F, arrowheads). H-J, CD68 immunoreactivity was present at levels comparable to controls (I) in KSS dentate white matter (H), where preferential loss of MAG was apparent. In contrast, CD68-positive cells were abundant in pattern III multiple sclerosis (MS) (n = 5), white matter stroke (WMS) (n

= 4), and progressive multifocal leukoencephalopathy (PML) (n = 3) lesions, reported disorders with distal "dying-back" oligodendrogliopathy (J). Error bars indicate SEM.
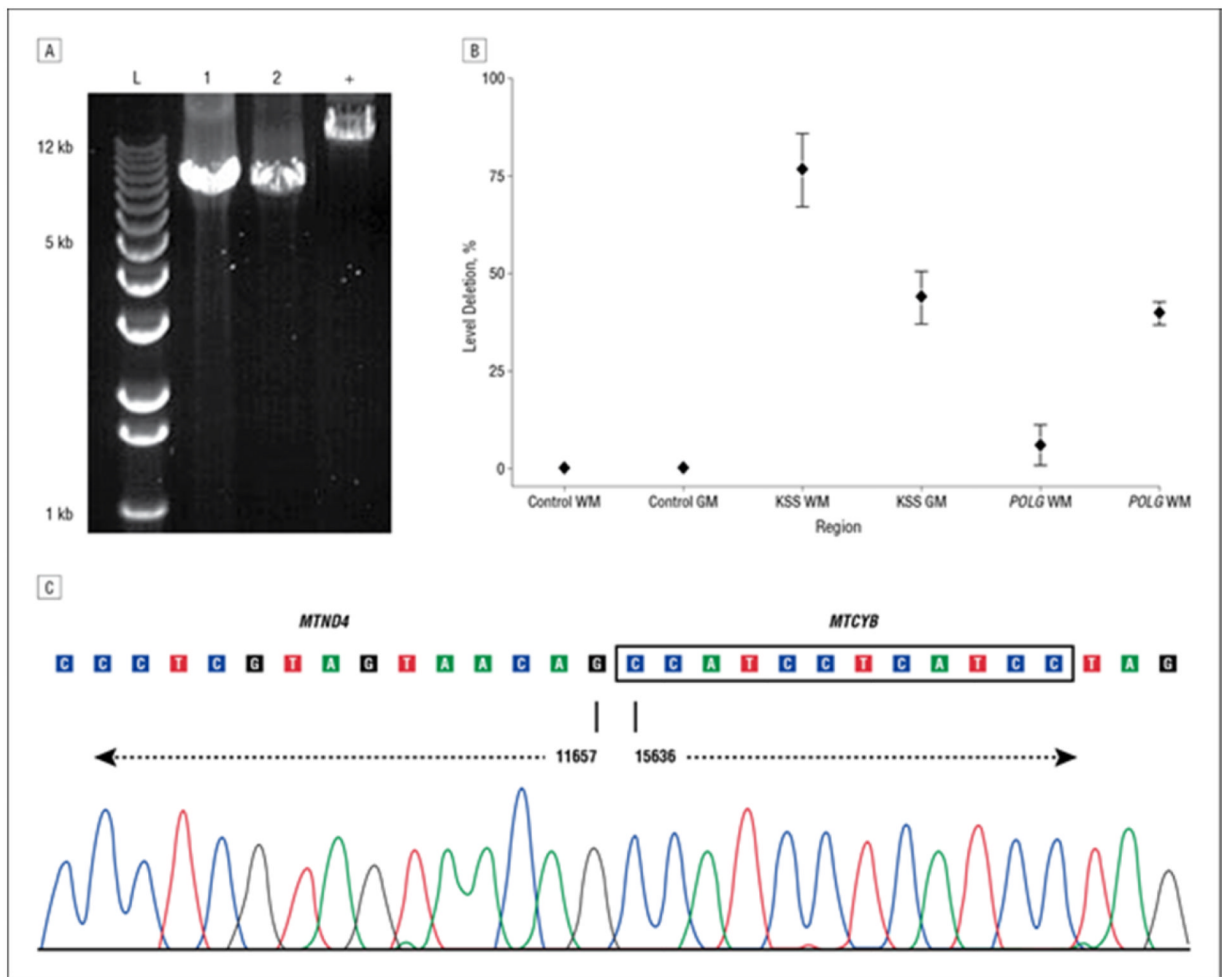
Figure 3.
The single deletion of mitochondrial DNA (mtDNA) within the white matter of the dentate nucleus in Kearns-Sayre syndrome (KSS) is pathogenic. A, A single deletion of mtDNA was detected in laser microdissected regions from KSS dentate nucleus white (lane 1) and gray (lane 2) matter using long-range polymerase chain reaction (PCR); lane 3 shows full-length amplified product from control DNA sample (meninges). B, Real-time PCR revealed significantly greater heteroplasmy levels in dentate white and gray matter ($P < .001$) than the corresponding regions in control tissue in 3 separate experiments. However, heteroplasmy levels in KSS dentate white matter (WM) were higher than the 60% threshold and significantly greater than in gray matter (GM). In primary mtDNA disorders without myelin-associated glycoprotein loss and due to *POLG* mutations (3 patients), the heteroplasmy level in dentate white matter was less than 60% and