A Pathogenic Peripherin Gene Mutation in a Patient with Amyotrophic Lateral Sclerosis

Conrad L. Leung[1*]; Cui Zhen He[1*]; Petra Kaufmann[2]; Steven S. Chin[1]; Ali Naini[2]; Ronald K. H. Liem[1]; Hiroshi Mitsumoto[2]; Arthur P. Hays[1]

[1] Departments of Pathology and [2]Neurology, College of Physicians and Surgeons, Columbia University, New York, NY.
*These 2 authors contributed equally to this work.

Corresponding author:
Hays A. P., 630 W. 168th Street, PH 15th Stem Room124, Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY 10032 (E-mail: aph1@columbia.edu)

Peripherin is a neuronal intermediate filament protein that is expressed chiefly in motor neurons and other nerve cells that project into the peripheral nervous system. Transgenic mice that over-express peripherin develop motor neuron degeneration, suggesting that mutations in peripherin could contribute to the development of motor neuron disease. In this paper, we report the identification of a homozygous mutation in the peripherin gene (PRPH) in a patient with amyotrophic lateral sclerosis (ALS). The mutation resulted in a substitution of aspartate with tyrosine at amino acid position 141, which is located within the first linker region of the rod domain. Immunocytochemical analysis of the spinal cord of the patient upon autopsy revealed distinctive large aggregates within the cell bodies of residual spinal motor neurons that contained peripherin and was also immunoreactive with antibodies to the neurofilament proteins. In order to study the effect of the mutation on peripherin assembly, we performed transient transfections. Unlike wild-type peripherin, which self-assembles to form a filamentous network, the mutant peripherin was prone to form aggregates in transfected cells, indicating that the mutation adversely affects peripherin assembly. Moreover, the neurofilament light (NF-L) protein was not able to rescue the mutant protein from forming aggregates. These data imply that mutation of PRPH is a contributing factor for ALS.

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by motor neuron death. Approximately 10% of ALS cases are familial and exhibit a clear inherited trait. Thus far, 6 familial ALS loci have been reported, and mutations of an identified gene have been found for 2 of these loci (12, 13). The first identified gene encodes the cytosolic enzyme, copper-zinc superoxide dismutase 1 (SOD1). Mutations of the SOD1 gene are responsible for an autosomal dominant form of ALS with an onset in middle life (12). Mutations in the second familial ALS gene, *ALS2*, are responsible for an autosomal recessive form of ALS with early onset (13, 32). The ALS2 gene product is called alsin and contains multiple guanine-nucleotide exchange factor domains. The protein is thought to activate Rab5, a small GTPase that participates in endosomal trafficking (24). How mutations of the *SOD1* and *ALS2* genes cause ALS is still not clear.

Cytologically, the degenerating motor neurons of ALS patients exhibit loss of dendrites, perturbations of the cytoskeleton and formation of cytoplasmic inclusions. Among the cytoskeletal derangements, abnormal accumulations of neurofilaments (NFs) in axonal spheroids of motor neurons represent one of the most dramatic pathological hallmarks of this disease. The spheroids are made up of neuronal intermediate filament (IF) proteins, including the neurofilament triplet proteins (NFTPs) and peripherin. The NFTPs are composed of three subunits called NF-L, NF-M and NF-H. These proteins are the building blocks of NFs, and share a tripartite structure, consisting of a highly conserved central α-helical rod domain of ~310 amino acid and globular N-terminal head and C-terminal tail domains. The rod domain contains long stretches of heptad repeats that mediate the formation of coiled-coil dimers, the first step in IF assembly. Heterozygous mutations of NF-H have been identified in 1% to 2% of sporadic ALS cases and in one ALS family (11). These mutations consist of small deletions located in the tail domain of NF-H and may affect the phosphorylation of NF-H and disrupt NF assembly in neurons. Mutations of the NF-L gene are responsible for another neurological disorder, Charcot-Marie-Tooth (CMT) 2E (10, 18, 22). Mutations of NF-L disrupt its self-assembly in transfected cells and perturb axonal transport of neurofilament proteins in cultured neurons (6, 26). These in vitro abnormalities correlate with the human disease.

The NFTPs are widely expressed in the nervous system. In contrast, peripherin is expressed in chiefly the peripheral nervous system and enteric neurons. During nerve regeneration, peripherin expression is up-regulated, while the expression of the NFTPs is down-regulated (30, 31). In transfected cells, over-expressed peripherin can self-assemble into homopolymeric filaments and also co-polymerize with NF-L to form heteropolymeric filaments (4). In the absence of NF-L, rodent NF-M or NF-H can disrupt peripherin filaments. However, in the presence of NF-L, NF-H and NF-M co-assemble with peripherin to form heteropolymeric filament network.

