Characterization of *DCTN1* genetic variability in neurodegeneration

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ABSTRACT

Objective: Recently, mutations in *DCTN1* were found to cause Perry syndrome, a parkinsonian disorder with TDP-43-positive pathology. Previously, mutations in *DCTN1* were identified in a family with lower motor neuron disease, in amyotrophic lateral sclerosis (ALS), and in a family with ALS/frontotemporal dementia (FTD), suggesting a central role for *DCTN1* in neurodegeneration.

Methods: In this study we sequenced all *DCTN1* exons and exon-intron boundaries in 286 samples diagnosed with Parkinson disease (PD), frontotemporal lobar degeneration (FTLD), or ALS.

Results: This analysis revealed 36 novel variants (9 missense, 5 silent, and 22 noncoding). Segregation analysis in families and association studies in PD, FTLD, and ALS case–control series did not identify any variants segregating with disease or associated with increased disease risk.

Conclusions: This study suggests that pathogenic mutations in *DCTN1* are rare and do not play a common role in the development of Parkinson disease, frontotemporal lobar degeneration, or amyotrophic lateral sclerosis. ***Neurology*® 2009;72:2024–2028**

GLOSSARY

AD = Alzheimer disease; **ALS** = amyotrophic lateral sclerosis; **CAP-Gly** = cytoskeleton-associated protein-glycine-rich; **FTD** = frontotemporal dementia; **FTLD** = frontotemporal lobar degeneration; **MND** = motor neuron disease; **PD** = Parkinson disease.

Axonal transport machinery is central to neuronal health and survival, with dysfunction implicated in several neurodegenerative disorders including Alzheimer disease (AD), frontotemporal lobar degeneration (FTLD), motor neuron disease/amyotrophic lateral sclerosis (MND/ALS), and Parkinson disease (PD).[1]

The dynactin protein complex plays a crucial role in axon maintenance, regulating vesicle and organelle transport via direct binding to microtubules, the molecular motor dynein, and various cargoes.[2] The *dynactin 1* gene (*DCTN1*; NM_004082) encodes the p150^{Glued} protein, which is the major subunit of the dynactin protein complex. In 2003, Puls and colleagues[3,4] reported a single base-pair change in *DCTN1* leading to a glycine-59-serine (p.G59S) substitution which was observed to segregate with disease in a family with a slowly progressive lower MND (autosomal dominant distal spinal and bulbar muscular atrophy). In vitro evidence demonstrated that the p.G59S substitution results in reduced microtubule binding which may cause either a loss or toxic gain of function.[3,5] Other *DCTN1* mutations have been identified in

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Medical Devices: Agencourt bead technology (Beverly, MA); Agilent technology (Santa Clara, CA); Applied Biosystems High Capacity Archive Kit (Applied Biosystems, Foster City, CA); Biomek FX automation (Beckman Coulter, Fullerton, CA); Sequenom MassArray iPLEX (San Diego, CA); TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA).

Table Demographics for the neurodegenerative series

	Control	PD	FTLD	ALS
Sample size	435	440	374	372
Gender ratio (M:F)	1.1:1	1.2:1	1:1	1.5:1
Age at onset, y				
Mean ± SD	NA	61.9 ± 12.2	60.0 ± 10.8	55.1 ± 12.9
Range	NA	16-85	20-80	19-87
Age, y				
Mean ± SD	72.2 ± 10.7	72.2 ± 11.0	—	57.9 ± 12.3
Range	34-90	30-92	—	24-88

patients with ALS and in a family with ALS and frontotemporal dementia (FTD) although definitive evidence of pathogenicity is lacking.[6,7]

Recently, our group identified mutations in *DCTN1* as the cause of Perry syndrome, an autosomal dominantly inherited condition clinically characterized by parkinsonism, depression, weight loss, and central hypoventilation.[8,9] These *DCTN1* mutations (p.G71R/A/E, p.T72P, and p.Q74P) are located within or immediately adjacent to the ^{67}GKNDG^{71} microtubule binding motif. Neuropathologic studies in Perry syndrome have shown severe neuronal loss in the substantia nigra and the locus ceruleus, and characteristic transactive-response DNA-binding protein-43 (TDP-43)-positive neuronal and glial inclusions.[10]

The recent identification of *DCTN1* mutations in a parkinsonian phenotype with pathologic TDP-43-positive inclusions indicates a neuropathologic overlap between PD, FTLD, and ALS, suggesting a central role for dynactin p150^{Glued} in neuronal health. In this study we performed comprehensive screening for *DCTN1* genetic variability and evaluation of pathogenicity in patients with PD, FTLD, and ALS.

