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Biallelic *COX10* Mutations and *PMP22* Deletion in a Family With Leigh Syndrome and Hereditary Neuropathy With Liability to Pressure Palsy

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Abstract

Objectives

Leigh syndrome is a progressive encephalopathy characterized by symmetrical lesions in brain. This study aimed to investigate the clinicopathologic and genetic characteristics of a family with Leigh syndrome and hereditary neuropathy with liability to pressure palsy (HNPP).

Methods

Data from a Japanese family's clinical features, MRIs, muscle biopsy, and an autopsy were analyzed. A whole-exome sequence was performed, as well as real-time PCR analysis to determine copy number variations and Western blot analyses.

Results

The proband and her 2 siblings developed spastic paraplegia and mental retardation during childhood. The proband and her sister had peripheral neuropathy, whereas their father developed compression neuropathy. Leigh encephalopathy was diagnosed neuropathologically. Brain MRI revealed changes in cerebral white matter as well as multiple lesions in the brainstem and cerebellum. Muscle biopsy revealed type 2 fiber uniformity and decreased staining of cytochrome c oxidase. The *COX10* missense mutation was identified through whole-exome sequence. A 1.4-Mb genomic deletion extending from intron 5 of *COX10* to *PMP22* was detected.

Discussion

These findings suggest that in this family, Leigh syndrome is associated with a mitochondrial respiratory chain complex IV deficiency caused by biallelic *COX10* mutations coexisting with HNPP caused by heterozygous *PMP22* deletion.

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Leigh syndrome is a group of clinically heterogeneous conditions with widely varying clinical manifestations.[1-3] More than 75 causative genes have been identified in Leigh syndrome.[4] The diagnosis of Leigh syndrome is challenging due to the wide range of clinical manifestations. We present the clinicopathologic and genetic characteristics of a family with Leigh syndrome coexisting with hereditary neuropathy with liability to pressure palsy (HNPP).

Clinical Characteristics

Table 1 summarizes the family's clinical characteristics. Patients 1 (III-1, eFigure 1, links.lww.com/NXG/A545) and 3 (III-3) presented with spastic paraplegia, intellectual disability, cerebellar ataxia, and respiratory failure. Brain MRI for patient 1 revealed diffuse high-intensity lesions with coarsening and vacuolization in the white matter (eFigure 2, A–F) as well as multiple lesions in the brainstem and cerebellum (eFigure 2, G–I). Patients 1 and 3 had marked peripheral neuropathy. Patient 4 (II-5) developed compression neuropathy after surgery (eTable 1).

Histology and Respiratory Chain Enzyme Activity of Biopsied Muscle

A biopsy specimen from patient 1 revealed variation in muscle fiber diameter (eFigure 3A, links.lww.com/NXG/A545). A moderate increase in lipid was found in muscle fibers (eFigure 3B). Modified Gomori trichrome staining revealed no ragged-red fibers (eFigure 3C). COX activity was found to be reduced in a widespread and severe manner (eFigure 3, D and E). The uniform staining pattern of type 2 fibers was revealed by ATPase histochemistry (eFigure 3F).

Measurement of respiratory chain complex enzyme activity in biopsied muscle revealed that the levels of all enzymes were lower than those in normal tissues (eTable 2, links.lww.com/NXG/A545).[5] The enzyme activity in fibroblast revealed a selective decrease in complex IV (eTable 2). The results of the enzymes were consistent with complex IV deficiency.

Autopsy Findings

An autopsy was performed on patient 3 (III-3) at age 9 years. The brain showed diffuse and severe white matter degeneration (Figure 1, A and B). The loss of myelin and axons appeared to be similar in extent in the degenerated white matter (Figure 1, C and D), and vacuolar changes of the neuropil accompanied by gliosis were observed (Figure 1E). Atrophy and myelin pallor was also evident in the cerebellar white matter, middle cerebellar peduncles, and pontine base (Figure 1F). Spotty demyelinating lesions were found in the left medial lemniscus (Figure 1F) and the reticular formation of the medulla oblongata (Figure 1, G and H). Axons were relatively preserved in both lesions, as well as the ventral part of the medulla oblongata, despite the significant loss of myelin (Figure 1, I and J). Olivary hypertrophy with severe gliosis (Figure 1K), Purkinje cell loss in the cerebellar cortex, and gliosis in the dentate nucleus (Figure 1L) were evident.