In transgenic mice, over-expression of wild-type peripherin induces degeneration of spinal motor neurons during aging (3). Moreover, expression of mutant rodent peripherin in cultured cells interferes with the assembly of the protein into a filamentous network (15). These studies suggest that abnormal expression or mutations of peripherin in motor neurons could

contribute to the development of ALS. To test this hypothesis, we sequenced the peripherin gene (PRPH) of sporadic ALS patients and identified one individual with a homozygous mutation in exon 1. Large irregularly shaped-aggregates containing peripherin and NFTPs that differed from the morphology of other inclusions, including axonal spheroids, were found in the cell bodies of the remaining motor neurons of the patient. By transient transfections, we studied the assembly properties of this mutant peripherin and found that it is prone to form aggregates. Co-expression of wild-type peripherin, but not NF-L in transfected cells rescued the mutant protein from forming aggregates.

MATERIALS AND METHODS

Genotyping of PRPH gene. Genomic DNAs were extracted by Proteinase K treatment. DNA fragments that included exons and introns of the PRPH gene were PCR amplified using the primers shown in Table 1.

PCR products were purified and sequenced in both directions using BigDye™ sequencing kits and an ABI3700 automatic sequencer (Applied Biosystem).

Plasmid construction and site directed mutagenesis. The eukaryotic expression construct of human peripherin, pCMV-peripherin was obtained from I.M.A.G.E. consortium (GenBank accession no. BQ722318). The construction of human NF-L expression vector, pCI-NFL has previously been described (26). The peripherin D141Y mutation construct, pCMV-D141Y-peripherin was generated by site-directed mutagenesis using QuickChange Mutagenesis kit (Stratagene) following manufacturer's protocol.

Transient transfection and immunocytochemical studies. SW13vim$^-$ cells were grown at 37°C and 5% CO_2 in DMEM/F12 medium (Invitrogen) supplemented with 5% fetal bovine serum. Transient transfection experiments were done using Lipofectamine reagent (Invitrogen) according to the manufacturer's protocol. Forty-eight hours after transfection, coverslips with adherent cells were fixed in cold methanol at -20°C. After rinsing several times with PBS, adherent cells on coverslips were blocked with 5% normal goat serum and incubated with primary antibodies at room temperature for one hour. The primary antibody-treated cells were then washed with PBS and incubated with appropriate secondary antibodies (Molecular Probes) for 30 minutes. Subsequently, the coverslips were washed with PBS and mounted onto slides with Aquamount (Lerner Laboratories). Confocal microscopy was performed with a LSM 410 laser scanning confocal microscope (Zeiss). Statistical analysis of the phenotypes observed in cells transfected with wild-type and mutant peripherin constructs was carried out in four separate transfection experiments. Cells were immunostained with polyclonal anti-peripherin antibody and monoclonal anti-vimentin antibody. Immunostaining for vimentin was performed to eliminate counting of rare reverting cells that express vimentin.

Western blot analysis. To collect samples for Western blots, transient transfections were performed on SW13vim$^-$ cells in 60 mm dishes using one µg of a DNA construct in a single transfection experiment and 1+1 µg of two DNA constructs in a co-transfection experiment. Forty-eight

Exon1	Forward Primer: 5´-CCAGG CCCCG GCCTA GCTCT GCGAA CGG-3´ Reverse Primer: 5´-CCGCT TCGAC CTCGC CTCGA CGGCA GCG-3´
Exon2-3	Forward Primer: 5´-CTGGG CGCGA CCCCG CAGTT CAGCC TCT-3´ Reverse Primer: 5´-ACCAC CCCAC AACCT CAGAA ACCGC CCC-3´
Exon4-6	Forward Primer: 5´-GCGTG GAATT TGCGC CGCTG TCCAT CCT-3´ Reverse Primer: 5´-GCCCA GTCCC GACCC CGCCC TGCCC CGC-3´
Exon7-8	Forward Primer: 5´-CAGGG CGGGG CCTGG GCAGG GGCGC TGA-3´ Reverse Primer: 5´-ATAAT ATGGG AAATG GTGGG GTCCT GGC-3´
Exon9	Forward Primer: 5´-AGGGT GGCGC TGAAT GGCTT GTGCC CAT-3´ Reverse Primer: 5´-ACTTC CACAG AAACA AATGC ACTGG AGA-3´

Table 1. Sequences of primers used.

