THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 44, Issue of October 29, pp. 45951–45956, 2004

Printed in U.S.A.

A Frameshift Deletion in Peripherin Gene Associated with Amyotrophic Lateral Sclerosis*

Received for publication, July 19, 2004
Published, JBC Papers in Press, August 17, 2004, DOI 10.1074/jbc.M408139200

François Gros-Louis‡§, Roxanne Larivière¶, Geneviève Gowing¶, Sandra Laurent‡, William Camu‖, Jean-Pierre Bouchard‡‡, Vincent Meininger§§, Guy A. Rouleau‡, and Jean-Pierre Julien¶ ¶¶**

*From the ‡Centre for Research in Neuroscience, McGill University Health Centre, Montreal General Hospital Research Institute, Montreal, Québec H3G 1A4, Canada, the ¶Centre de Recherche du Centre Hospitalier de l'Université Laval, Département d'anatomie et physiologie de l'Université Laval, 2795 boul. Laurier, Québec G1V 4G2, Canada, the ‖Unité de Neurologie Comportementale et Dégénérative Molecular Unit, INSERM V336, Institute of Biology, 34295 Montpellier Cedex 5, France, the **Clinique du Motoneurone, Service d'Explorations Neurologiques, Hôpital Guy de Chauliac, 34295 Montpellier Cedex 5, France, the §§Federation of Neurology Mazarin, Hôpital Pitié Salpetrière Paris France, 47–83, Bd. de L'Hôpital, 75651 Paris, Cedex 13, France, and the ‡‡Département des Sciences Neurologiques, Centre Hospitalier Affilié Universitaire de Québec, Pavillion Enfant-Jésus, Québec G1J 1Z4, Canada*

Peripherin is a neuronal intermediate filament associated with inclusion bodies in motor neurons of patients with amyotrophic lateral sclerosis (ALS). A possible peripherin involvement in ALS pathogenesis has been suggested based on studies with transgenic mouse overexpressors and with a toxic splicing variant of the mouse peripherin gene. However, the existence of peripherin gene mutations in human ALS has not yet been documented. Therefore, we screened for sequence variants of the peripherin gene (*PRPH*) in a cohort of ALS patients including familial and sporadic cases. We identified 18 polymorphic variants of *PRPH* detected in both ALS and age-matched control populations. Two additional *PRPH* variants were discovered in ALS cases but not in 380 control individuals. One variant consisted of a nucleotide insertion in intron 8 ($PRPH^{IVS8-36insA}$), whereas the other one consisted of a 1-bp deletion within exon 1 ($PRPH^{228delC}$), predicting a truncated peripherin species of 85 amino acids. Remarkably, expression of this frameshift peripherin mutant in SW13 cells resulted in disruption of neurofilament network assembly. These results suggest that *PRPH* mutations may be responsible for a small percentage of ALS, cases and they provide further support of the view that neurofilament disorganization may contribute to pathogenesis.

Amyotrophic lateral sclerosis (ALS)[1] is a common, usually fatal, adult onset neurodegenerative disorder characterized by degeneration of motor neurons in the cortex, brainstem, and spinal cord (1). Dysfunction and death of these motor neurons lead to progressive muscle weakness, atrophy, and ultimately paralysis and death, usually within 3–5 years after disease onset (2). The disease occurs in sporadic (SALS) and familial (FALS) forms with an estimated incidence of ~2 per 100,000. Although most cases of ALS are sporadic, some families demonstrate a clinically indistinguishable form of ALS with clear Mendelian inheritance and high penetrance (FALS). Mutations in the copper/zinc superoxide dismutase gene (*SOD1*), a ubiquitously expressed and highly conserved metalloenzyme, are responsible for ~15% of FALS (3, 4). More recently, a second gene (*ALS2*) has been identified and has been associated to a recessive and juvenile onset form of the disease (5, 6). The *ALS2* gene is ubiquitously expressed, and it encodes a protein having multiple motifs with homology to guanine nucleotide exchange factor. To date, there are over 90 different missense mutations that have been reported (www.alsod.org) in the *SOD1* gene and up to eight described mutations in the *ALS2* gene (5–9). Transgenic mice overexpressing mutant SOD1 genes develop a neurodegenerative disease with many pathological features found in both familial and sporadic ALS (10–12). However, the mechanism by which mutant SOD1 induces motor neuron cell death remains unclear.

A hallmark of motor neurons in ALS is the presence of perikaryal and axonal inclusion bodies composed of intermediate filament (IF) proteins, namely neurofilament and peripherin proteins (13–15). Neurofilaments, the major type of IFs expressed in adult neurons, are formed by the co-assembly of three type IV IF proteins, the neurofilament light (NF-L), neurofilament medium (NF-M), and neurofilament heavy (NF-H) subunits. Peripherin is a type III IF protein expressed predominantly in the peripheral nervous system with low levels detectable in spinal motor neurons (16–18). Peripherin expression is increased after neuronal injury (19, 20). The peripherin gene (*PRPH*) comprises nine exons spanning a 3.5-kb region of chromosome 12. The full-length peripherin protein contains 471 amino acids. Peripherin shows similarity in sequence to proteins of the IF family. The IF proteins share a central rod domain comprising 310 residues characterized by the presence of coiled-coil α-helices (1A, 1B, 2A, and 2B) connected by short linkers. The rod domain is flanked by an N-terminal non-helical head domain of variable length and a C-terminal tail domain tail, which is also non-helical with great length variation between different IF proteins.

Currently, it remains unclear to what extent IF abnormalities contribute to ALS pathogenesis. Codon deletions or inser-

* This work was supported by the NINDS National Institutes of Health, by the Muscular Dystrophy Association, and by the ALS Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by the Canadian Institute for Health Research.

