NUBPL mutations in patients with complex I deficiency and a distinct MRI pattern

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ABSTRACT

Objective: To identify the mutated gene in a group of patients with an unclassified heritable white matter disorder sharing the same, distinct MRI pattern.

Methods: We used MRI pattern recognition analysis to select a group of patients with a similar, characteristic MRI pattern. We performed whole-exome sequencing to identify the mutated gene. We examined patients' fibroblasts for biochemical consequences of the mutant protein.

Results: We identified 6 patients from 5 unrelated families with a similar MRI pattern showing predominant abnormalities of the cerebellar cortex, deep cerebral white matter, and corpus callosum. The 4 tested patients had a respiratory chain complex I deficiency. Exome sequencing revealed mutations in *NUBPL*, encoding an iron-sulfur cluster assembly factor for complex I, in all patients. Upon identification of the mutated gene, we analyzed the MRI of a previously published case with *NUBPL* mutations and found exactly the same pattern. A strongly decreased amount of *NUBPL* protein and fully assembled complex I was found in patients' fibroblasts. Analysis of the effect of mutated *NUBPL* on the assembly of the peripheral arm of complex I indicated that *NUBPL* is involved in assembly of iron-sulfur clusters early in the complex I assembly pathway.

Conclusion: Our data show that *NUBPL* mutations are associated with a unique, consistent, and recognizable MRI pattern, which facilitates fast diagnosis and obviates the need for other tests, including assessment of mitochondrial complex activities in muscle or fibroblasts. *Neurology*® 2013;80:1577-1583

GLOSSARY

BN = blue native; **PAGE** = polyacrylamide gel electrophoresis; **SDS** = sodium dodecyl sulfate.

There are many rare childhood leukoencephalopathies and currently a high percentage of cases remain without a specific diagnosis.¹ Consequently, the diagnostic process is challenging. In mitochondrial leukoencephalopathies, elevated lactate in body fluids often points in the right direction, generally followed by analysis of respiratory chain function in muscle tissue, and DNA analysis guided by the results. The extreme clinical and genetic heterogeneity of mitochondrial disorders, however, makes the final diagnosis frequently hard or impossible to achieve.²⁻⁴ MRI pattern recognition can greatly facilitate this diagnostic process by providing a rapid diagnosis in patients with known white matter disorders¹ and allowing identification of groups of patients with the same novel disorder among the unsolved cases.⁵ Formerly, definition of novel disorders was followed by genetic linkage studies if numerous patients or highly informative families were available.⁶⁻⁹ The recent introduction of whole-exome sequencing has created the opportunity to identify the mutated gene in small groups of patients with a rare mendelian disorder.¹⁰⁻¹²

Supplemental data at
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METHODS Patients. We identified 6 patients from 5 unrelated families from our MRI database of more than 3,000 cases with an unclassified leukoencephalopathy using MRI pattern recognition analysis.⁵ Patients 3 and 4 are affected siblings. Inclusion criteria were 1) extensive cerebellar cortex signal abnormalities; 2) signal abnormalities in the corpus callosum; and 3) absence of signal abnormalities in the basal ganglia, thalami, and cerebral cortex. Patient 2 was previously published by Wolf et al.¹³ In none of the patients a molecular diagnosis was achieved.

S.H.K. and M.S.v.d.K. evaluated the MRIs according to a previous protocol.⁵ We retrospectively reviewed the clinical information and laboratory investigations. Upon identification of the mutated gene, we included the MRI of a previously published case (patient 7) in our analysis to confirm consistency of our findings.^{14,15}

Standard protocol approvals, registrations, and patient consents. We received approval of the ethical standards committee for our research on patients with unclassified leukoencephalopathies. We received written informed consent for exome sequencing from all guardians of the patients participating in the study.

Whole-exome sequencing. We performed whole-exome sequencing in DNA of patients 2 and 4, using SeqCap EZ Human Exome Library v3.0 kit (Nimblegen) on Hiseq2000 (Illumina, San Diego, CA; detailed information in e-Methods on the *Neurology*[®] Web site at www.neurology.org).

NUBPL mutation analysis. We amplified the 11 exons and intron-exon junctions of the human *NUBPL* gene (NG_028349.1) by PCR using suitable primers (available upon request) and analyzed these by Sanger sequencing.