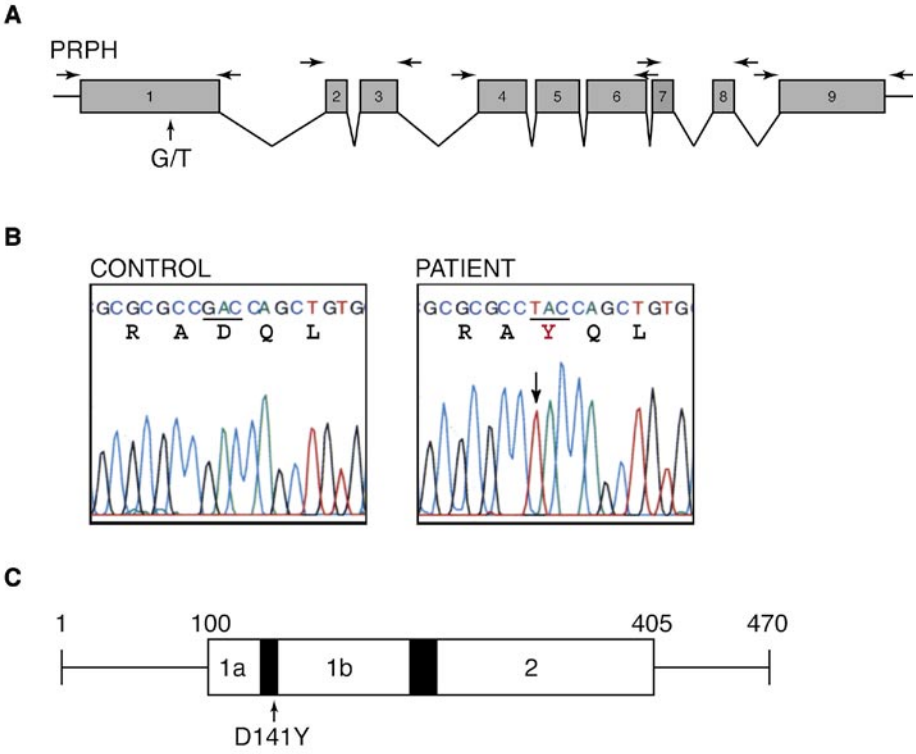

Figure 1. Analyses of a PRPH mutation. **A.** The structure of the peripherin gene is illustrated. Horizontal arrows indicate the relative positions of PCR primers used for genotyping. **B.** Electropherogram sequencing profiles of D141Y mutation in the patient and an unrelated control individual. The G→T transversion mutation is marked by an arrow and the affected codon is underlined. **C.** A schematic representation of the peripherin proteins. Coil 1a, 1b and 2 of the rod domain are labeled in the diagram and the linker regions are colored in black. The numbers of the amino acids that mark the boundaries of each domain are also shown. The arrow indicates the position of the D141Y mutation.