¶¶ A Canada Research Chair in Neurodegeneration. To whom correspondence should be addressed: Centre de Recherche de CHUL, 2705 boul. Laurier, Québec G1V 4G2, Canada. E-mail: Jean-Pierre.Julien@crchul.ulaval.ca.

[1] The abbreviation used are: ALS, amyotrophic lateral sclerosis; FALS, familial ALS; SALS, sporadic ALS; PRPH, peripherin; IF, intermediate filament; NF-H, neurofilament heavy; NF-L, neurofilament light; NF-M, neurofilament medium.

TABLE I
Clinical information of the studied ALS cohort

Patient	Total	Male/Female	Age of onset[a]	Spinal onset[b]	Bulbar onset[b]	Duration
SALS	150	98/52	56 (23–82)	62	23	4.5
FALS	40	18/22	49 (37–60)	9	4	4.5

[a] Mean age of onset (with lowest and highest values in parentheses).
[b] Spinal/bulbar onset information were not available for all the patient.

tions in the KSP phosphorylation domain of NF-H have been detected in a small number of sporadic cases of ALS, ~1% of total cases (21–23). However, it is unknown how these NF-H variants might predispose to ALS. There is growing evidence for potential peripherin involvement in human disease. Overexpression of peripherin induced motor neuron disease in transgenic mice (24), and it provoked the death of cultured motor neurons (25). Moreover, an up-regulation of peripherin mRNA has recently been detected in a familial ALS case (26). Under certain conditions, including in mice expressing a mutant form of superoxide dismutase linked to ALS, the mouse peripherin gene was found to produce a splicing peripherin species that is assembly-incompetent and toxic when expressed in cultured neuronal cells (26). However, the involvement of peripherin in ALS has recently been called into question because neither up-regulation nor suppression of all peripherin isoforms had any effect on disease onset, mortality, and loss of motor neurons in mice expressing mutant SOD1 (27).

To further examine the possible involvement of peripherin in ALS, we screened the peripherin gene (*PRPH*) for sequence variations in sporadic and familial ALS cases from North American and European origins. Out of 20 identified *PRPH* variants, two were detected only in ALS cases and not in age-matched controls. Of particular interest is the discovery of a *PRPH* frameshift deletion mutant, the expression of which disrupted neurofilament assembly in cultured cells.

MATERIALS AND METHODS

Patient and Control Samples—All patients were assessed by expert clinicians, and they gave written informed consent. Diagnosis of ALS was made according to El Escorial ALS diagnostic criteria (35). The FALS panel comprised French (n = 23) samples as well as French-Canadian (n = 10) and North American (n = 7) patients. The SALS sample set comprised French (n = 95) and French-Canadian (n = 54) samples. The FALS cohort exhibited a mean age of onset of 49 years, and the SALS cohort exhibited a mean age of onset of 56 years. Both SALS and FALS cohorts showed a mean disease duration of 4.5 years (see Table I). An initial set of age-match control samples (n = 95) came from non-ALS French individuals. Additional control samples were screened to increase to the power of our analysis and comprised another set of age-match French controls (n = 95).

Variant Detection and Genotyping—DNA was extracted from whole blood and/or transformed lymphoblastoid cell lines using standard procedures. Primers were designed using Lazergene software and synthesized by Invitrogen. Thirteen overlapping primer pairs were designed from genomic DNA to amplify each intron and exon of the *PRPH* gene including the flanking splice sites and promoter as well of the 5′- and 3′-untranslated regions (see Fig. 1). Products were PCR-amplified, checked on agarose gels, and then sequenced using the forward primer for all of the amplicons. The PCR fragment sequence variations were also sequenced on the reverse strands. The PCR primer pair sequences will be given upon request.

Restriction Digest Analysis of Variants—Genotyping of the IVS1 + 69G → C and IVS2 − 78A → C variants was performed by restriction enzyme digestion analysis. The presence of the IVS1 + 69G → C and IVS2 − 78A → C variants creates new restriction sites, and amplified PCR fragments were digested with MboII and BamHI, respectively, to identify each of these two variations in the control population.

Statistical Analysis—We assessed differences in the distribution of each genotype between the groups (sporadic ALS, familial ALS, and control) by calculating the χ-square contingency test, from which we derived p values with one degree of freedom.

DNA Constructs—A 4.1-kb fragment was obtained by PCR amplification of the human *PRPH* gene. PCR was performed on DNA extracted from a patient blood sample by using PROOFSTART *Taq* (Qiagen) and the primers 5′-untranslated region, 5′-TGGGGCCTAAGCCAGGTCAACTCT-3′ and 3′-untranslated region, 5′-CTTTGAGCAGGCCTAGGGCAGAGT-3′ for 35 cycles at 94 °C for 15 s, 58 °C for 30 s, and 72 °C for 3 min. PCR product was cloned using TOPO TA cloning kit (Invitrogen). The cloned PRPH PCR fragment was then subcloned into the expression vector pcDNA^{TM}3.1 (Invitrogen) between the NotI site and the HindIII site of the multiple cloning sites. The cloned PRPH PCR fragments were direct sequenced using the primers described above.

Transient Transfection Assays—Sw13vim(−) cells lacking cytoplasmic IFs were grown in Dulbecco's minimal essential medium (Invitrogen) supplemented with 10% v/v fetal bovine serum at 37 °C and 5% CO_2. The cells were transiently transfected using LipofectAMINE Plus (Invitrogen) as described in the manufacturer's protocol. One μg of DNA from each of these constructs was used: peripherin^{mouse}, peripherin^{228delC}, peripherin$^{IVS8-36insA}$. Human NF-L and rat NF-M described by Beaulieu *et al.* (28) were also co-transfected into the cells with each of the peripherin clones to investigate their co-assembly properties with the peripherin gene product.

