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Insertion of Mutant Proteolipid Protein Results in Missorting of Myelin Proteins

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Abstract

Two brothers with a leukodystrophy, progressive spastic diplegia, and peripheral neuropathy were found to have proteinaceous aggregates in the peripheral nerve myelin sheath. The patients' mother had only subclinical peripheral neuropathy, but the maternal grandmother had adult-onset leukodystrophy. Sequencing of the proteolipid protein (PLP) gene showed a point mutation IVS4 + 1 G→A within the donor splice site of intron 4. We identified one transcript with a deletion of exon 4 (ex4, 169bp) encoding for PLP and DM20 proteins and lacking two transmembrane domains, and a second transcript with exon 4 + 10bp encoding three transmembrane domains. Immunohistochemistry showed abnormal aggregation in the myelin sheath of MBP and P0. Myelin-associated glycoprotein was present in the Schmidt–Lanterman clefts but significantly reduced in the periaxonal region. Using immunogold electron microscopy, we demonstrated the presence of mutated PLP/DM20 and the absence of the intact protein in the patient peripheral myelin sheath. We conclude that insertion of mutant PLP/DM20 with resulting aberrant

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distribution of other myelin proteins in peripheral nerve may constitute an important mechanism of dysmyelination in disorders associated with PLP mutations.

The PLP gene maps to the long arm of the human X chromosome at Xq21–q22 and encodes for the two major myelin proteins of the central nervous system (CNS), the proteolipid protein (PLP), and its spliced isoform DM20.¹ PLP is the major structural component of CNS myelin, whereas DM20, which is produced earlier in CNS development, may be involved in oligodendrocyte differentiation and survival,² as well as in the maintenance of myelin compaction.³ Additional alternative splicing products with a soma-restricted expression (sr-PLP and sr-DM20) has been found in mice. In addition to oligodendrocytes, these sr-PLP/DM20 proteins also are expressed by several classes of neurons.⁴ In humans, the clinical findings associated with PLP mutations are spread over a wide continuum, extending from the most severe form of Pelizaeus–Merzbacher disease to the relatively mild, late-onset spastic paraplegia, leading to the concept of PLP-related disorders.^{5–7}

A demyelinating peripheral neuropathy is frequently observed in PLP1 null mutations⁸ and mutations which truncate PLP1 expression within the PLP1-specific domain without alteration of DM20 expression.⁹ In our investigation of patients with leukodystrophies of unknown cause, we identified a novel PLP mutation associated with aggregation and abnormal distribution of myelin proteins in the peripheral nervous system (PNS).

Patients and Methods

The family pedigree is described in Figure 1. The father (II-3) of the two affected boys had a progressive macular degeneration considered as Stargardt's disease. The history and clinical characteristics of the affected members of the family are described in Table 1.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of the brain of Patients 1 and 2 showed normal T1-weighted images contrasting with diffusely increased signal on T2-weighted images and on fluid-attenuated inversion recovery images. The corpus callosum, internal capsules, and cerebellar white matter had normal low signal on T2-weighted images. No cortical or cerebellar atrophy was present. No change was observed in successive MRI performed at age 7, 10, and 11 years. MRI of Patient 3 showed mild ventricular enlargement, with increased periventricular signal on T2-weighted and fluidattenuated inversion recovery images. Patients 1 to 3 had on ¹HMRSI (Bonavita and colleagues¹⁰) a significant reduction of *N*-acetyl aspartate and choline-containing compounds in the brainstem and hemispheric cortex and white matter (not shown).

Nerve Conduction Studies

Nerve conduction studies showed mild to moderate slowing of motor conduction velocity, decreased amplitude, and prolongation of distal and F-wave latencies (Table 2). These findings were sometimes asymmetric and almost always spared the sensory fibers (see Table 2). Electromyography was normal in all patients.