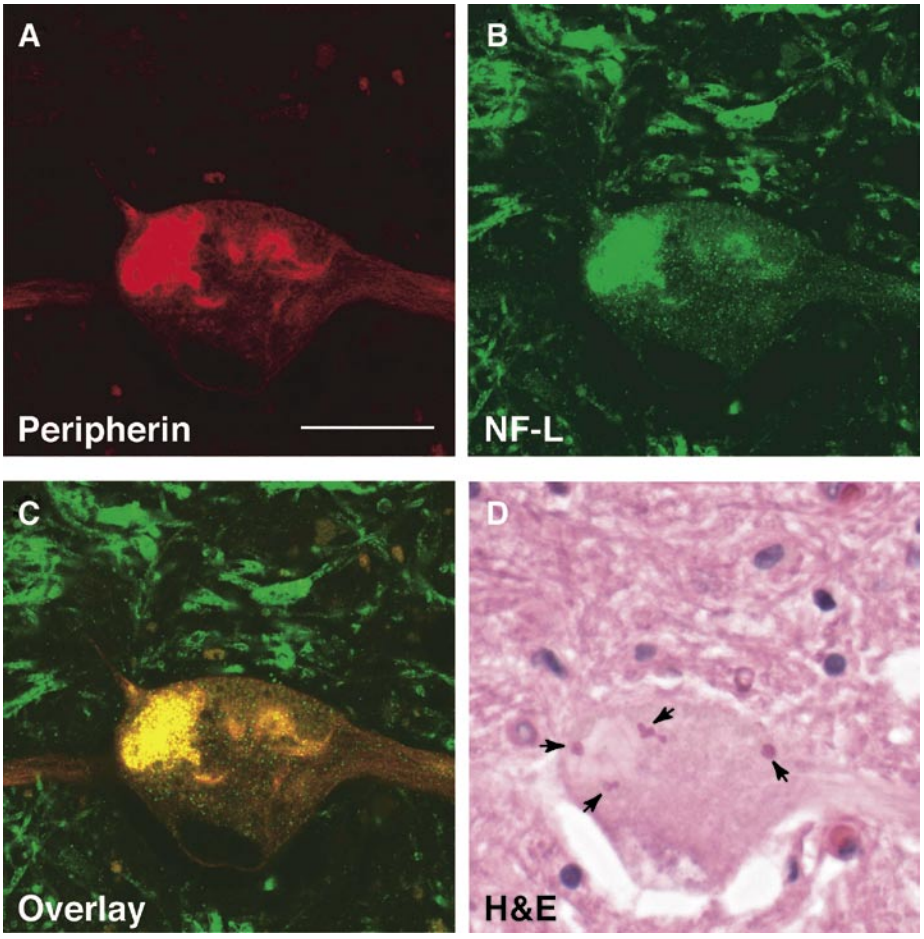

Figure 2. Histopathological analyses of the patient with D141Y mutation. Transverse section of the spinal cord obtained from the patient was stained with rabbit polyclonal anti-peripherin AB1530 antibody (**A**) and mouse monoclonal anti-NFL NR4 antibody (**B**). Images were taken with a confocal microscope on one optical section (**A**, **B**) and the superimposed images are also shown in panel **C**. After immunohistochemical analysis, the same section was retrieved and subjected to hematoxylin and eosin (H&E) staining (**D**). Inclusions that express both peripherin and NF-L were detected in cell bodies of the remaining motor nerve cells. H&E staining reveals that the inclusion is eosinophilic and hyaline and located in a ballooned or chromatolytic neuron. In the cell that is presented, the inclusion is located near four Bunina bodies (arrows in **D**). Bar = 25 μm.

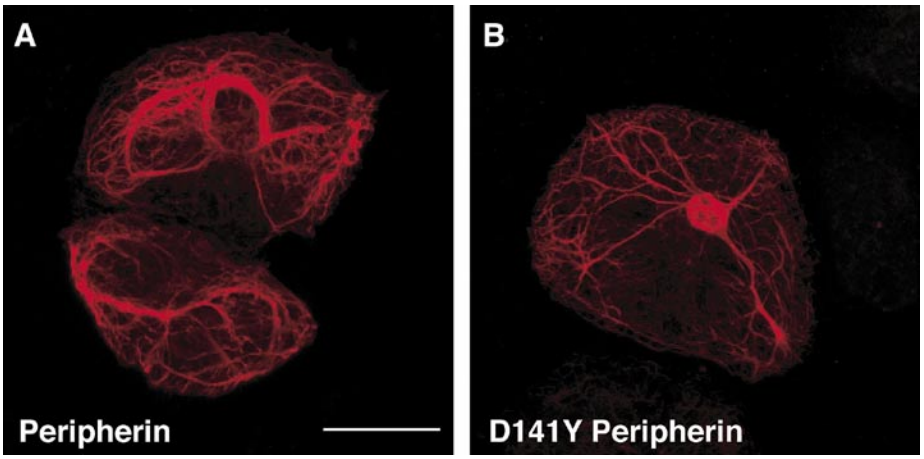

Figure 3. Self-assembly of human wild-type and D141Y mutant peripherin. SW13vim$^-$ cells from transient transfection of pCMV-peripherin (**A**) and pCMV-D141Y-peripherin (**B**) were labeled with polyclonal anti-peripherin Ab6264 and observed under a confocal microscope. Each of these composite figures was created by stacking 6 images from one-μm optical sections. The majority of transfected cells that expressed wild-type peripherin formed an extensive homopolymeric filament network. In contrast, cells that were transfected with the D141Y mutant peripherin construct demonstrate a less extensive filamentous network with a compact filamentous aggregate of the protein. Bar = 20 μm.

hours after transfection, cells were lysed in 75-μl lysis buffer containing 6.25mM Tris-HCL (pH7.5), 1% SDS, 5 mM EDTA and a cocktail of proteases inhibitors. Standard procedures for SDS-PAGE, protein-transfer onto PVDF membranes (Immobilon-P; Millipore), immunoblotting, and chemiluminescence (ECL; Amersham Life Sciences) were employed. One tenth of the volume of cell lysate collected from a 60 mm dish was used to blot with monoclonal anti-peripherin antibody (Chemicon) and half of this amount was used to blot with monoclonal anti-β-tubulin antibody (Sigma) as a loading control. The relative amounts of peripherin were determined by scanning the autoradiograph and quantified using NIH Image 1.63 software.

Histological analyses. Histological staining procedures on paraffin sections have been described previously (14). Briefly, antigen retrieval was performed by microwaving samples in citrate buffer (pH 6.0) for 20 minutes. After several washes with modified PBS buffer (pH 7.4) that contained 0.1% Triton X-100, the samples were blocked with 5% normal goat serum for 30 minutes, incubated with primary antibodies overnight, washed with PBS and incubated with the appropriate secondary antibodies (Molecular Probes) for 30 minutes, washed with PBS and mounted with coverslips. After being examined under a confocal microscope (Zeiss), immuno-stained samples were retrieved for hematoxylin and eosin staining by soaking in PBS overnight.