SW13vim(−) cells grown on glass coverslips were fixed in 4% (w/v) paraformaldehyde in phosphate-buffered saline for 20 min and blocked for 1 h in 5% (w/v) bovine serum albumin, 0.1% Triton X-100 in phosphate-buffered saline at room temperature. Immunocytochemistry was performed using antibodies recognizing peripherin (monoclonal antibody MAB1527; polyclonal antibodies AB1530) and polyclonal antibodies to NF-L (AB1983) and NF-M (AB1982), all from Chemicon International, Inc., and used at 1:1000 diluted in blocking solution overnight at 4 °C. Antibody recognizing vimentin intermediate filament from BD Biosciences (catalog number 550513) was used at 1:500. Antibody distribution was visualized by epifluorescence microscopy after incubation with secondary antibodies, anti-mouse or anti-rabbit IgG conjugated to Alexa Fluor 488 (green) or 594 (red) (Molecular Probes) diluted 1:250 for 2 h at room temperature and mounted in Prolong (Molecular Probes).

RESULTS

Genetic Screen of Peripherin Gene—DNA samples from 40 unrelated FALS cases (non-SOD1 and non-ALS2 cases) and 54 unrelated SALS cases were initially screened for peripherin sequence variations. Another set of samples from 95 non-related SALS cases was also examined to increase the power of our analysis when it was not possible to confirm or exclude association between *PRPH* and ALS. Clinical information of the studied ALS cohort is provided in Table I. A total of 20 sequence variations were detected within the peripherin gene (*PRPH*) of 189 ALS patients (Fig. 1*A* and Table II). Seven sequence variations occurred within coding regions , and one occurred in the 5′-untranslated mRNA region. The remaining 12 sequence variants were found within introns or within the promoter region of the gene. Seventeen sequence variants consisted of single nucleotide polymorphisms. Of the remaining *PRPH* variants, one had a deletion of a single cytosine in exon 1, and two had a single nucleotide insertion of either guanine or adenine in the 5′-untranslated region and intron 8, respectively.

Of the seven variants occurring in the coding sequence (coding single nucleotide polymorphisms), three are predicted to result in amino acid substitution at residues that are highly conserved between human, mouse, and rat peripherin proteins. These variants are 25G → A (R9Q), 420G → T (D141Y), and 829G → A (A277T). Arginine to glutamine is a non-conservative change occurring in the N terminus head domain of the peripherin protein. Aspartic acid to tyrosine is also a non-conservative change substituting an acidic residue for a polar aromatic residue within the rod domain of the protein. The

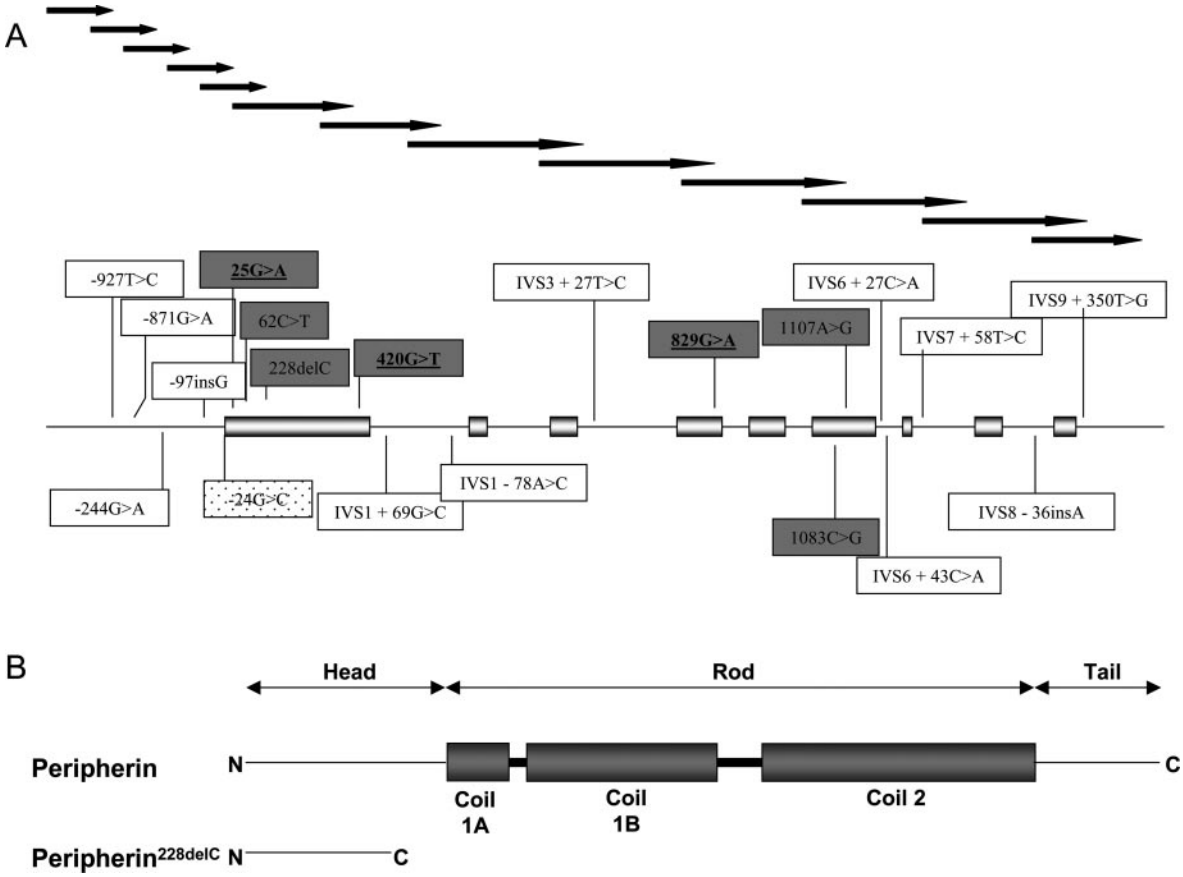

FIG. 1. **Schematic diagram showing the *PRPH* sequence variants in ALS.** In *A*, the *green arrows* represent the locations of PCR primer pairs that have been used to amplify and sequence the *PRPH* fragments (not scaled). The exons are shown in *blue boxes* along the *horizontal line*. The location of each variant is indicated with coding sequences in *gray boxes*. The variants leading to an amino acid change are in *bold*. The variants in the untranslated region are shown in *clear boxes with dots*. The intronic and promoter variants are shown in *clear boxes*. *B* shows the protein structure of normal peripherin and of truncated peripherin^{228delC}. The *orange boxes* represent α-helical regions, and the *thick black lines* represent connecting linkers.