Genetic Analysis

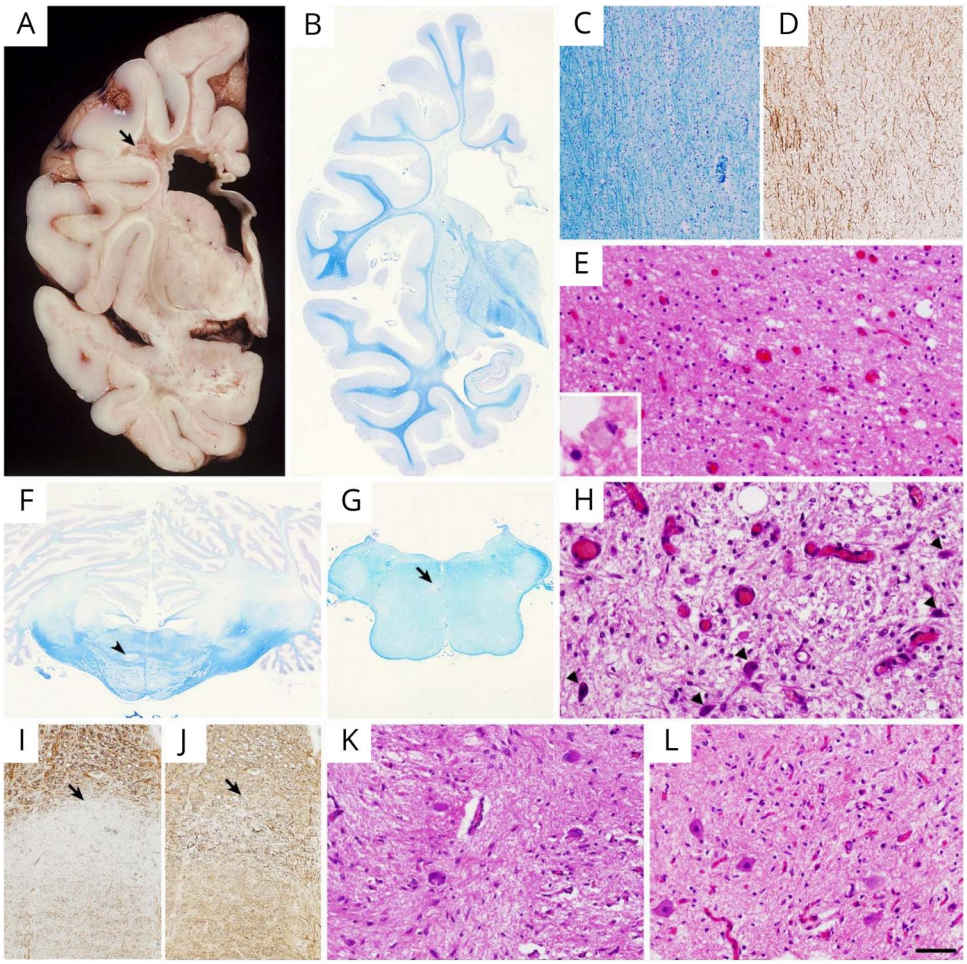
Whole-exome sequence analysis showed *COX10* variants of p.Arg159Gln and novel p.Pro295Leu in patients 1 and 3 (Figure 2A). An in silico analysis suggested that the p.Pro295Leu variant was pathogenic, whereas the p.Arg159Gln was

Table 1 Summary of Clinical and Genetic Findings of the Affected Member of the Family

	Patient 1 (III-1)	Patient 2 (III-2)	Patient 3 (III-3)	Patient 4 (II-5)
Clinical diagnosis	Leigh syndrome	Cerebral palsy	Leigh syndrome	Pressure palsy
Age at onset	4 y	15 mo	2 y	79 y
Age (current status)	54 y (alive)	4 y (deceased)	9 y (deceased)	80 y (alive)
Initial symptoms	Gait disturbance and mental and motor retardation	Gait disturbance and mental and motor retardation	Gait disturbance and mental and motor retardation	Numbness and weakness of upper extremities
Other symptoms	Spastic tetraplegia, nystagmus, vertigo, cerebellar ataxia, dysphagia, and respiratory failure	N/A	Spastic tetraplegia, dysphagia, and respiratory failure	None
Peripheral neuropathy	Present	N/A	Present	Present
Serum lactic acid (mg/dL)	Normal (17.4)	N/A	Normal (12.4)	N/A
CSF lactic acid (mg/dL)	Elevated (43.8)	N/A	N/A	N/A
***COX10* mutation(s)**	p.P295L/partial deletion	N/A	p.P295L/partial deletion	Partial deletion
***PMP22* mutation**	Deletion	N/A	Deletion	Deletion

Abbreviation: N/A = data not available.