Antibodies. The following primary antibodies were used for this study: rabbit polyclonal anti-peripherin Ab6264 antibody (2); rabbit polyclonal anti-peripherin AB1530 antibody (Chemicon); and mouse monoclonal antibodies that recognize peripherin (8G2 antibody, Chemicon), NF-L (NR4 antibody, Sigma), NF-M (NN18 antibody, Boehringer-Mannheim), NF-H (SMI-31 and SMI-32 antibodies, Sternberger Monoclonals), vimentin (V9 antibody, Sigma), β-tubulin (2-28-33 antibody, Sigma), ubiquitin (MAB1510, Chemicon) and a phosphorylated epitope of tau (AT8 antibody, Research Diagnostics, Inc.).

RESULTS

Sequence analysis of PRPH in ALS. We sequenced the PRPH gene from a cohort of 30 individuals who were diagnosed with ALS by neurological examination and autopsy. Genomic DNA was extracted from autopsy samples and subjected to PCR amplification of 9 exons and 4 introns (number 2, 4, 5 and 7) of PRPH (Figure 1A). PCR products were sequenced in both directions. We identified one patient with a homozygous guanine to thymine mutation in exon 1 (Figure 1B). Analysis of 100 non-ALS controls (200 alleles) from the same white United States population as the patient did not reveal the same sequence variant, suggesting that it is not a common polymorphism. The mutation resulted in a substitution of aspartate with tyrosine at amino acid position 141 (D141Y). This amino acid is highly conserved in other species and is located within the linker region between coil 1a and coil 1b of the rod domain of peripherin (Figure 1C). The SOD1 gene of this patient was also sequenced and no mutations were found.

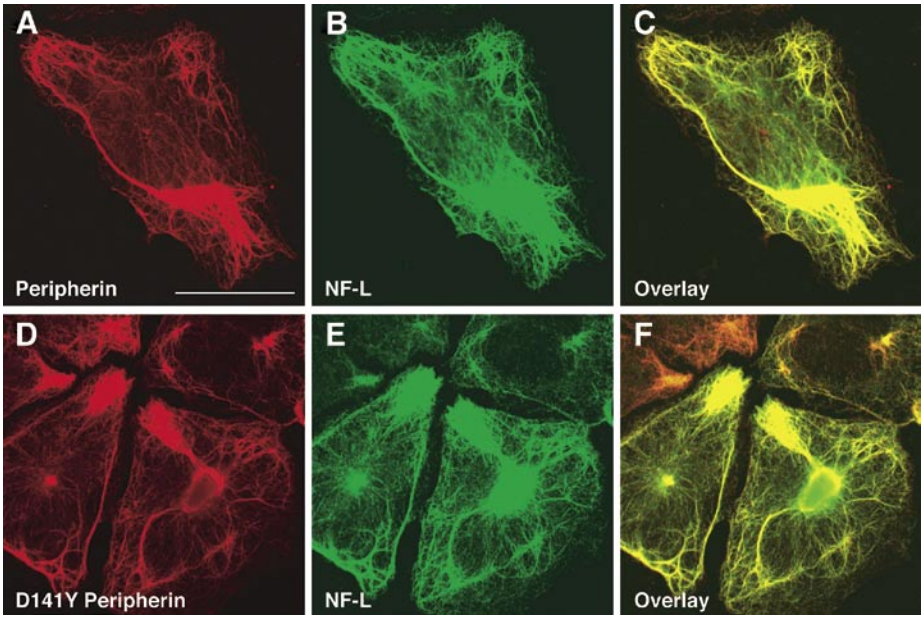

Figure 4. Co-assembly of human NF-L with wild-type and D141Y mutant peripherin. Transient transfections of pCI-NFL with pCMV-peripherin (**A**, **B**, **C**) and pCMV-D141Y-peripherin (**D**, **E**, **F**) were performed on SW13vim$^-$ cells. Transfected cells were stained with rabbit polyclonal anti-peripherin AB6264 antibody (**A**, **D**) and monoclonal mouse anti-NFL NR4 antibody (**B**, **E**). Images were taken with a confocal microscope on one optical section. Superimposed images of peripherin and NF-L stainings were illustrated in panel **C** and **F**. Wild-type human peripherin co-assemble with NF-L to form an extensive heteropolymeric network. When D141Y peripherin and wild-type NF-L are co-expressed in the same cells, the 2 proteins are co-localized in filaments and compact aggregates. NF-L cannot rescue mutant peripherin from forming aggregates. Bar = 20 μm.