TABLE II
Details, location and genomic context of each sequence variants

Variant	Exon or Intron No.	Amino Acid Changes	Frequency, No. (%)			*P* Value^{FALS}	*P* Value^{SALS}	*P* Value^{combined}
			FALS	SALS	Control			
				%				
Coding								
25G → A	1	Arg → Gln	1/80 (1.3)	1/108 (1.0)	8/380 (2.1)	0.616	0.421	0.374
62C → T	1	No	1/80 (1.3)	2/108 (1.9)	7/380 (1.8)	0.713	0.995	0.834
228delC	1	No	0/80 (0)	1/300 (0.3)	0/380 (0)			
420G → T	1	Asp → Tyr	0/80 (0)	3/108 (1.5)	2/380 (0.5)	0.515	0.233	0.427
829G → A	4	Ala → Thr	1/80 (1.3)	2/108 (1.9)	4/190 (2.1)	0.634	0.881	0.757
1083C → G	6	No	1/80 (1.3)	1/108 (2.8)	4/190 (2.1)	0.634	0.446	0.449
1107A → G	6	No	11/80 (13.8)	18/108 (16.7)	38/190 (20)	0.224	0.479	0.244
5′UTR								
-24G → C	1	NA	1/80 (1.3)	0/108 (0)	3/190	0.838	0.189	0.320
Promoter region								
-927T → C	NA	NA	20/80 (25)	17/108 (15.7)	37/190 (19.5)	0.310	0.421	0.960
-871G → A	NA	NA	6/80 (7.5)	7/108 (6.5)	16/190 (8.4)	0.801	0.546	0.582
-244G → A	NA	NA	0/80 (0)	1/108 (1.0)	2/380 (0.5)	0.515	0.639	0.993
-97insG	NA	NA	0/80 (0)	1/108 (1.0)	1/380 (0.3)	0.646	0.341	0.611
Intronic								
IVS1 + 69G → C	1	NA	17/80 (21.3)	17/108 (15.7)	32/190 (16.8)	0.391	0.805	0.750
IVS2 − 78A → C	1	NA	0/80 (0)	1/108 (1.0)	9/190 (4.7)	0.048	0.079	0.011
IVS3 + 27T → C	3	NA	16/80 (20)	18/108 (16.7)	38/190 (20)	0.999	0.479	0.959
IVS6 + 27C → A	6	NA	1/80 (1.3)	3/108 (2.8)	2/190 (1.1)	0.888	0.265	0.403
IVS6 + 43C → A	6	NA	3/80 (3.8)	4/108 (3.7)	7/190 (3.7)	0.979	0.993	0.984
IVS7 + 58T → C	7	NA	14/80 (17.5)	16/108 (14.8)	34/190 (17.9)	0.938	0.494	0.616
IVS8 − 36insA	8	NA	0/80 (0)	1/108 (1.0)	0/380 (0)			
IVS9 + 350T → G	NA	NA	12/80 (15)	12/108 (11.1)	34/190 (17.9)	0.563	0.119	0.167

change of alanine to threonine at amino acid 277 within the rod domain is also a non-conservative substitution of a neutral amino acid for a polar residue. To test whether any of these three *PRPH* variants may be causative mutations or susceptibility factors for ALS, we compared the frequency of each variant in ALS patients *versus* normal controls. The variant

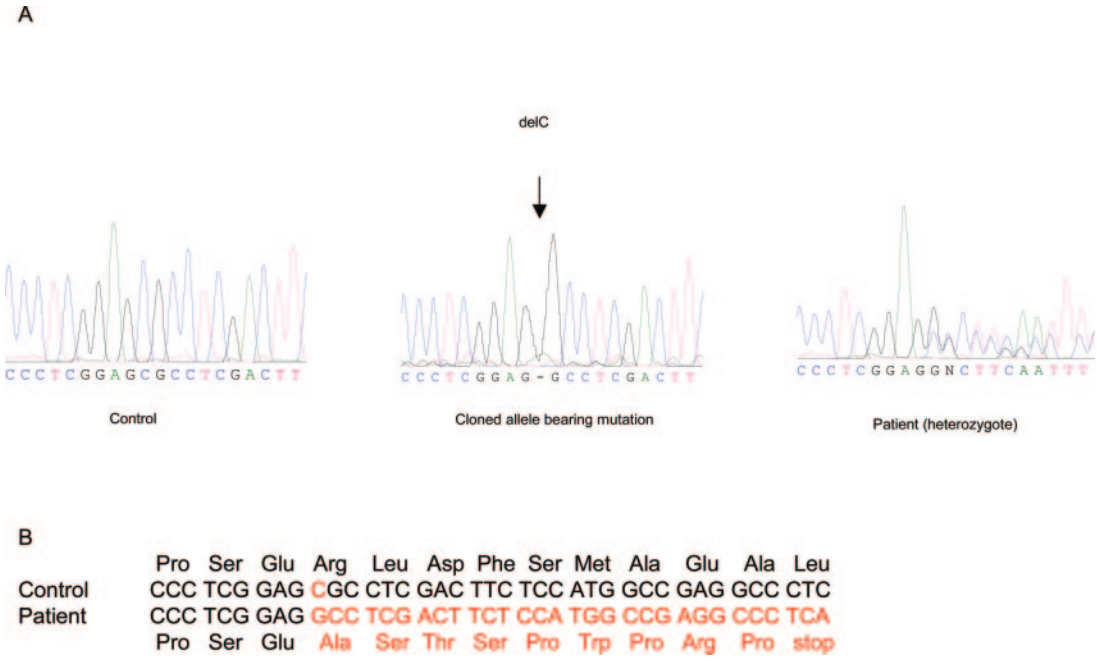

FIG. 2. **Deletion mutation in a patient with ALS.** *A* shows the sequence chromatograms of exon 1 from the normal *PRPH,* from the cloned mutant allele of the ALS patient, and from the DNA of the affected individual (heterozygote) using the forward primer of exon 1. *B*, the deletion shown in *panel A* leads to a frameshift mutation and premature stop at 10 codons after the C deletion site (indicated as *delC* in *panel A*).