Biochemical analysis. Skin fibroblasts of patients 2, 3, 4, and 6 were available and cultured in M199 medium supplemented with 10% fetal calf serum and antibiotics. We measured the enzyme activity of complexes I, II, III, IV, and V, and citrate synthase spectrophotometrically in mitochondria-enriched fractions isolated from fibroblasts and muscle as described.^{16–18} We performed biochemical analysis of *NUBPL* and complex I assembly with the fibroblasts of patients 3 and 4. We performed 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) and 1- and 2-dimensional 5% to 15% blue native (BN)-PAGE as previously described.¹⁹ Lanes were loaded with 40 µg (SDS analysis) or 80 µg (BN analysis) of solubilized mitochondrial protein. After electrophoresis, the gels were further processed for in-gel activity assays and Western blotting, as described.¹⁹ We incubated the Western blots using antibodies against *NUBPL* (kind gift of Prof. R. Lill, Marburg, Germany), complex I subunits *NDUFA9* and *NDUFS3*, and complex II subunit *SDHA*/70 kDa (MitoSciences, Eugene, OR).

RESULTS MRI findings. Detailed MRI findings are provided in table e-1 and illustrated in figure 1. Initial MRIs (figure 1, A–D, patient 7; I–L, patient 4) of patients 2, 4, 5, 6, and 7, obtained early in the disease course, showed confluent or multifocal cerebral white matter lesions, predominantly affecting the deep frontal and parietal white matter and sparing the U-fibers, internal and external capsules, and central part of the corona radiata under the pericentral cortex. Early MRIs showed prominent signal abnormalities and swelling of the corpus callosum. Callosal fibers connecting the pericentral cortex were relatively preserved (figure 1D). Extensive signal abnormalities were present in the cerebellar cortex and subcortical

white matter, whereas the cerebellar deep white matter and hilus of the dentate nucleus were spared (figure 1, A, D, I, and L). The inferior part of the cerebellum was less severely affected (figure 1, D and L). The corpus callosum and abnormal cerebral hemispheric white matter were rarefied (figure 1S). Contrast enhancement was seen in small areas of the abnormal cerebral white matter and corpus callosum (figure 1T). Restricted diffusion was present at the edges of the affected cerebral white matter and corpus callosum (figure 1, Q and R).

Late MRIs (figure 1, E–H, patient 2; M–P, patient 4) were available in patients 1, 2, 3, and 4. In patients 2 and 4, substantial improvement of the cerebral white matter and corpus callosum abnormalities were seen with decrease in both white matter swelling and extent of the white matter abnormalities, whereas the cerebellar abnormalities had worsened (figure 1, E–H, M, N, and P). In patients 1 and 3, only a late MRI was available. In patient 3, subtle signal changes were exclusively seen in the corpus callosum; patient 1 had severe atrophy of the corpus callosum and limited cerebral white matter abnormalities. The cerebellar white matter and cortex were now extensively affected and atrophic in all patients. Prominent lesions were present in the basis pontis and pyramids of the medulla (figure 1, E, H, M, and P). Contrast enhancement was seen in the pons and cerebellar cortex (Figure 1O). No diffusion restriction was observed.

Clinical profiles and laboratory results. Detailed clinical characteristics and laboratory results are provided in tables e-2 and e-3. In contrast to the other patients, patient 7 was the only patient in which the early motor development was delayed. Patients presented with insufficient gain or loss of motor skills and signs of cerebellar dysfunction at the end of the first or in the second year of life. On follow-up, signs of continuing development were observed in most patients. Patient 1 experienced continuous slow regression; patients 4, 6, and 7 had episodes of regression of speech and mobility with partial recovery. Five patients developed spasticity. At the most recent clinical follow-up, patients 2 and 3 were able to walk without support. All patients had motor problems due to ataxia. Patient 1 died at 9 years of age of respiratory complications. Cognitive capabilities varied between normal and significantly deficient. No involvement of internal organs was noted.

Plasma and CSF lactate was elevated in most patients. Respiratory chain enzyme assays in muscle and fibroblasts revealed a complex I deficiency in all tested patients, ranging between 27% and 83% of the lowest reference value. The other respiratory chain complexes and complex V showed a normal activity in all patients (tables e-3 and e-4). The diagnosis of complex I deficiency was accomplished between 6 months and 2.5 years after the first MRI.