Clinical and pathological examination of the patient with a homozygous D141Y peripherin mutation. The patient was a 42-year-old man, who first noticed difficulty manipulating small objects with his left hand. Weakness progressed to involve all four limbs, and he developed dysarthria and dysphagia. Weakness of the limbs was accompanied by wasting of the muscles in both hands and upper motor signs including spasticity, brisk tendon reflexes (with spread and clonus at the jaw and ankles) and bilateral Hoffmann signs but down-going toes. The patient did not have dementia and died of respiratory failure nearly 3 years after the onset of symptoms. There was no reported consanguinity in the family and no other member had been diagnosed with ALS. The maternal grandmother of the patient was reported to die of a neurological disorder, but no clinical or autopsy data were available to establish a diagnosis. Family members declined to have further genotyping performed.

Examination of the brain and spinal cord of the patient confirmed the diagnosis of ALS with no occult tumor or other major pathology. There was loss of Betz cells in the precentral gyrus and severe degeneration of the pyramidal tracts. The tract degeneration was most pronounced at the level of the spinal thoracic segments and consisted of loss of myelinated fibers and many macrophages. Moderate loss of motor neurons and astrocytosis were evident in the hypoglossal nucleus and ventral horns of the spinal cord. A few surviving motor nerve cells exhibited Bunina bodies or ubiquitinated skein-like inclusions but no Lewy body-like inclusions. A few motor neurons also had large filamentous inclusions of irregular shape that were strongly immunoreactive for peripherin (Figure 2A). Double-labeling of these inclusions showed that they also expressed NF-L (Figure 2B, C), NF-M and NF-H (data not shown). The inclusions were eosinophilic and hyaline (Figure 2D) and were not ubiquitinated. They tended to be located in ballooned cells with peripherally located nucleus. Morphologically, this kind of inclusion differed from all other inclusion bodies in a recent autopsy study of peripherin in 40 ALS patients (14). The inclusion closely resembles hyaline conglomerate inclusions, which are found in certain SOD1 mutations in ALS patients, but they are less compact in texture, more clearly fibrillar in substructure and only weakly argyrophilic based on the Bielschowsky stain. Rare neurofibrillary tangles that were immunoreactive for phosphorylated tau were found in cortical neurons (data not shown); they were strongly argyrophilic and did not express peripherin. The inclusions were flame-shaped or had a pre-tangle morphology and were located more commonly in layers 2 and 3 of the cortex than elsewhere. They affected all lobes of the cerebral hemispheres and were least common in the hippocampal formation and the entorhinal cortex. The tangles were accompanied by tau-containing threads in the adjacent neuropil. Betz cells did not exhibit neurofibrillary tangles and did not express peripherin.

In vitro studies of D141Y peripherin mutation. To determine whether the mutation affected the assembly properties of peripherin, transient transfections were performed on human SW13vim$^-$ cells that do not contain cytoplasmic IFs. Like rodent peripherin (9, 16), over-expressed wild-type (wt) human peripherin can self-assemble into an extensive homopolymeric network (Figure 3A). The majority of cells that were transfected with mutant peripherin contained filamentous aggregates that

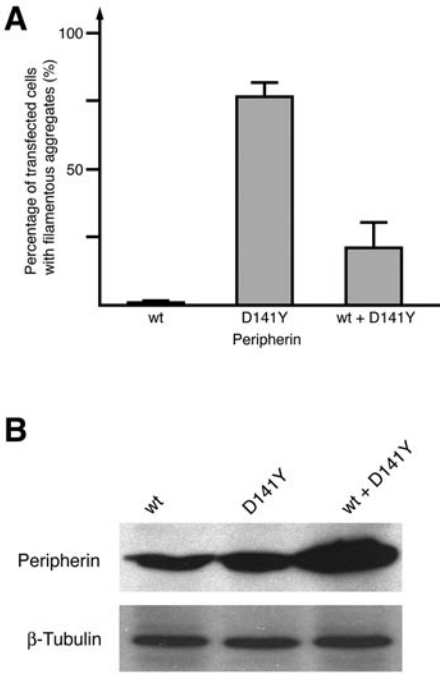

Figure 5. Rescue of D141Y peripherin aggregates by wild-type peripherin. SW13vim$^-$ cells were transfected with pCMV-peripherin, pCMV-D141Y-peripherin, or both constructs together. Immunostainings with anti-peripherin antibody were performed on the transfected cells and the percentage of transfected cells displaying filamentous aggregates were measured in four separate experiments and plotted onto a graph (**A**). Cell lysates collected from the transfection experiments were subjected to western blots with monoclonal anti-peripherin antibody and monoclonal β-tubulin antibody (**B**). Cells transfected with both constructs showed higher expression levels of peripherin. β-Tubulin was blotted as a loading control.