Evoked Potentials

Brainstem auditory-evoked potentials of Patient 1 performed at 9 years of age for both right and left ears showed a normal peak I without subsequent reproducible peaks. Patient 2 had normal brainstem auditory-evoked potentials. Somatosensory-evoked potentials performed at age 11 years for Patient 1 confirmed the severe impairment of CNS conduction without reproducible cortical peaks with posterior tibial stimulation of both legs. Median nerve stimulation showed a prolonged N20 at 23 and 24m/sec after right and left median stimulation, respectively.

Materials

GENOMIC DNA ANALYSIS—Patients' genomic DNA was extracted from peripheral blood lymphocytes. Each exon of the *PLP1* gene was sequenced using primers flanking the coding region and the splicing sites according to the conditions previously reported.⁵ After amplification, the polymerase chain reaction (PCR) products were purified and sequenced on an ABI 377 automated DNA sequencer (PE Biosystems, Warrington, UK), using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems).

TRANSCRIPTS ANALYSIS—Total RNA was extracted either from sural nerve biopsy tissue of patient and normal controls using Rneasy Mini Kit (Qiagen, Valencia, CA) followed by Dnase I treatment. RNA extract subsequently was reverse transcribed. The complete coding cDNA sequence has been amplified using F1 (5' CCG AAG AAG GAG GCT GGA GAG AC 3') and R1 (5' GGC CCC TAT AGA TGG CAA GAG GAC 3') primers spanning the region from start codon in exon 1 to stop codon in exon 7. PCR was performed in a volume of 50μl on 1μl of reverse transcription reaction with 1 × Gold buffer, 1.5mM MgCl₂, 200M dNTPs, 40pmol of each primer, 3U Taq Gold (Perkin-Elmer, Boston MA). PCR was performed on a GeneAmp PCR System 9700 (Perkin-Elmer) under the following conditions: 95°C for 12 minutes; 35 cycles of 94°C for 20 seconds, 63°C for 30 seconds, and 72°C for 2 minutes 30 seconds; and 72°C for 3 minutes. PCR products then were run on 2% agarose gel. Each PCR product was then isolated by cutting the gel, purified using the Gel Extraction kit (Qiagen) and cloned into the pGEM-TEasy vector (Promega, Madison, WI). The clones were sequenced with either the F1 or R1 primer on an ABI 377 automated DNA sequencer (PE Biosystems), using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems). The presence of PLP transcript containing exon 4 in F1/R1 PCR product was confirmed using the following primers: PLP Ex3bF (5' GTT CCA GAG GCC AAC ATC AA 3') and PLP Ex4R (5' GCA ATA GAC TGG CAG GTG GT 3'). PCR was performed in a volume of 50μl on 1μl F1/R1 PCR product diluted (1/10th) with 1 × buffer (Invitrogen, Carlsbad, CA), 2mM MgCl₂, 200M dNTPs, 40 pmol of each primer, 3U Taq DNA Polymerase (Invitrogen). PCR was performed under the following conditions: 95°C for 5 minutes; 35 cycles of 94°C for 20 seconds, 61°C for 15 seconds, and 72°C for 20 seconds; and 72°C for 5 minutes.

NERVE BIOPSY—Specimens were obtained under general anesthesia in the presence of the examining neuropathologist who received and prepared specimens for study. Portions were prepared for electron microscopy by immersion in a 2.5% solution of glutaraldehyde in 0.025M cacodylate buffer (pH 7.35–7.45).

Sections for immunohistochemistry were preserved in formalin and embedded in paraffin.

Specimens for embedding in LR White medium and examination with immunogold were immersed in phosphatebuffered saline (PBS) with 4% paraformaldehyde. Additional tissue was cryopreserved by immersion in isopentane suspended in liquid nitrogen. Paraffin-embedded and plastic-embedded tissues samples were processed, prepared, and stained at both the National Institutes of Health and the Armed Forces Institute of Pathology.