Figure 1 Illustrations of the characteristic MRI pattern

MRIs of patient 7 (A-D, T), patient 2 (E-H), and patient 4 (I-S) obtained in the early (A-D, I-L, Q-T) and late stages (E-H, M-P) are shown. Axial T2-weighted images (A-C, E-G, I, J, M, N), sagittal T1-weighted (D, H, L) and T2-weighted (P) images, contrast-enhanced coronal (O) and axial (T) T1-weighted images, axial diffusion-weighted image (DWI) (Q), apparent diffusion coefficient (ADC) (R), and fluid-attenuated inversion recovery (S) images are shown. The initial MRIs (A, D, I, L) show diffuse signal abnormalities of the cerebellar cortex with sparing of the cerebellar deep white matter and pons, T2 hyperintensity of the corpus callosum with swelling (B, J), and variable deep cerebral white matter abnormalities (C, K). The inferior part of the cerebellum is relatively less affected (D, L) and the callosal fibers connecting the pericentral sulcus are spared (D). Contrast enhancement is seen in the corpus callosum (T). DWI and ADC images show restricted diffusion at the edges of the corpus callosum lesions (Q, R). The abnormal cerebral white matter is rarefied surrounded by a rim of abnormal but solid tissue (S). The late MRIs (F, G, N, P) show significant improvement or disappearance of the corpus callosum and deep white matter changes. The cerebellar abnormalities worsen and there are additional signal changes in the cerebellar deep white matter and basis pontis (E, H, M, P). Contrast enhancement is seen in the cerebellar cortex (O).

Genetic analysis. We performed whole-exome sequencing in DNA of patients 2 and 4. After filtering of the raw data under the hypothesis of a mitochondrial disorder, *NUBPL* (MIM*613621), encoding a Fe/S protein involved in complex I assembly, was the only remaining candidate gene with mutations (table e-5). Both patients harbored a heterozygous c.166G>A transition, predicting p.Gly56Arg. Patient 4 also harbored a c.313G>T change, predicting p.Asp105Tyr. Manual analysis of the intron data additionally revealed a heterozygous c.815–27T>C change in both patients. Sanger sequencing confirmed these mutations and revealed a frameshift mutation in patient 2: c.667_668insCCTTGTGCTG/p.Glu223Alafs*4. Subsequently, we identified *NUBPL* mutations in all 4 other patients (see table 1 for all identified *NUBPL* mutations). The c.166G>A missense mutation and the intronic c.815–27T>C branch-site mutation were present on the same allele in all patients. These 2 mutations were

previously reported in patient 7 of this report by Calvo et al.¹⁴ within a large exome sequencing project of complex I-deficient patients. We analyzed all identified exonic missense mutations with Polyphen-2, which showed prediction scores of ≥ 0.99 . The amino acids involved are moderately to highly conserved. The mutations were therefore presumed to be pathogenic. The intronic c.815–27T>C mutation was previously shown to cause aberrant splicing of *NUBPL* mRNA.¹⁵ This mutation is found in the heterozygous state in 1 of 60 controls in the 1000 Genomes database. None of the other identified mutations are present in the public single nucleotide polymorphism databases, including the dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>) and 1000 Genomes database.

Biochemical analysis. We performed biochemical analysis of *NUBPL* and complex I assembly with the fibroblasts of patients 3 and 4. The *NUBPL* signals obtained in

Table 1 Overview of *NUBPL* mutations^a

Patient	Country of origin	c.DNA ^b	Protein	Exon	State	Inheritance
1	Argentina	c.166G>A	p.Gly56Arg	2	Homozygous ^c	Unknown
		c.815–27T>C ^d		Intron 9	Homozygous ^c	Unknown
2	Germany	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
		c.667_668insCCTTGTGCTG	p.Glu223Alafs*4	8	Heterozygous	Maternal
		c.815–27T>C ^d		Intron 9	Heterozygous	Paternal
3 and 4 (sibs)	Canada	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
		c.313G>T	p.Asp105Tyr	4	Heterozygous	Maternal
		c.815–27T>C ^d		Intron 9	Heterozygous	Paternal
5	United States	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
		693+1G>A ^e	p.?	Intron 8	Heterozygous	Unknown ^f
		815–27T>C ^d		Intron 9	Heterozygous	Paternal
6	Netherlands	c.166G>A	p.Gly56Arg	2	Heterozygous	Maternal
		c.579A>C	p.Leu193Phe	7	Heterozygous	Paternal
		c.815–27T>C ^d		Intron 9	Heterozygous	Maternal
7 ^g	Australia	c.166G>A	p.Gly56Arg	2	Heterozygous	Paternal
		240-kb deletion (exons 1–4); 137-kb duplication (exon 7)		1–4 and 7	Heterozygous	Maternal
		c.815–27T>C ^d		Intron 9	Heterozygous	Paternal

^aNomenclature according to Human Genome Variation Society (<http://www.hgvs.org/mutnomen>).