METHODS

Study population. Sequencing of *DCTN1* was performed in 159 familial PD samples from North America, Europe, and North Africa. To enrich our sequencing population for patients with TDP-43 positive pathology, we further selected 127 patients ascertained at the Mayo Clinics in Jacksonville and Rochester: 100 patients with pathologically confirmed TDP-43-positive inclusions (79 FTLD and 21 AD) and 27 patients with clinical FTLD or ALS (8 FTD-ALS, 5 FTLD and 14 ALS) with a documented positive family history in 9 patients. Segregation analysis was performed when possible. Association of variants was further evaluated in PD, FTLD (88% clinical FTD and 22% pathologically confirmed FTLD), and ALS cases from the United States and ethnically matched controls free of disease and no family history of neurologic disorders; demographics are shown in the table. For population-specific variants, ethnically matched PD cases from Norway, France, and Tunisia were used. All sites obtained local ethics committee approval before beginning subject recruitment. Subjects were informed of all aspects pertaining to their participation in the study, and gave either written or proxy consent prior to recruitment. Physical examinations were performed by neurologists specialized in movement disorders, dementia, or MND.

DNA sequencing of *DCTN1*. Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. Primer pairs (available on request) for *DCTN1* were used to sequence all 32 exons and exon-intron boundaries by PCR using standard protocols. PCR products were purified from unincorporated nucleotides using Agencourt bead technology (Beverly, MA) with Biomek FX automation (Beckman Coulter, Fullerton, CA). Sequence analysis was performed as previously described.[11]

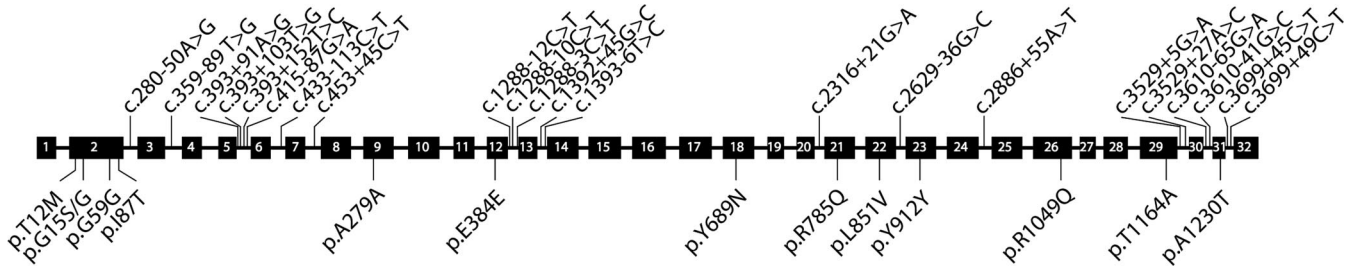
Genetic association analysis. The population frequency of reported pathogenic variants and all coding and noncoding variants within 25 bases from exon-intron boundaries identified in our sequencing effort were assessed in a case–control series of PD, FTLD, and ALS. Genotyping was performed by a combination of exon 2 sequencing, TaqMan probes, and Sequenom MassArray iPLEX (San Diego, CA); all primer sequences are available on request. For each variant genotyping error was assessed by deviation from Hardy-Weinberg equilibrium expectation. Association between variants and disease was investigated using Fisher exact test.

cDNA sequencing. Frozen tissue was regionally dissected to include material from cerebellar cortex. Total human RNA was isolated using TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). High-quality, nondegraded RNA was confirmed by RNA integrity number (arbitrary values are on a scale from 0 to 10, higher values indicating the best quality RNA) using Agilent technology (Santa Clara, CA). One microgram of tissue RNA was reverse transcribed using Applied Biosystems High Capacity Archive Kit (Foster City, CA) according to manufacturer's instructions. PCR amplification and sequencing of cDNA spanning two exons on either side of each variant was performed.

RESULTS

Sequencing analysis of *DCTN1* in 286 samples diagnosed with PD, FTLD, or ALS identified 36 novel variants (9 missense, 5 silent, and 22 noncoding); for each variant, the location within the gene is given in figure 1 and the dbSNP identification numbers and diagnosis of the patient in which the variant was identified is given in table e-1 on the *Neurology*® Web site at www.neurology.org. In addition, 19 known polymorphisms were also detected (4 missense, 2 silent, and 13 noncoding) (table e-2). Segregation analysis was performed for exonic and exon-intron boundary variants when additional family members were available. Conclusive cosegregation with disease was not observed for any variant; therefore, they are unlikely to be pathogenic. However, a number of pedigrees are small and we cannot rule out the possibility of disease phenocopies or low penetrance as the reason for lack of segregation (figure