were never observed in cells transfected with *wt* peripherin (Figure 3B). When *wt* peripherin was co-expressed with NF-L in the same cell, they co-assembled into a filamentous network (Figure 4A, B, C); however, NF-L could not prevent the mutant D141Y peripherin from forming aggregates (Figure 4D, E, F). To determine whether mutant peripherin exerted a dominant effect over *wt* peripherin, co-transfection experiments were carried out with both *wt* and mutant peripherin constructs. Since we were not able to distinguish cells that were expressing only one or both proteins, we counted the number of cells that contained aggregates and found that it was dramatically reduced (22% in cells transfected with both constructs compared to 79% in cells transfected with the mutant protein alone), suggesting that *wt* peripherin rescued the assembly deficits of D141Y peripherin (Figure 5A). To determine the expression levels of *wt* and mutant peripherin in transfected cells, we performed Western blot analyses. Forty-eight hours post-transfection, the total amount of peripherin in cells transfected with both *wt* and mutant peripherin was 2-fold and 1.8-fold compared to cells transfected with *wt* or mutant peripherin respectively, illustrating that both *wt* and mutant peripherin were expressed together in the co-transfection experiments (Figure 5B). This finding is consistent with the autosomal recessive inheritance that we observed, as neither parent developed ALS even though both of them survived the death of their son. In summary, the D141Y mutation did not abolish the ability of peripherin to assemble into filaments, but led to the formation of aggregates. The mechanisms underlying aggregate formation of the mutant protein is not known.

DISCUSSION

ALS was first described by the French physician Jean-Martin Charcot as early as 1869. Although the etiology of this disease is still poorly understood, advances in molecular genetics and pathological examinations have revealed a few compelling disease mechanisms. The proposed mechanisms include damage of the motor neuron by oxidative stress, glutamate-mediated excitotoxicity, and aberrant protein aggregation. Regardless of the disease origin, aberrant protein aggregation in motor neurons is always observed during the course of the disease. In this report, we describe the first case of ALS related to a pathogenic mutation in PRPH. The mutation caused the aggregation of peripherin in cultured cells and may account for the aberrant peripherin aggregates found in the remaining motor neurons of the patient. We hypothesize that the formation of peripherin aggregates is a contributing factor to the development of ALS in this clinical case.

Cytoplasmic inclusions in the proximal axon and cell body of degenerating motor neurons are a universal pathological finding in ALS and are also evident in transgenic mice that over-express mutant SOD1. Moreover, prolonged glutamate treatment can induce accumulation of NFs in proximal axons of cultured neurons (1). Because NFs are a major component of the neuronal cytoskeleton and are expressed in axonal spheroids of ALS patients, their relationship with ALS has been studied extensively. To study the possible NF related neurotoxic effects, various knockout and transgenic mice have been generated, including mice that over-express single or combinations of NFTPs. These mice have been crossed also with SOD1 mutant mice to investigate whether NFTPs are involved in the deleterious pathway of mutant SOD1 (7). Together, these studies have painted a complex picture of NF-mediated neurodegeneration (for review, see 7, 19, 21). The main lesson learned from the transgenic models is that axonal accumulations of NFs are sufficient to cause neuronal death.

Although a lot of attention has focused on NFs in ALS, recent evidence has suggested that peripherin, another neuronal IF protein, may play an important role in the development of ALS. First, Lewy body-like inclusions and spheroids (axonal swellings) in motor neurons of sporadic ALS patients contain peripherin along with NFTPs (14). Second, over-expression of peripherin in transgenic mice resulted in selective motor neuron loss with IF aggregates in residual motor neurons (3). Moreover, high expression of peripherin also caused apoptotic death in cultured embryonic neurons (27). Third, a neurotoxic peripherin splice variant has been described in a mouse model of ALS (28) although this splice variant may not be present in humans because the length of intron 4 differs between the human (91 bp) and mouse (96 bp) PRPHs. However, upregulation of *wt* peripherin by transgene over-expression or suppression of peripherin by gene knock-out did not alter the course of pathology of SOD1 mutant transgenic mice, implying that the *wt* protein does not contribute to motor neuron death associated with mutant SOD1 toxicity (20). Nonetheless, it is possible that mutations of peripherin can be a contributing factor for ALS. Our findings of a PRPH mutation in an ALS patient who exhibits large peripherin inclusions in the dying motor neurons and the effect of this mutation on peripherin assembly in transiently transfected cells strongly support this notion.