^b*NUBPL* reference sequence: NM_025152.2.

^cWe could not confirm whether this mutation was homozygous or hemizygous because no parental DNA or fibroblasts of the patient were available for study.

^dFor the 3 transcripts generated in the presence of the c.815–27T>C mutation, see Tucker et al., 2012.¹⁵

^eAffects an essential nucleotide of the splice donor site, which probably leads to skipping of exon 8.

^fMaternal DNA not available.

^gPreviously published patient.^{14,15}

both patients were severely decreased as compared with the control (figure 2A). Both complex I in-gel activity and the amount of fully assembled complex I were decreased in both patients as compared with the control (figure 2B), which is compatible with the 50% to 60% reduction of complex I activity measured spectrophotometrically (table e-4). We performed 2-dimensional BN/SDS-PAGE analysis to investigate the effect of mutated NUBPL on the assembly of the peripheral arm of complex I, which contains the Fe-S clusters. We observed that the amount of fully assembled complex I was decreased in both patients (figure 2C), consistent with the findings from the BN analysis (figure 2B). Moreover, no subassemblies of the peripheral arm of complex I were present in the patients' fibroblasts, in contrast to the control cells (figure 2C).

DISCUSSION In this study, we show that the combination of disease definition by MRI pattern analysis and whole-exome sequencing can provide a rapid molecular diagnosis in a small group of patients with the same rare, unclassified leukoencephalopathy of suspected mitochondrial origin. All of our patients harbor mutations in *NUBPL*, involved in mitochondrial respiratory chain complex I assembly.^{20,21}

The MRI pattern observed in the present patients is unique and has not been described in any other

disorder of mitochondrial or other origin.²² The early stages are characterized by a combination of abnormalities involving the cerebellar cortex and deep cerebral white matter and corpus callosum. On follow-up, the corpus callosum and cerebral white matter abnormalities improve and disappear in some patients. In contrast, the cerebellar abnormalities are progressive and brainstem abnormalities appear. One patient with *NUBPL* mutations has been published before with little clinical information and no MRIs (patient 7).¹⁴ We were able to retrieve his MRI and confirmed that the abnormalities fit perfectly within the pattern observed in the other patients. While preparing this manuscript, an additional patient with *NUBPL* mutations was published.²³ His MRI at 23 years showed severe cerebellar abnormalities and signal changes of the basis pontis and pyramids of the medulla, characteristic for the late disease stage.²³

We used whole-exome sequencing as a first genetic approach to identify the associated gene. Although highly sensitive in detecting genetic variants, filtering and interpretation of gene variants remain challenging. To selectively reduce the number of variants, specific clues can be used for choosing the correct filters. In our patients, MRI features suggested a mitochondrial disease: multifocal and confluent white matter lesions, rarefied at the center, surrounded by a rim of abnormal but solid tissue,

Figure 2 Biochemical analysis of mutated NUBPL

NUBPL and complex I protein levels and assembly were analyzed in fibroblasts of patients 3 (P3) and 4 (P4) and control (C). Western blot analysis showed a decreased amount of NUBPL in patients' fibroblasts (A). Complex I analysis in patients' fibroblasts by in-gel activity assay (CI-IGA) (upper panel) and BN-PAGE, by using an antibody against complex I subunit NDUFA9 (middle panel), or against complex II subunit SDHB, serving as a loading control (lower panel). Patients had a reduced activity of complex I compared with controls (upper panel), and a reduced amount of complex I (middle panel) (B). Analysis of complex I assembly by 2-dimensional BN/SDS-PAGE in fibroblasts of the patients and control, using complex I antibodies against NDUFS3 and NDUFA9. Fully assembled complex I (CI) was reduced in the patients. Complex I assembly intermediates (CI-subassemblies) of the peripheral arm were not detected in the patients, in contrast to the control (C). BN = blue native; PAGE = polyacrylamide gel electrophoresis; SDS = sodium dodecyl sulfate.