significantly less than in KSS. C, Sequencing of the single, large-scale mtDNA deletion extracted from the long-range PCR gel confirms the 3978–base pair (bp) deletion. The mtDNA deletion removed several key genes and occurs at the site of an imperfect 12-bp repeat (highlighted).
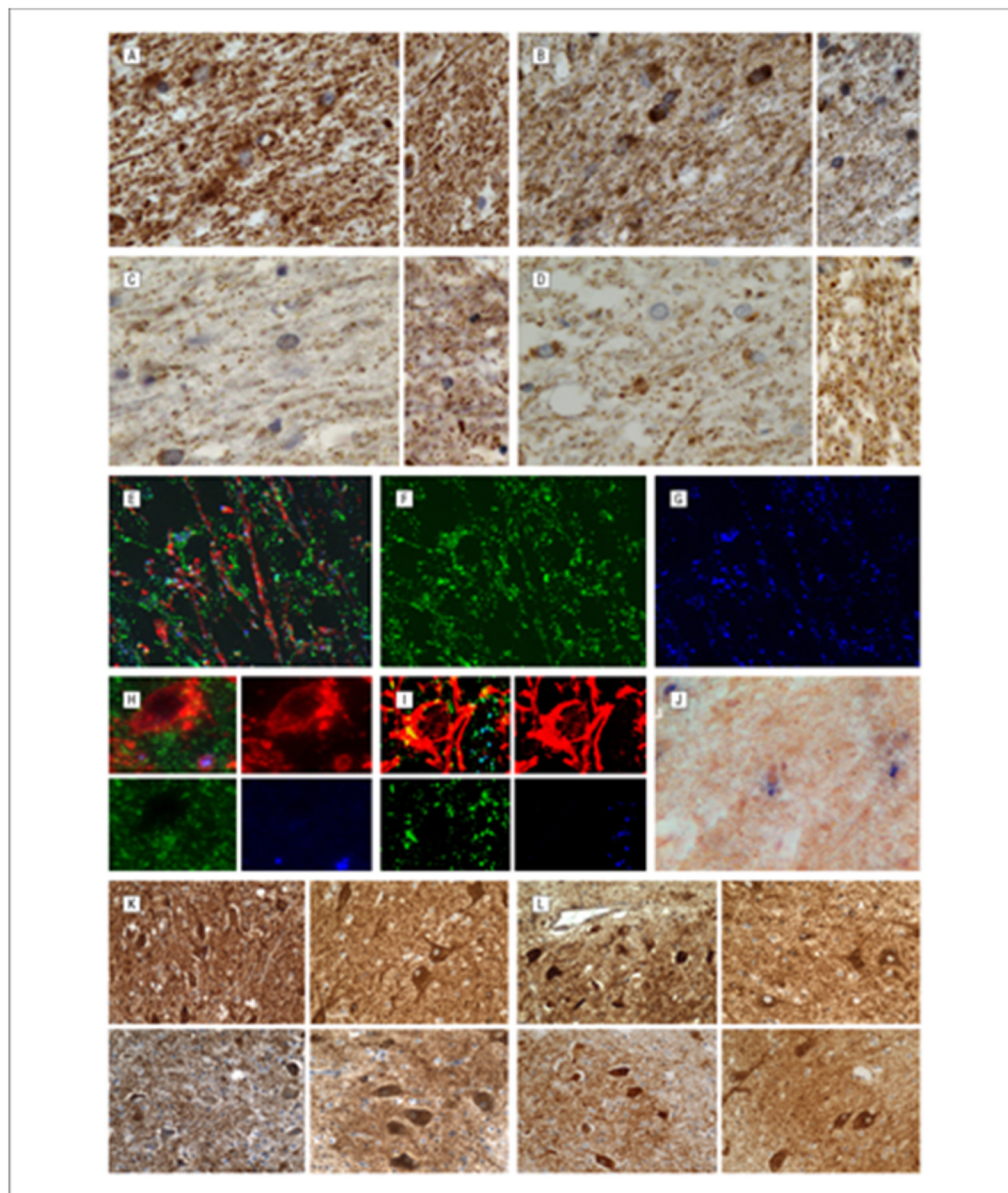
Figure 4.
Mitochondrial respiratory chain complex subunit expression and complex IV (COX) activity in Kearns-Sayre syndrome (KSS) dentate white (WM) and gray (GM) matter. A-D, Immunohistochemical detection of mitochondrial proteins within dentate nucleus WM showed comparable density of porin (A) and complex II (SDH) 70-kDa (B) in KSS (Ai and Bi) and controls (Aii and Bii). There was a notable reduction in complex I 30-kDa (C) and complex IV subunit-I or COX-I (D) in KSS (Ci and Di) compared with controls (Cii and Dii). Densitometric analysis showed a significant (P<.001) decrease in complex I 30-kDa (by 30.7%) and COX-I (by 31.4%) immunoreactivity in KSS (mean [SD], 100.3 [4.4] and 79.9 [5.0] for complex I 30-kDa and COX-I, respectively) compared with controls (144.7 [6.8] and 116.4 [8.0] for complex I 30-kDa and COX-I, respectively). E, Immunofluorescent labeling of phosphorylated neurofilament (axons, red), mitochondria (porin, green) and Cox-I (blue) identified a striking loss of COX-I in the nonaxonal population of mitochondria. In contrast, COX-I is relatively spared in axonal mitochondria. F and G, Immunofluorescent