ANTIBODIES USED IN IMMUNOHISTOCHEMISTRY, IMMUNOELECTRON MICROSCOPY, AND WESTERN BLOTS

—The mouse monoclonal antibody to MAG (B11F7) was purified by affinity chromatography from its hybridoma supernatant.¹¹ A mouse monoclonal antibody to myelin protein zero (P0) was from a hybridoma kindly provided by Christopher Linnington (Martinsreid, Germany). Rabbit polyclonal antibodies to myelin basic protein (MBP) were obtained from DAKO Corporation (Carpinteria, CA). Mouse monoclonal antibodies recognizing residues 40 to 59 of both PLP and DM20 were generously provided by Drs V. Kuchroo and E. Greenfield. Rabbit polyclonal antibodies to PLP1-specific residues 117 to 129 were kindly given by Dr I. Griffiths. For the C-terminal of PLP, AA3 monoclonal antibody for residues 264 to 276 was a gift of Dr W. Macklin.

IMMUNOHISTOCHEMISTRY—Immunohistochemistry for MAG, P0, and MBP were standardized on mouse tissue and normal human peripheral nerve biopsies over 10 dilutions ranging from 40 to 2,000 and seven time intervals ranging from 1 to 36 hours. In Patients 1 and 2, as well as the normal and pathological controls, immunohistochemistry was performed with anti-P0 at 1 to 20 dilution and incubated for 4 hours; MBP was used at 1 to 400 dilution and incubated for 1 hour; and anti-MAG was diluted 1 to 100 and incubated for 4 hours.

WESTERN BLOT—Sural nerves from the patient and controls were homogenized in PBS containing a cocktail of protease inhibitors (Pierce, Rockford, IL). Protein concentrations were determined by bicinchonic acid assay (Pierce). Total nerve homogenate (20µg protein/lane) was loaded onto 14 % Tris-glycine polyacrylamide minigels (Novex, San Diego, CA), transferred to nitrocellulose, and immunostained with antibodies to MAG (1:100), MBP (1:500), or P0 (undiluted hybridoma supernatant). After incubation with peroxidase-labeled anti-mouse or anti-rabbit secondary antibodies, the proteins were detected with chloronaphtholdiaminobenzidine (Pierce) or enhanced chemiluminescence (NEN Life Sciences Products, Boston, MA).

IMMUNOELECTRON MICROSCOPY—Peripheral nerve tissue, fixed in 4% paraformaldehyde/PBS, was embedded in LR White resin (SPI, West Chester, PA). Ultrathin sections were mounted on 150-microns mesh uncoated nickel grids. Grids were floated on blocking solution (PBS, 0.1% Tween 20, 0.5% cold water fish gelatin; Ted Pella, Redding, CA) for 20 minutes, incubated for 1 hour with the same primary antibodies used in conventional immunohistochemistry above, rinsed in blocking buffer for 5 minutes, and then incubated with 10nm gold-conjugated secondary antibody (Ted Pella), rinsed in PBS, and air dried. Sections were stained with uranyl acetate and examined with a Phillips CM10

electron microscope. Concurrent staining with secondary antibody only was routinely performed with each sample. Virtually no back-ground labeling was seen in any case.

Results

PLP1 Is Mutated in the Affected Patients

The early-onset spastic paraplegia associated with a diffusely abnormal signal on hemispheric white matter exclusively on T2-weighted MRI in two affected brothers, and the late-onset spastic paraplegia associated severe alteration of CNS conduction and dementia in the grandmother of these patients suggested an X-linked form of spastic paraplegia related to *PLP1* mutations (SPG2). Sequence analysis of genomic DNA amplified with primers flanking exon 4 demonstrated a G to A substitution in the splice donor site at the beginning of intron 4 (IVS4 + 1 G → A) in both affected brothers (see Fig 1). Both the clinically normal mother and the affected grand-mother were heterozygotes for the G to A substitution (see Fig 1).