How does a mutation of PRPH contribute to the development of ALS? In transfection assays, peripherin is able to self-assemble into a filamentous network and co-assemble with NFTPs to form heteropolymeric filaments (4). Immunofluo-

rescence and immunoelectron microscopy studies on PC12 cells and sciatic nerves have illustrated that some neurons contain predominantly either NFTPs or peripherin, whereas others express both proteins within the same IF (25). Therefore, a pathogenic mutation of PRPH would affect not only peripherin self-assembly, but also the organization of the whole neuronal IF network. As the D141Y mutation induces aggregates of peripherin that cannot be rescued by NF-L in transfected cells (Figure 4D, E, F), the NF arrays in peripherin-containing neurons of the patient may be disrupted also and form inclusion bodies composed of both NFTPs and peripherin (Figure 2). Potentially, these inclusion bodies could perturb axonal transport and lead to death of neurons although the precise mechanism of how the protein aggregates trigger cell injury and death remains to be elucidated. Similar theory has been proposed for NF-L mutations that are responsible for the degeneration of peripheral nerve axons observed in CMT2E. NF-L mutations have been shown to cause defects in NF assembly and disrupt axonal transport (6, 26). It is important to note that some of the autopsy findings in the patient, including loss of Betz cells in the precentral gyrus and neurofibrillary tangles formation in cortical neurons, cannot be easily explained by the mutation of PRPH because peripherin was not detected in these cells. Therefore, this aspect of the pathology could be a secondary effect due to the mutation or caused by another unknown mechanism. It is possible that peripherin is expressed also during early development of these cells or its expression is just too low to be detected by immunohistochemistry.

How did the PRPH mutation arise in the ALS patient? Transient transfection assays illustrated that mutant peripherin has a great tendency to form filamentous aggregates and that *wt* peripherin could rescue the defect (Figure 5). These data suggest that if formation of peripherin aggregates were the major cause of ALS in this patient, individuals carrying heterozygous D141Y PRPH mutation would not develop the disease. Since there was no family history of ALS and the identified mutation was homozygous in the patient, we speculate that the patient's parents were heterozygous carriers. Nevertheless, we cannot rule out the unlikely possibility of a homozygous spontaneous mutation, as family members of the patient have declined further genetic analysis. Without genetic information of the family, it is also impossible to study the segregation of the disease and the mutation. Hence, we cannot exclude the possibility that other unknown factors are responsible for the disease and that the gene mutation is a risk factor.

Many autosomal recessive disorders exhibit loss-of-function gene mutations whereas autosomal dominant inheritance often has mutations that produce altered or gain of function of the encoded protein, particularly in neurodegenerative diseases. If the ALS case presented here is due to the PRPH mutation, it represents an unusual mechanism of recessive mutation. On one hand, the mutant D141Y peripherin formed aggregates, indicating a gain-of-function. On the other hand, as demonstrated by transient transfection, the formation of abnormal aggregates could be blocked by co-expression of *wt* peripherin, suggesting a recessive inheritance pattern, at least at the level of the cell. Indeed, parents of the patient, presumably heterozygous carriers, have not been found suffering from ALS. Although recessive gain-of-function mutations are uncommon, they have been reported in genetic diseases linked to other IF gene mutations. For example, desmin-related myopathy usually shows dominant inheritance as a result of mutations of the desmin gene, but recessive inheritance has been reported. In one case, the patient had a homozygous deletion that shortened desmin by seven amino acids in coil 1b of the rod domain (23). He or she displayed severe generalized myopathy and aberrant intra-sarcoplasmic accumulation of desmin IFs. In transfected cells, overexpression of mutated desmin resulted in abnormal filaments and aggregate formation that could be rescued by co-expression of *wt* desmin. These results are reminiscent of our findings of a PRPH mutation in an ALS patient.

In fact, mutations of genes encoding many other IF proteins are linked to human genetic diseases. Mutations of many different keratin genes are responsible for a host of skin disorders (29); mutations of the phakinin gene cause congenital cataracts (8, 17); mutations of the glial fibrillary acidic protein gene result in Alexander disease (5); and as mentioned above, mutations of the NF-L gene are responsible for CMT2E (22). As in desmin myopathy, mutations of IF proteins frequently disrupt the ability of the mutant proteins to form filaments and consequently lead to perturbation of the whole IF network in affected cells. In many cases, mutations also cause IF protein to form aggregates. It has been suggested that the disruption of the IF network and the formation of aggregates are responsible for the development of the disease. As each IF gene exhibits distinctive tissue-specific expression of the encoded protein, each mutation determines a unique pattern of disease in different organs. Our findings of a pathogenic PRPH mutation in an ALS patient can further strengthen this concept. Peripherin is highly expressed in neurons of the peripheral nervous system and may contribute to the selective loss of motor neurons in ALS. Future studies will further elucidate the role of peripherin in the pathogenesis of ALS.

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