restricted diffusion in these solid rims, additional gray matter abnormalities, patchy contrast enhancement, and elevated lactate in affected brain areas.²² Our suspicion was substantiated by biochemical evidence of complex I deficiency in all tested patients. In suspected mitochondrial disorders, public mitochondrial databases, such as Mitocarta,²⁴ can substantially reduce the number of candidate genes. However, filtering should be done with caution because not all variants may be detected, such as the insertion of patient 2. Our filter included all genes that contained just one or more variants in both patients. If the filter had been more stringent, *NUBPL* would have been missed.

Besides the c.166G>A missense mutation, the intronic c.815–27T>C branch-site mutation was present on the same allele in all patients. Functional in vitro studies have shown that the c.815–27T>C variant possesses more pathogenic qualities than the c.166G>A missense mutation. Overexpression of *NUBPL* protein carrying the missense mutation is able to fully complement complex I activity in an *NUBPL*-deficient cell line, while the intronic branch-site variant results in aberrant splicing of *NUBPL* mRNA,¹⁵ which was also confirmed by us (data not shown). The intronic branch-site variant has been found in approximately 1 of 60 controls (1000 Genomes database). Considering the rarity of the disease, it remains inconclusive whether the c.815–27T>C variant is pathogenic in its own right, or acts in synergy with the c.166G>A variant.

All tested patients have a complex I deficiency in muscle and fibroblasts, ranging between 83% and 27% of the lowest reference value. There appears to be no correlation between the residual complex I activity levels and the severity of the clinical phenotype, as has been documented before for other complex I defects.³

Protein analysis of fibroblasts of patients 3 and 4 shows that the mutations in *NUBPL* result in severely decreased *NUBPL* protein levels. Furthermore, consistent with the proposed role in complex I assembly, the decreased *NUBPL* protein levels result in a decreased complex I activity. *NUBPL* is a chaperone involved in the iron-sulfur cluster assembly in complex I.²⁰ Complex I contains 7 iron-sulfur clusters, associated with 5 different subunits (NDUFS1, -S7, -S8, -V1, and -V2), which are located in the peripheral arm of the complex.²⁵ NDUFS7 and -S8 assemble relatively early in the assembly pathway, and defects in these subunits usually do not result in accumulation of assembly intermediates, in contrast to defects in NDUFV1, -V2, and -S1. We observed no accumulation of assembly intermediates of the peripheral arm in our patients and conclude that *NUBPL* is at least involved in early assembly of the iron-sulfur clusters within the subunits NDUFS7 or -S8 in the peripheral arm of complex I.

The 2 previously reported patients with *NUBPL* mutations were published as single cases with little clinical

information and in the first case without MRIs.^{14,23} The consequence of such publications is that the disease phenotype remains uncertain and diagnosis in new patients is difficult. In this study, we demonstrate that *NUBPL* mutations are associated with a consistent and recognizable MRI pattern that is pathognomonic for the disease. If present in a new patient, no further studies are warranted; sequence analysis of *NUBPL* is the single necessary test.

AUTHOR CONTRIBUTIONS

Sietske H. Kevelam interpreted the MRIs, collected patient data, analyzed and interpreted the exome data, and participated in drafting the manuscript. Richard J. Rodenburg and Leo G. Nijtmans performed and interpreted the biochemical analyses, and participated in drafting the manuscript. Nicole I. Wolf, Patrick Ferreira, Roelineke J. Lunsing, Anne Mitchell, Hugo A. Arroyo, Dietz Rating, and Adeline Vanderver helped collect patient data and revised the manuscript for intellectual content. Carola G.M. van Berkel performed the DNA analyses and revised the manuscript for intellectual content. Truus E.M. Abbink supervised the DNA analyses and revised the manuscript for intellectual content. Peter Heutink supervised the exome analyses and revised the manuscript for intellectual content. Marjo S. van der Knaap conceptualized the study, has access to all data, takes responsibility for the data, participated in the design of the study, interpreted the MRIs, and participated in drafting the manuscript.

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REFERENCES

1. Schiffmann R, van der Knaap MS. Invited article: an MRI-based approach to the diagnosis of white matter disorders. *Neurology* 2009;72:750–759.
2. Loeffen JL, Smeitink JA, Trijbels JM, et al. Isolated complex I deficiency in children: clinical, biochemical and genetic aspects. *Hum Mutat* 2000;15:123–134.