Various PLP1 and DM20 Transcripts Are Expressed in the Sural Nerve of the Mutated Male Patients

To identify the PLP and DM20 transcripts encoding by the patient's mutated gene, we performed reverse transcription PCR using primers designed to amplify the full-length (1,365bp) open reading frame cDNA of PLP from RNA extract of a sural nerve biopsy. In nerve biopsy from the control, two bands corresponding to the PLP and DM20 transcripts were visualized by gel electrophoresis, DM20 transcript being more abundant than the full-length PLP transcript. The PCR products in Patient 1 resolved on gel electrophoresis into three fragments: two shorter than those isolated from the control and one of similar size than the wild-type DM20 (Fig 2A). Sequence analysis obtained after cloning of the fragments showed that the two shorter transcripts correspond to DM20 and PLP transcripts with a 169bp deletion within exon 4 (ex4, 169bp), whereas the longer transcript corresponds to a DM20 transcript containing a normal exon 4 with an additional 10bp of the intron 4 (Ex4 + 10bp; see Fig 2C). Subsequent amplifications using specific primers of, respectively, exon 3b and exon 4 sequences (Ex 3bF/Ex4R) demonstrated that a PLP transcript with Ex4 + 10bp is also expressed (see Fig 2B). The predicted PLP1 and DM20 proteins resulting from the two ex4 transcripts would lack the C and D transmembrane domains, the extracytoplasmic C–D loop, and the C-terminal region. The proteins resulting from Ex4 + 10bp transcripts are expected to have a normal C transmembrane domain and part of the C–D loop (see Fig 2D).

Myelin Structure of Sural Nerve from PLP1 Mutated Male Is Altered

Sural nerve specimens and muscle biopsy was performed on Patient III-2 only. Muscle biopsy was normal. Neuropathology on toluidine blue semithin sections showed a moderate to severe loss of large and small myelinated axons, abundant, thinly myelinated axons, and near absence of axons with normal myelin-sheath-thickness to axon-diameter ratio (Fig 3A, B). Many of the large myelinated axons had disrupted and lightly stained myelin lamellae with granular, darkstaining deposits on the toluidine blue semithin sections (see Fig 3B). Onion bulbs were rare and poorly formed.

The Presence of Mutant Proteolipid Protein/DM20 Disrupts the Distribution of Myelin Proteins in the Sural Nerve

Immunohistochemical analysis showed that MBP (see Fig 3C, D) and P0 (see Fig 3E, F) immunoreactivity was particulate and distributed in punctate aggregates within the myelin sheath, in contrast with the normal homogeneous, immunoreactivity in the control tissues. MAG immunoreactivity in the patient was strong in the Schmidt–Lanterman clefts and paranodes but substantially reduced or undetectable at the normal periaxonal location in virtually all the axons examined (see Fig 3G, H). Western blot showed substantial reduction of MBP, P0, and MAG in the nerve of the patient compared with control (see Fig 3I). Neither wild-type nor mutated PLP/DM20 was detectable in extract from the sural nerve biopsy using the Western blot technique.

Immunoelectron microscopy examination with both the monoclonal antibody recognizing residues 40 to 59 of PLP and DM20 and the polyclonal antibody for the PLP-specific residues 117 to 129 demonstrated that the mutant PLP/DM20 were present in the normally compacted myelin and in the disrupted layers (Fig 4A, B). To confirm that normal PLP/DM20 was not present in the patient's myelin sheath, we stained sural nerve tissue using an antibody for the C-terminal of PLP.¹² Contrary to its presence in the normal control, we found no immunogold labeling in the myelin sheath of the patient (not shown).