frequencies and results of statistical analysis are shown in Table II. None of the variant frequencies were found to be significantly increased in ALS patients when compared with the control population.

However, we identified two additional variants that were not detected among the control population. One of these consists of a nucleotide deletion ($PRPH^{228delC}$) resulting in a frameshift within the exon 1 and a premature stop codon (Fig. 2). The predicted truncated protein of 8.8 kDA has 85 amino acids, the first 76 residues corresponding to the normal protein and the other extra 9 derived from the frameshift mutation (Fig. 1*B*). The female ALS patient having this mutant *PRPH* died at the age of 65 years old after disease duration of 5 years that had started at the left proximal lower limb. The other insertion variant $PRPH^{IVS8-36insA}$ is unlikely to have a functional impact on the protein, albeit an effect on pre-mRNA processing cannot be excluded as the A insertion could affect the branch point during lariat formation or the transcript regulation.

Disruption of Neurofilament Assembly by Expression of Mutant $PRPH^{228delC}$—To test the effects of the two variants $PRPH^{228delC}$ and $PRPH^{IVS8-36insA}$ on the assembly of IFs, we carried out transfection experiments with the cloned *PRPH* genes into SW13 cells that lack endogenous IF structures. We have subcloned the *PRPH* variant alleles into an expression vector driven by the cytomegalovirus promoter. As shown in Fig. 3, single transient transfection of vectors expressing human NF-L, normal peripherin (mouse or human), or $PRPH^{IVS8-36insA}$ resulted in self-assembly of proteins into the typical IF network. However, single transfection of the vector for $PRPH^{228delC}$ yielded only weak and diffuse anti-peripherin immunoreactivity in SW13 cells with the absence of the IF network (Fig. 3*D*).

Since peripherin can co-assemble with NF-L (28) and it is co-expressed with neurofilaments in motor neurons, we investigated the effects of co-expression of the *PRPH* species with either neurofilament NF-L or NF-M proteins in SW13 cells. As expected, the co-expression of normal mouse or human peripherin with NF-L led to the formation of the typical filamentous network immunoreactive for NF-L and peripherin (Fig. 4, *A* and *B*). Similar results were obtained by co-transfecting the variant $PRPH^{IVS8-36insA}$ with NF-L (Fig. 4*C*). However, the co-transfection of $PRPH^{228delC}$ with human NF-L led to the disruption of the IF network (Fig. 4, *D–F*). Note that the intensity of anti-peripherin immunostaining was much higher in the presence of NF-L than in the single $PRPH^{228delC}$ transfection

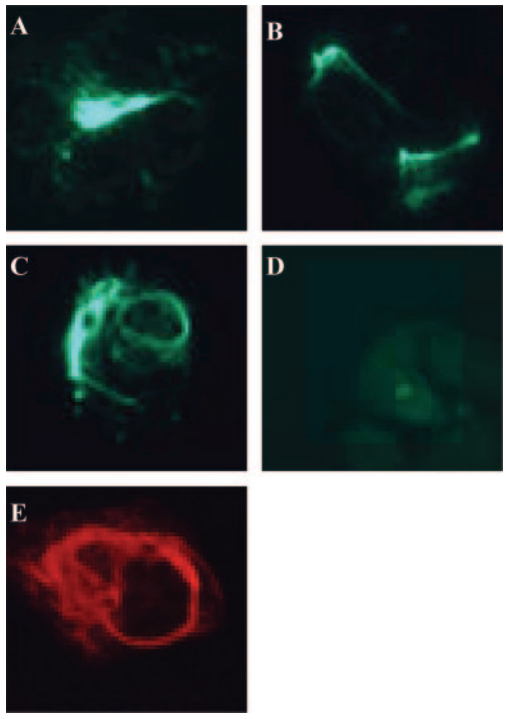

FIG. 3. **Single transfection of peripherin species and of NF-L in SW13 cells.** The cells were transfected with vectors coding for normal mouse peripherin (*A*), normal human *PRPH* (*B*), $PRPH^{IVS8-36insA}$ (*C*), $PRPH^{228delC}$ (*D*), and human NF-L (*E*) NF-L. The immunofluorescence detection of peripherin (*green*) and NF-L (*red*) was done with anti-peripherin monoclonal antibody (MAB1527) and with anti-NF-L polyclonal antibody (AB1983).