3. Distelmaier F, Koopman WJ, van den Heuvel LP, et al. Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease. *Brain* 2009;132:833–842.
4. Tucker EJ, Compton AG, Calvo SE, Thorburn DR. The molecular basis of human complex I deficiency. *IUBMB Life* 2011;63:669–677.
5. van der Knaap MS, Breiter SN, Naidu S, et al. Defining and categorizing leukoencephalopathies of unknown origin: MR imaging approach. *Radiology* 1999;213:121–133.
6. Leegwater PA, Vermeulen G, Konst AA, et al. Subunits of the translation initiation factor eIF2B are mutant in leukoencephalopathy with vanishing white matter. *Nat Genet* 2001;29:383–388.
7. Leegwater PA, Yuan BQ, van der Steen J, et al. Mutations of MLC1 (KIAA0027), encoding a putative membrane protein, cause megalencephalic leukoencephalopathy with subcortical cysts. *Am J Hum Genet* 2001;68:831–838.
8. Scheper GC, van der Klok T, van Andel RJ, et al. Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. *Nat Genet* 2007;39:534–539.
9. Zara F, Biancheri R, Bruno C, et al. Deficiency of hyccin, a newly identified membrane protein, causes hypomyelination and congenital cataract. *Nat Genet* 2006;38:1111–1113.
10. Steenweg ME, Ghezzi D, Haack T, et al. Leukoencephalopathy with thalamus and brainstem involvement and high lactate 'LTBL' caused by EARS2 mutations. *Brain* 2012;135:1387–1394.
11. Steenweg ME, Pouwels PJ, Wolf NI, et al. Leukoencephalopathy with brainstem and spinal cord involvement and high lactate: quantitative magnetic resonance imaging. *Brain* 2012;134:3333–3341.
12. Ku CS, Naidoo N, Pawitan Y. Revisiting Mendelian disorders through exome sequencing. *Hum Genet* 2011;129:351–370.
13. Wolf NI, Seitz A, Harting I, et al. New pattern of brain MRI lesions in isolated complex I deficiency. *Neuropediatrics* 2003;34:156–159.
14. Calvo SE, Tucker EJ, Compton AG, et al. High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. *Nat Genet* 2010;42:851–858.
15. Tucker EJ, Mimaki M, Compton AG, et al. Next-generation sequencing in molecular diagnosis: NUBPL mutations highlight the challenges of variant detection and interpretation. *Hum Mutat* 2012;33:411–418.
16. Janssen AJ, Trijbels FJ, Sengers RC, et al. Spectrophotometric assay for complex I of the respiratory chain in tissue samples and cultured fibroblasts. *Clin Chem* 2007;53:729–734.
17. Janssen AJ, Trijbels FJ, Sengers RC, et al. Measurement of the energy-generating capacity of human muscle mitochondria: diagnostic procedure and application to human pathology. *Clin Chem* 2006;52:860–871.
18. Rodenburg RJ. Biochemical diagnosis of mitochondrial disorders. *J Inher Metab Dis* 2011;34:283–292.
19. Calvaruso MA, Smeitink J, Nijtmans L. Electrophoresis techniques to investigate defects in oxidative phosphorylation. *Methods* 2008;46:281–287.
20. Sheftel AD, Stehling O, Pierik AJ, et al. Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I. *Mol Cell Biol* 2009;29:6059–6073.
21. Bych K, Kerscher S, Netz DJ, et al. The iron-sulphur protein Ind1 is required for effective complex I assembly. *EMBO J* 2008;27:1736–1746.
22. Van der Knaap MS, Valk J. *Magnetic Resonance of Myelination and Myelin Disorders*, 3rd ed. Heidelberg: Springer; 2005.
23. Tenisch EV, Lebre AS, Grevett D, et al. Massive and exclusive pontocerebellar damage in mitochondrial disease and NUBPL mutations. *Neurology* 2012;79:391.
24. Pagliarini DJ, Calvo SE, Chang B, et al. A mitochondrial protein compendium elucidates complex I disease biology. *Cell* 2008;134:112–123.
25. Sazanov LA, Hinchliffe P. Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*. *Science* 2006;311:1430–1436.

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