Discussion

We describe here a family with a novel splice site mutation in intron 4 of the *PLP1* gene, which alters *PLP1* RNA splicing, predicted to produce truncated PLP and DM20 proteins. The age of presentation of the two index patients with a spastic paraparesis with relative preservation of cognition and the paucity of other CNS deficits suggest the SPG2 rather the Pelizaeus–Merzbacher form of *PLP1* mutants.⁵ Using PLP/DM20 antibodies against different part of the proteins, we demonstrated that at least one form of the mutant protein is present in the PNS myelin sheath despite a predicted loss of at least the fourth transmembrane domain and the C-terminal of both PLP and DM20. This alteration of PLP/DM20 caused a progressive myelinopathy affecting both the CNS and PNS in hemizygous affected males as well as in heterozygotes females. The presence of mutant PLP/DM20 in the myelin sheath led to an abnormal distribution of other major myelin proteins in the PNS. A similar abnormal distribution of proteins may occur in the CNS. The findings on MRI and ¹HMRSI indicate that axonal damage and hypomyelination occurs in the CNS as a result of the PLP mutation.¹⁰ The mechanism of mutation in our patients resembles a previous report, in which a G to T transition in the donor splice site of intron 6 results in skipping of exon 6.¹³ That mutation, unlike this one, was an in-frame deletion that would not be expected to prematurely terminate protein translation.

Dysmyelinating peripheral neuropathy has been reported in patients with *PLP1* mutations.^{8,9} Its occurrence as a moderate multifocal motor and sensory demyelinating neuropathy has been associated primarily with null mutations caused by either a deletion in the genomic DNA,¹⁴ a premature stop codon generated by a frameshift mutation after an early single base deletion,¹⁵ a substitution in the initiation codon,¹⁶ or a nonsense mutation affecting PLP, but sparing DM20.^{8,9} Unlike other instances with demonstrated PNS involvement, the

mutation in this case resulted in a severe phenotype with onset of clinical signs in the neonatal period. The PNS signs and electrodiagnostic findings in the other reported clinical cases and kindreds were more consistent with a lower motor neuron disease, rather than a dysmyelinating peripheral neuropathy. PNS integrity has been formally addressed in a limited number of cases.^{8,15–24} Alteration of the PLP-specific transcript, but not DM20, is necessary to cause peripheral neuropathy in *PLP1* mutations, suggesting that the PLP-specific domain plays an important role in normal peripheral nerve function.⁹ Because, as in our patients, clinical evidence of peripheral neuropathy can be relatively subtle, it is certainly possible that there are other *PLP1* mutations in which existing PNS dysfunction was not detected or evaluated. The relative preservation of sensory nerve conduction and amplitude indicates that, despite the disruption of myelin structure, a sufficient number of fast-conducting myelinated nerve fibers remained.

Although its role in the PNS is not yet clear, PLP is thought to stabilize the intraperiod line in the CNS and facilitate development or maintenance of compact myelin.^{25,26} The normal periodicity of the compacted portion of the myelin sheaths in our patient either confirms the critical role of the cysteine residues present in the first three hydrophilic loops of the protein or indicates that PLP plays a minor role in myelin periodicity of the PNS. Additional proposed functions of PLP include formation of ionophores or proton channels and maintenance of important interactions between myelinating cells and axons.²⁷ Studies seeking to link disturbances in specific residues or domains with disease severity have uncovered correlations^{5,28–31} but have not firmly established the functional significance of individual domains. Phenotypic severity of PLP mutants reflects *in vivo* and *in vitro* variations in transport and folding of PLP isoproteins.^{30,31} Loss of function as a result of absence of transcript,^{14,16} translational failure,¹⁵ early truncation that eliminates potentially essential domains,^{5,15,32–35} or, possibly, transcript instability usually is associated with relatively mild phenotypes, as in our patients. Clinical disease was evident only when the terminal exon was affected.^{5,35}

We demonstrated, using immunoelectron microscopy, that PLP/DM20 mutant proteins were present in the PNS myelin sheaths. The inability to detect PLP/DM20 on Western blot is most likely caused by the insensitivity of the method to detect even the normal protein in nerve homogenates. It is likely that the resulting polypeptide (Ex4 + 10bp) is preferentially inserted into the myelin sheath rather than the ex4, 169bp that lacks the third transmembrane domain. The C terminus, mutated in our patients' PLP, may be an important functional domain.^{36,37} It is difficult to determine the relative contribution to the dysmyelination of the insertion of the mutant PLP/DM20 and the abnormal distribution of other myelin proteins.³⁸ As in our patients, mutations of individual myelin genes often cause reduction in levels of other myelin proteins,^{39–42} and abnormal distribution of myelin protein secondary to mutations in MPB, P0, connexin32, and PMP22 has been described.^{25,41,43,44}