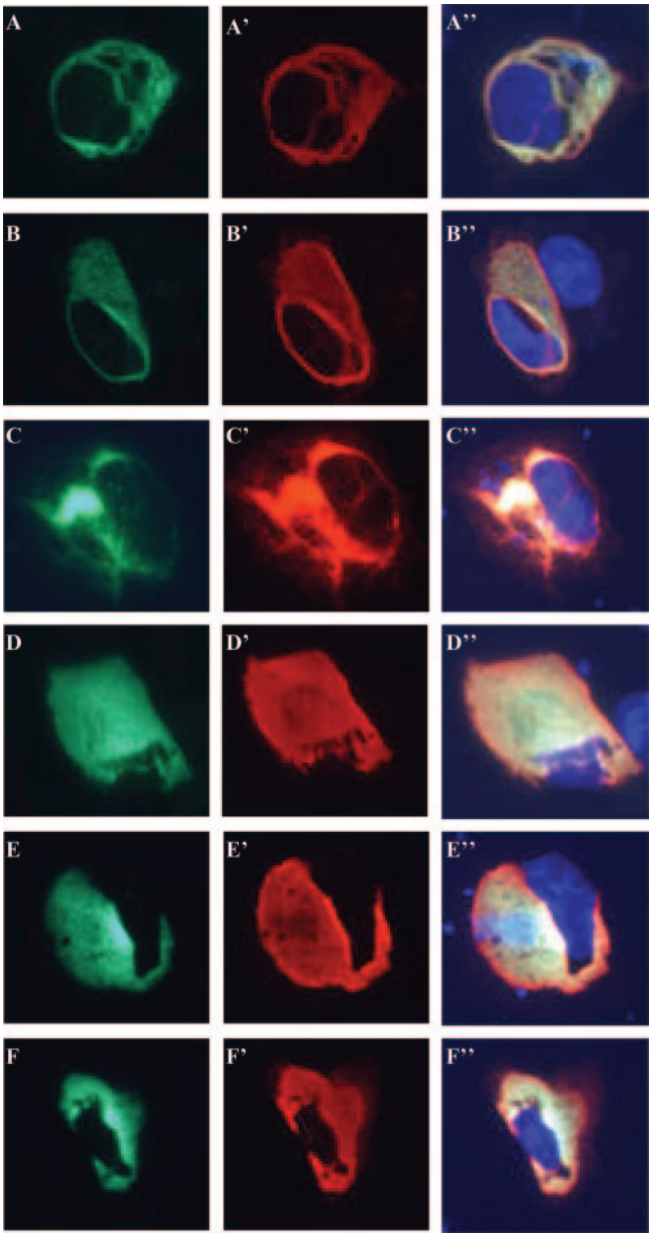

FIG. 4. **Disruption of human NF-L assembly by the peripherin frameshift mutant.** SW13 cells were co-transfected with human NF-L (*A′–F′*) and mouse peripherin (*A*), human peripherin (*B*), variant $PRPH^{IVS8-36insA}$ (*C*), and frameshift mutant $PRPH^{228delC}$ (*D–F*). The peripherin species were detected (*green*) with the monoclonal antibody (MAB1527) and the NF-L (*red*) with polyclonal antibody (AB1983). Overlay and 4′,6-diamidino-2-phenylindole-stained cells from adjacent pictures are shown in *A″–F″*).

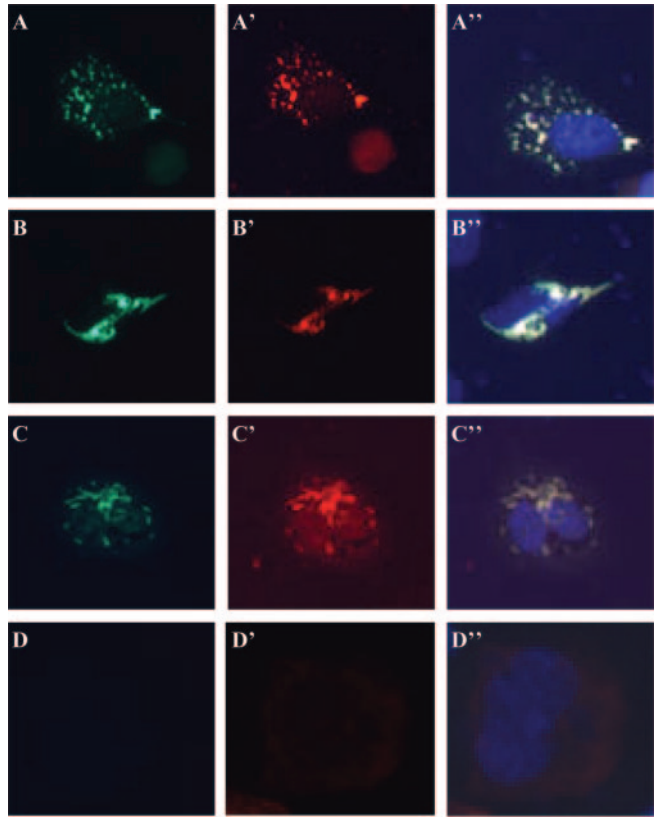

FIG. 5. **Effects of peripherin species on assembly of human NF-M in SW13 cells.** Panels show detection of protein aggregates in cells that co-express NF-M (*A′–D′*) with mouse peripherin (*A*), normal human *PRPH* (*B*), and variant $PRPH^{IVS8-36insA}$ (*C*). In contrast, no IF protein aggregates were detected in cells co-transfected with NF-M and the frameshift mutant $PRPH^{228delC}$ (*D*). Peripherin (*green*) was detected with monoclonal antibody (MAB1527) and for NF-M with polyclonal antibody (AB1982). Overlay and 4′,6-diamidino-2-phenylindole-stained cells from adjacent pictures are shown in *A″–D″*.

assay (Fig. 3*D*), suggesting a stabilization of the mutant peripherin through interaction with NF-L proteins.

It is well established that the NF-M and NF-H proteins require the NF-L subunit for neurofilament network formation (28). The co-transfection in SW13 cells of normal peripherin (mouse or human) with NF-M resulted in the formation of punctuate aggregates (Fig. 5, *A* and *B*) as reported previously (28). This shows that although peripherin can interact with NF-M to form heteropolymers, the presence of NF-M prevents IF network formation. Similar results were obtained by co-expressing the $PRPH^{IVS8-36insA}$ variant gene with NF-M vector (Fig. 5*C*). However, no IF protein aggregates have been detected in any SW13 cells after co-transfection of NF-M with the frameshift mutant $PRPH^{228delC}$ (Fig. 5*D*). A plausible explanation is that this may result from interference of NF-M oligomerization into high molecular weight complexes by interaction with the mutant peripherin consisting of the short N-terminal head region.