Figure 1 Neuropathologic Findings of the Autopsied Brain (Patient 3)



(A) The brain weight was 1,100 g. A coronal section of the left cerebral hemisphere showed marked volume loss and grayish discoloration of the white matter. Severely softened deep white matter with cavity-like changes (arrow). (B) Diffuse myelin pallor in the affected white matter. U-fibers are mostly spared. (C and D) Myelin (C) and axons (D) are depleted to similar extents accompanied by some swelling axons in the moderately affected parietal white matter. (E) Neuropil vacuolar changes with gliosis and macrophage infiltration (inset) in relatively preserved white matter of the frontal lobe. (F) Atrophy and myelin pallor of the cerebellar white matter, middle cerebellar peduncles, and pontine base. A spotty demyelinating lesion in the left medial lemniscus (arrowhead). (G) Diffuse myelin pallor of the ventral part of medulla oblongata with bilateral olivary hypertrophy. A spotty demyelinating lesion crossing the midline raphe (arrow). (H) A high-magnification image was taken from the demyelinating lesion indicated by an arrow in (G). Tissue rarefaction and neuropil vacuolation are visible, and capillary prominence, gliosis, and macrophage infiltration are evident. Some neurons are relatively preserved in the lesion (triangles). (I and J) Images taken from the same lesion pointed with an arrow in (G). In the myelin basic protein (MBP) severely depleted lesion (I arrow), SMI-31-labeled axons are relatively preserved (J arrow). (K) Neuronal loss with marked gliosis in the inferior olivary nucleus. (L) Mild neuropil vacuolar changes accompanied by capillary prominence and gliosis in the dentate nucleus. (B, C, F, and G) Klüver-Barrera, and (E, H, K, and L) hematoxylin and eosin staining, (D and J) SMI-31, and (I) MBP immunohistochemistry. Bar = 145 μm for C and D, 50 μm for E, K, and L, 16 μm for inset in E, 43 μm for H, and 400 μm for I and J.

predicted to be benign (eTable 3, links.lww.com/NXG/A545). CNV analysis of *COX10* revealed a decreased gene dosage, indicating the presence of a deletion in patients 1, 3, and 4 (Figure 2B). Furthermore, CNV analysis revealed a decreased gene dosage in *PMP22* (Figure 2C). A DNA microarray analysis revealed an approximately 1.4-Mb deletion spanning from intron 6 of *COX10* to *PMP22* (Figure 2D).