Trafficking of PLP/DM20 appeared to be independent of other myelin proteins such as MOG, MAG, or MBP. PLP/DM20 was found to colocalize with MBP and MOG at the level of the plasma membrane, but the trafficking to the endosomal/lysosomal compartment appeared unique to PLP.⁴⁵ The association of PLP with myelin lipids such as

galactocerebroside and cholesterol in the Golgi may be critical for the efficient sorting of this protein. PLP may assemble into a “myelin raft” which directs sorting and trafficking of myelin components.⁴⁵ Recent studies demonstrated that overexpression of PLP leads to the formation of endosomal/lysosomal accumulations of cholesterol and PLP accompanied by the mistrafficking of myelin raft components which can be responsible for impairment in oligodendrocytes viability.⁴⁶ Mutant PLP may have abnormal interaction with myelin lipids causing changes in the conformation of the cell membrane resulting in abnormal insertion of other myelin proteins.⁴⁷ Aberrant distribution of MAG may interfere with its normal function in glia–axon interactions.⁴⁸ Aging mice deficient for MAG expressed a peripheral neuropathy with mild reductions in conduction velocity and in the muscle action potential amplitude.⁴⁹

Because our patients have demonstrated abnormalities in other PNS myelin proteins (MAG, P0, MBP), this PLP mutation may contribute to our knowledge of the way in which genetic abnormalities in one myelin protein influences the disposition of other myelin constituents. Based on our findings, the pathogenic mechanisms of some mutations in the PLP gene deserve further investigation.

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Fig 1.

The pedigree of the affected family. The carrier status of Patient III-1 is not known.

Fig 2.

Aberrant splicing resulting from the mutation IVS4 + 1 G → A. (A) Reverse transcription polymerase chain reaction (PCR) products from total RNA extracted from sural nerve biopsy of patient and control with F1/R1 primers. Both proteolipid protein (PLP) and DM20 (missing 105bp of exon 3b) transcripts were found in the control, with DM20 more abundant than the full-length PLP transcript. In the patient sample, three different transcripts were found, one corresponding in size to wild-type DM20, and two shorter products. Sequencing showed that the two smaller products correspond to PLP and DM20 with deletion of exon 4

(169bp), whereas the larger one corresponded to DM20 containing exon 4 plus 10bp from intron 4. (B) Ex3bF/Ex4R PCR amplification on F1/R1 PCR product from patient shows that a PLP transcript containing exon 4 is also expressed. (C) Schematic overview showing the position of IVS4 + 1 G → A mutation in the patient and the different resulting splice forms. Donor and acceptor splice sites are indicated in capital letters. The underlined GT sequence in intron 4 corresponds to the new splice donor site used to splice exon 4 + 10bp with exon 5. (D) Two types of mutated proteins were expressed in the patient; one containing 178 amino acids of PLP (or 143 amino acids of DM20) with an additional 27 frameshifted amino acid sequence (marked in black) at the C terminus, and the other containing 208 amino acids of PLP (or 173 amino acids of DM20) with an additional 26 frame-shifted amino acid sequence at the C terminus.

Fig 3.