labeling of CNPase (oligodendrocytes, red) or glial fibrillary acidic protein (astrocytes, red), mitochondria (porin, green), and COX-I (blue) confirmed the lack of COX-I–positive elements within oligodendrocytes (F) and astrocytes (G). Supplementary eFigure 3 shows COX-I and porin labeling within astrocytes and oligodendrocytes in control tissue. H, COX and succinate dehydrogenase immunohistochemical analysis identified numerous respiratory-deficient cells in the KSS dentate nucleus WM (H), however, such cells were absent in KSS GM and WM from control cases (Supplementary eFigure 3, C-D). I-L, Porin (I), SDH 70-kDa (J), and COX-I (L) immunoreactivity were not diminished within KSS dentate nucleus neurons (Ii, Ji, and Li) compared with controls (Iii, Jii, and Lii). However, complex I 30-kDa was apparently decreased within KSS patient 1 dentate nucleus neurons (Ki) compared with controls (Kii). Scale bar represents 100 μm.

Table

Details of Study Patients With Primary Mitochondrial Disorders and Controls

Patient No./ Sex/Age,y	PMI, h	Genetic Defect	Clinical Presentation	No. of Blocks	Myelin Loss	Pattern of MAG Loss
1/F/40	58	Single deletion	KSS	5[a]	Yes	+
2/F/22[14]	3	Single deletion	KSS	3	Yes	+
3/M/18[14]	4	Single deletion	KSS	4[a]	Yes	+
4/M/57	4	m.8344A>G	MERRF	3[a]	Yes	E
5/M/36[12]	2	m.8344A>G	MERRF	4[a]	Yes	E
6/F/42	59	m.8344A>G	MERRF	2	Yes	E
7/F/60	10	m.3243A>G	MELAS	2	Yes	E
8/F/57	36	m.3243A>G	MELAS	2	Yes	E
9/M/45	24	m.3243A>G	MELAS	2	Yes	E
10/F/42	24	m.3243A>G	MELAS	2	Yes	E
11/F/34	38	m.13094T>C	MELAS	5	Yes	E
12/M/55	10	m.14709T>C	Ataxia	4	Yes	E
13/F/39[15]	46	m.3460G>A	LHON plus	3[a]	Yes	E
14/NR/32[16]	17	1504C >T/A3371C	MNGIE	4[a]	Yes	E
15/M/50	24	*POLG* mutation; p.A467T, p.X1240Q	Ataxia, neuropathy	4[a]	Yes	E
16/F/24	83	*POLG* mutation; P.A467T, p. W748S	Ataxia	3[a]	Yes	E
17/M/59	67	*POLG* mutation; p.G848S, P.W748S	Ataxia, dementia	2	Yes	E
18/M/64	64	Control	NA	5[a]	No	No
19/M/55	24	Control	NA	3[a]	No	No
20/M/86	43	Control	NA	3[a]	No	No
21/M/27	24-48	Control	NA	3	No	No
22/F/50	82	Control	NA	2	No	No
23/M/54	<24	Control	NA	2	No	No
24/M/47	15	Control	NA	2	No	No

Abbreviation: E, equal loss of all myelin components; KSS, Kearns-Sayre syndrome; LHON, Leber hereditary optic neuropahty; MAG, myelin-associated glycoprotein; MELAS, mitochondrial encephalopathy lactic acidosis and strokelike episodes; MERRF, myoclonic epilepshy with ragged red fibers; MNGIE, mitochondrial neurogastrointerstinal encephalomyopathy; NA, not available; PMI, postmortem interval; + loss of MAG and 2′, 3′-cyclic nucleotide phosphodiesterase, with relative sparing of myelin basic protein and muelin oligodendrocyte glycoprotein immunoreactivity (preferential MAG loss).

[a] Cases where spinal cord sections were investigated.