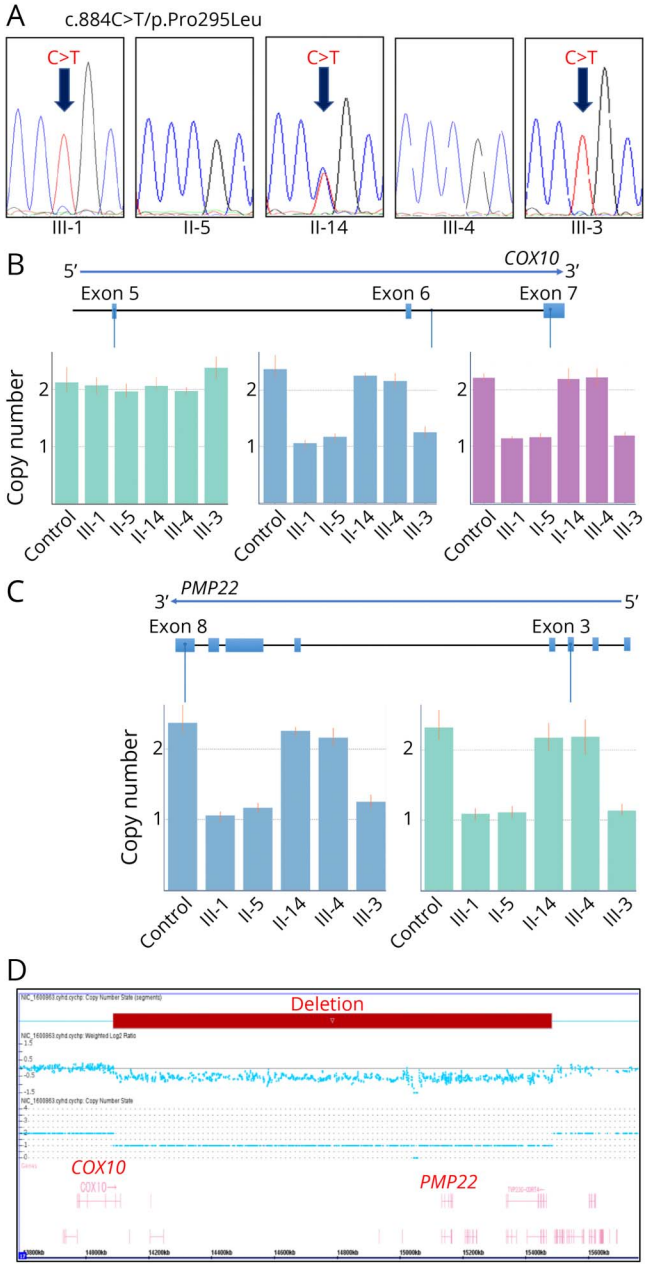
Protein Analysis

Proteins were extracted from the frozen tissue (cerebral cortex and white matter) in patient 3. In both mitochondrial and total lysate fractions, Western blot analysis revealed decreased expression levels of the COX1/MT-CO1, COX2/MT-CO2, and COX4 complex IV subunits (eFigure 4, A and B, links.lww.com/NXG/A545).

Discussion

In this study, we identified a family with Leigh syndrome coexisting with HNPP caused by biallelic *COX10* mutations and heterozygous *PMP22* deletion. An enzyme activity analysis revealed that mitochondrial respiratory chain complex IV deficiency is the cause of Leigh syndrome in this family, which is most likely caused by biallelic *COX10* mutations composed of novel missense mutation and partial deletion (eFigure 5, links.lww.com/NXG/A545).

Figure 2 Genetic Analysis



(A) Electropherograms of the p.Pro295Leu missense mutation in exon 6 of *COX10* in patients 1 and 3 and their mother are shown. There were no pathogenic mitochondrial mutations in this family. (B) A CNV analysis of *COX10* revealed a decreased gene dosage in intron 6 and exon 7 of *COX10* in patients 1 (III-1), 3 (III-3), and 4 (II-5). The results are presented as the mean ± SEM (n = 3). (C) Decreases in CNV in *PMP22* in patients 1 (III-1), 3 (III-3), and 4 (II-5). The results are presented as the mean ± SEM (n = 3). (D) Microarray analysis of patient 1 (III-1) revealed an approximately 1.4-Mb deletion extending from *COX10* intron 5 to *PMP22* (hg19 chr17: 14,087,933–15,484,859; 1,397 kbp). The deletion is predicted to result in a truncated COX10 lacking 130 amino acids at its C-terminus. CNV = copy number variant.

Peripheral neuropathy was observed in patients 1 and 3, and compression neuropathy was noted in their father (patient 4). We identified a deletion of *PMP22*, which is cosegregated with neuropathy in the family (eFigure 5, links.lww.com/NXG/A545). We report the patients of Leigh syndrome with neuropathy associated with *PMP22* deletion and *COX10* mutation. *COX10* is located in the vicinity of *PMP22* and has a CMT1A-repeat sequence that is highly similar to that of *PMP22* (eFigure 6).[6] The presence of such a sequence suggests a mechanism for homologous recombination.[7] Genetic rearrangements resulting in HNPP deletion disrupted 1 copy of *COX10* on the recombinant allele. If the pathologic mutation of *COX10* coexists in the other allele, a patient with HNPP could develop Leigh syndrome, as was observed in our family.

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Disclosure

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Appendix *(continued)*

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References

1. Leigh DJ. Subacute necrotizing encephalomyelopathy in an infant. *J Neurol Neurosurg Psychiatry*. 1951;14(3):216-221. doi:10.1136/jnnp.14.3.216.
2. Rahman S, Blok RB, Dahl HH, et al. Leigh syndrome: clinical features and biochemical and DNA abnormalities. *Ann Neurol*. 1996;39(3):343-351. doi:10.1002/ana.410390311.
3. Baertling F, Rodenburg RJ, Schaper J, et al. A guide to diagnosis and treatment of Leigh syndrome. *J Neurol Neurosurg Psychiatry*. 2014;85(3):257-265. doi:10.1136/jnnp-2012-304426.
4. Lake NJ, Compton AG, Rahman S, et al. Leigh syndrome: one disorder, more than 75 monogenic causes. *Ann Neurol*. 2016;79(2):190-203. doi:10.1002/ana.24551.
5. Ogawa E, Shimura M, Fushimi T, et al. Clinical validity of biochemical and molecular analysis in diagnosing Leigh syndrome: a study of 106 Japanese patients. *J Inherit Metab Dis*. 2017;40(5):685-693. doi:10.1007/s10545-017-0042-6.
6. Reiter LT, Murakami T, Koeuth T, et al. A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. *Nat Genet*. 1996;12(3):288-297. doi:10.1038/ng0396-288.
7. Reiter LT, Murakami T, Koeuth T, et al. The human COX10 gene is disrupted during homologous recombination between the 24 kb proximal and distal CMT1A-REPs. *Hum Mol Genet*. 1997;6(9):1595-1603. doi:10.1093/hmg/6.9.1595.