DISCUSSION

In this study, we have identified 20 *PRPH* variants in ALS cases, including three variants predicted to cause non-conservative amino acid changes and one frameshift deletion in exon 1. To assess the possible involvement of these variants in ALS, we compared the allelic frequency of each variant in populations of ALS patients *versus* controls and examined the co-segregation of the variants with disease status in families. Except for the two variants $PRPH^{228delC}$ and $PRPH^{IVS8-36insA}$ found exclusively in ALS cases, the data did not reveal significant frequency differences between ALS and control individuals for any of the other 18 variants (Table II). We conclude that these latter *PRPH* variants represent polymorphisms that do not predispose to ALS. As for the $PRPH^{IVS8-36insA}$ variant identified in ALS but not in 380 control chromosomes, there is no evidence based on *in vitro* transfection assays that this variant had any effects on peripherin processing and assembly into the IF network (Figs. 3*C*, 4*C*, and 5*C*). Therefore, this sequence variation is probably a rare polymorphism.

In contrast, we obtained compelling evidence for the deleterious effects of the frameshift mutant $PRPH^{228delC}$. This mutation in exon 1 is predicted to cause a premature stop codon leading to a truncated protein comprising 76 amino acids of the peripherin head domain (Fig. 1*B*). As expected, this truncated peripherin species that lacks rod coiled-coil sequences was un-

able to self-assemble into IF structures (Fig. 3*D*). It is somewhat surprising that this short peripherin head mutant in SW13 cells was able to disrupt NF-L assembly into IF network (Fig. 4, *D–F*) and to impede assembly of NF-M into oligomeric structures (Fig. 5*D*). Although interactions of the peripherin N-terminal head domain with IF proteins have never been documented, studies with the yeast two-hybrid system on domain interactions of vimentin, another type III IF protein, revealed relatively weak interactions between the head domain and other vimentin regions (29). However, it is generally agreed that the head domain of IF proteins is essential for filament assembly and that transient phosphorylation of the head domain inhibits neurofilament polymerization and IF network formation (30, 31). Our observation of IF assembly disruption by the *PRPH* mutant expression in SW13 cells suggests that the detrimental effect of this mutation is likely related to a disorganization neurofilament network rather than to a 50% reduction of peripherin content resulting from the loss of one intact allele. Moreover, this would be in line with the observation that the peripherin knockout mice do not develop motor neuron disease (32).

Compelling evidence for the involvement of neurofilaments in pathogenesis came from the recent discovery of neurofilament gene mutations linked to neurodegenerative diseases. Missense mutations and a 3-bp in-frame deletion in the NF-L gene subunit have been reported in patients with Charcot-Marie-Tooth type 2 (33, 34). Codon deletions and insertion in the KSP repeat region of the neurofilament NF-H gene (*NEFH*) have been detected in a small number of sporadic ALS cases, ~1% of total cases (21–23). However, the functional consequences of these *NEFH* phosphorylation variants are unknown, and these variants are being viewed as risk factors for the disease.

Our discovery of a frameshift mutation in *PRPH* that can disrupt neurofilament assembly provides the first direct evidence for peripherin involvement in ALS, and it suggests that *PRPH* mutations may be responsible for a small number of ALS cases. So far, the pathways to neuronal death caused by neurofilament abnormalities remain unknown. Future studies with transgenic mice expressing this *PRPH* frameshift mutant might provide new insights into the molecular mechanisms of motor neuron degeneration.

Acknowledgments—We acknowledge Mélanie Benard and Ana Simoes Lopes for help in the selection of samples included in this study.