Light microscopy, ultrastructure, and immunohistochemistry of biopsied sural nerve. Light microscopy of toluidine blue-stained 1 μ m sections of control (A) with the normal thickness of myelin sheaths (arrow) and the patient (B), showing many thinly myelinated axons (arrow), loss of compaction of myelin sheaths and possible intramyelinic particulate material (B, arrowhead). (C) Immunohistochemical staining for P0 (C, control; D, patient) and myelin basic protein (E, control; F, patient) showing uneven and aggregated/particulate distribution (arrows) of these myelin proteins in the patient compared with the uniform

staining in the control (arrows). (G) control and (H) patient nerves stained for myelin-associated glycoprotein showing normal continuous periaxonal (arrows) and Schmidt-Lanterman cleft staining (arrowheads) in the control. Myelin-associated glycoprotein reactivity is absent from the periaxonal region (arrows) and is present only in the Schmidt-Lanterman clefts (arrowheads) in the patient's nerve. (I) Western blot of myelin proteins in patient (Pt) and control (Con) showing a marked reduction of these proteins in the patient. Proteolipid protein and DM20 were undetectable using this method (data not shown). Original magnification $\times 200$ in A and B; $\times 400$ in C to F; and $\times 1,000$ oil immersion lens in G and H.

Fig 4.

Immunoelectron microscopy. Immunoelectron microscopy for proteolipid protein (PLP) protein of nerve biopsy tissue of control (A) and patient (B) using secondary antibodies tagged with either 5nm gold particles for PLP-specific residues 117 to 129 (small arrow in both main image and inset) and 10nm gold particles recognizing residues 40 to 59 of both PLP and DM20 (large arrow in both main image and inset). (B) The mutant protein is identified both in the normal and in the poorly compacted myelin.

Table 1

Clinical Description of Patients with PLP1 Mutation

Patient No.	Early Development	Age at Examination (yr)	Cognitive Examination	Neurological Findings
1 (III-2)	No independent gait; spastic diplegia Kyphosis No independent gait	9.5	FSIQ 56; VIQ 73; PIQ 47	Dysarthria, mild optic atrophy, nystagmus. Weakness: mild UE, moderate-severe LE, hyperreflexia, Babinski, mild dysmetria
2 (III-3)	Motor delay, hypotonia, kyphosis	8	FSIQ 73; VIQ 94; PIQ 55, impaired auditory, verbal organization	Dysarthria, mild optic atrophy, nystagmus, Weakness mild UE, moderate-severe LE, hyporeflexia, Babinski, mild dysmetria
3 (I-2)	Onset age 18 yr: Dementia, spastic diplegia	52	VIQ 59; PIQ 63 (estimate)	Diplegia, hyporeflexia, dysmetria, tremor, Babinski, Ataxia, distal sensory deficit
4 (II-2)	Healthy	29	Normal	Normal

UE = upper extremities; LE = lower extremities; FSIQ = full-scale IQ; VIQ = verbal IQ; PIQ = performance IQ.

Table 2

Nerve Conduction Studies

Nerve	Patient				Reference Range
	1	2	3	4	
Sensory Nerves					
Superficial radial					
Amplitude			10µV	27µV	15µV
Velocity			53m/s	52m/s	50m/s
Sural					
Amplitude	24µV	21µV	8µV	7µV	6µV
Velocity	49m/s	44m/s	49m/s	38m/s	40m/s
Motor nerves					
Median					
APB amplitude		7.6mV	10.3mV	12.1mV	4.5mV
Velocity		31m/s	45m/s	44m/s	>50m/s
F-wave latency		35ms	30ms	37.6ms	<31ms
Tibial					
H amplitude	9.2mV	8.1mV	1.7mV	6.1mV	2.5mV
Velocity	34m/s	26m/s	34m/s	28m/s	>40m/s
F-wave latency	51ms ^a	52ms ^a	69ms	78.8ms	<61ms (adults)
Peroneal					
EDB amplitude	1.7mV	1.6mV	4.5mV	1.4mV	2.5mV
Velocity	34m/s	26m/s	34m/s	28m/s	40m/s
F-wave latency	47ms ^a	56ms ^a	64ms	81.5ms	<61ms (adults)

Abnormal values in are shown in boldface.

APB = abductor pollicies brevis; EDB = extensor digitorum brevis.

^aProlonged for height.