REFERENCES

1. Tandan, R., and Bradley, W. G. (1985) *Ann. Neurol.* **18,** 271–280
2. Mulder, D. W., Kurland, L. T., Offord, K. P., and Beard, C. M. (1986) *Neurology* **36,** 511–517
3. Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J. P., Deng, H. X., Rahmani, Z., Krizus, A., McKenna-Yasek, D., Cayabyab, A., Gaston, S. M., Berger, R., Tanzi, R. E., Halperin, J. J., Herzfeldt, B., Van den Bergh, R., Hung, W. Y., Bird, T., Deng, G., Mulder, D. W., Smyth, C., Laing, N. G., Soriano, E., Pericak-Vance, M. A., Haines, J., Rouleau, G. A., Gusella, J. S., Horvitz, H. R., and Brown, R. H. (1993) *Nature* **362,** 59–62
4. Pramatarova, A., Figlewicz, D. A., Krizus, A., Han, F. Y., Ceballos-Picot, I., Nicole, A., Dib, M., Meininger, V., Brown, R. H., and Rouleau, G. A. (1995) *Am. J. Hum. Genet.* **56,** 592–596
5. Hadano, S., Hand, C. K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R. S., Miyamoto, N., Showguchi-Miyata, J., Okada, Y., Singaraja, R., Figlewicz, D. A., Kwiatkowski, T., Hosler, B. A., Sagie, T., Skaug, J., Nasir, J., Brown, R. H., Jr., Scherer, S. W., Rouleau, G. A., Hayden, M. R., and Ikeda, J. E. (2001) *Nat. Genet.* **29,** 166–173
6. Yang, Y., Hentati, A., Deng, H. X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W. Y., Ouahchi, K., Yan, J., Azim, A. C., Cole, N., Gascon, G., Yagmour, A., Ben-Hamida, M., Pericak-Vance, M., Hentati, F., and Siddique, T. (2001) *Nat. Genet.* **29,** 160–165
7. Eymard-Pierre, E., Lesca, G., Dollet, S., Santorelli, F. M., di Capua, M., Bertini, E., and Boespflug-Tanguy, O. (2002) *Am. J. Hum. Genet.* **71,** 518–527
8. Devon, R. S., Helm, J. R., Rouleau, G. A., Leitner, Y., Lerman-Sagie, T., Lev, D., and Hayden, M. R. (2003) *Clin. Genet.* **64,** 210–215
9. Gros-Louis, F., Meijer, I. A., Hand, C. K., Dube, M. P., MacGregor, D. L., Seni, M. H., Devon, R. S., Hayden, M. R., Andermann, F., Andermann, E., and Rouleau, G. A. (2003) *Ann. Neurol.* **53,** 144–145
10. Gurney, M. E., Pu, H., Chiu, A. Y., Dal Canto, M. C., Polchow, C. Y., Alexander, D. D., Caliendo, J., Hentati, A., Kwon, Y. W., Deng, H. X., Chen, W., Zhai, P., Sufit, R. L., and Siddique, T. (1994) *Science* **264,** 1772–1775
11. Wong, P. C., Pardo, C. A., Borchelt, D. R., Lee, M. K., Copeland, N. G., Jenkins, N. A., Sisodia, S. S., Cleveland, D. W., and Price, D. L. (1995) *Neuron* **14,** 1105–1116
12. Bruijn, L. I., Becher, M. W., Lee, M. K., Anderson, K. L., Jenkins, N. A., Copeland, N. G., Sisodia, S. S., Rothstein, J. D., Borchelt, D. R., Price, D. L., and Cleveland, D. W. (1997) *Neuron* **18,** 327–338
13. Carpenter, S. (1968) *Neurology* **18,** 841–851
14. Corbo, M., and Hays, A. P. (1992) *J. Neuropathol. Exp. Neurol.* **51,** 531–537
15. Migheli, A., Pezzulo, T., Attanasio, A., and Schiffer, D. (1993) *Lab. Investig.* **68,** 185–191
16. Escurat, M., Djabali, K., Gumpel, M., Gros, F., and Portier, M. M. (1990) *J. Neurosci.* **10,** 764–784
17. Brody, B. A., Ley, C. A., and Parysek, L. M. (1989) *J. Neurosci.* **9,** 2391–2401
18. Parysek, L. M., Chisholm, R. L., Ley, C. A., and Goldman, R. D. (1988) *Neuron* **1,** 395–401
19. Troy, C. M., Muma, N. A., Greene, L. A., Price, D. L., and Shelanski, M. L. (1990) *Brain Res.* **529,** 232–238
20. Wong, J., and Oblinger, M. M. (1990) *J. Neurosci. Res.* **27,** 332–341
21. Figlewicz, D. A., Krizus, A., Martinoli, M. G., Meininger, V., Dib, M., Rouleau, G. A., and Julien, J. P. (1994) *Hum. Mol. Genet.* **3,** 1757–1761
22. Al-Chalabi, A., Andersen, P. M., Nilsson, P., Chioza, B., Andersson, J. L., Russ, C., Shaw, C. E., Powell, J. F., and Leigh, P. N. (1999) *Hum. Mol. Genet.* **8,** 157–164
23. Tomkins, J., Usher, P., Slade, J. Y., Ince, P. G., Curtis, A., Bushby, K., and Shaw, P. J. (1998) *Neuroreport* **9,** 3967–3970
24. Beaulieu, J.-M., Nguyen, M. D., and Julien, J.-P. (1999) *J. Cell Biol.* **147,** 531–544
25. Robertson, J., Beaulieu, J. M., Doroudchi, M. M., Durham, H. D., Julien, J. P., and Mushynski, W. E. (2001) *J. Cell Biol.* **155,** 217–226
26. Robertson, J., Doroudchi, M. M., Nguyen, M. D., Durham, H. D., Strong, M. J., Shaw, G., Julien, J. P., and Mushynski, W. E. (2003) *J. Cell Biol.* **160,** 939–949
27. Lariviere, R. C., Beaulieu, J. M., Nguyen, M. D., and Julien, J. P. (2003) *Neurobiol. Dis.* **13,** 158–166
28. Beaulieu, J. M., Robertson, J., and Julien, J. P. (1999) *Biochem. Cell Biol.* **77,** 41–45
29. Meng, J. J., Khan, S., and Ip, W. (1996) *J. Biol. Chem.* **271,** 1599–1604
30. Ching, G. Y., and Liem, R. K. (1999) *J. Cell Sci.* **112,** 2233–2240
31. Zheng, Y. L., Li, B. S., Veeranna, and Pant, H. C. (2003) *J. Biol. Chem.* **278,** 24026–24032
32. Lariviere, R. C., Nguyen, M. D., Ribeiro-da-Silva, A., and Julien, J. P. (2002) *J. Neurochem.* **81,** 525–532
33. De Jonghe, P., Mersivanova, I., Nelis, E., Del Favero, J., Martin, J. J., Van Broeckhoven, C., Evgrafov, O., and Timmerman, V. (2001) *Ann. Neurol.* **49,** 245–249
34. Mersiyanova, I. V., Perepelov, A. V., Polyakov, A. V., Sitnikov, V. F., Dadali, E. L., Oparin, R. B., Petrin, A. N., and Evgrafov, O. V. (2000) *Am. J. Hum. Genet.* **67,** 37–46
35. Brooks, B. R. (1994) *J. Neurol. Sci.* **124,** (suppl.) 96–107

A Frameshift Deletion in Peripherin Gene Associated with Amyotrophic Lateral Sclerosis

François Gros-Louis, Roxanne Larivière, Geneviève Gowing, Sandra Laurent, William Camu, Jean-Pierre Bouchard, Vincent Meininger, Guy A. Rouleau and Jean-Pierre Julien

J. Biol. Chem. 2004, 279:45951-45956.
doi: 10.1074/jbc.M408139200 originally published online August 17, 2004