Figure 1 Schematic of *DCTN1*

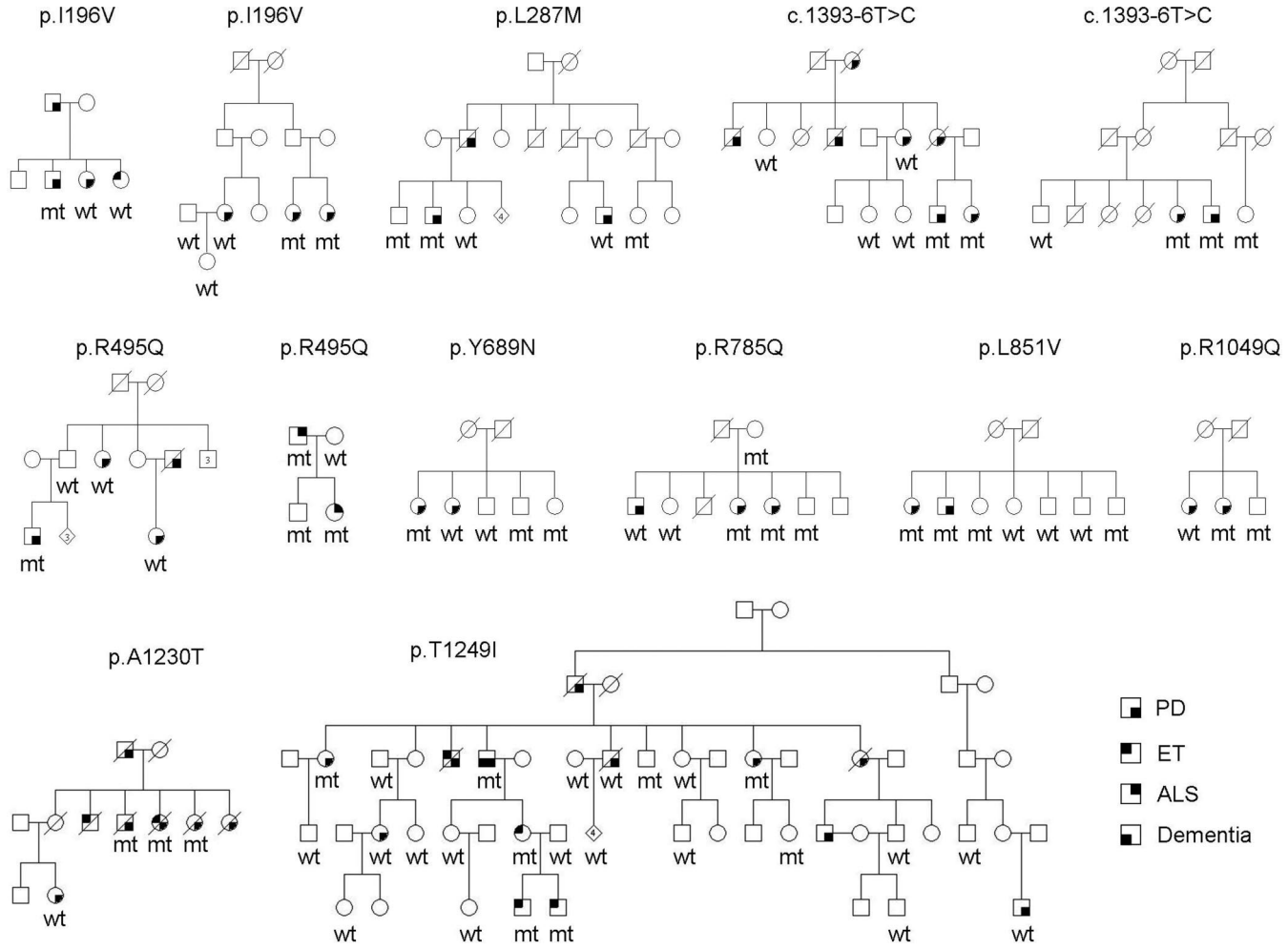


Exons are represented as black boxes and drawn to scale. Positions of all novel variants are indicated; coding variants are given below the gene and noncoding variants above.

2). In addition, all known and novel coding variants identified in our sequencing cohort, all intronic variants within 25 bases from exon-intron boundaries, and all previously reported pathogenic *DCTN1* mutations for MND, ALS/FTD, and Perry syndrome were genotyped in three independent US Caucasian PD, FTLD, and ALS case–control series (table). These analyses showed that *DCTN1* variants are rare and are not associated with an increased risk of developing PD, FTLD, or ALS (table e-3).

Through our *DCTN1* sequencing, we observed three variants in patients with PD from North Africa and Europe (c.1288-10C>T, p.R785Q, and p.A1230T) that were found neither in samples of US Caucasian ethnicity nor in the case–control series. To assess the role of those variants in the specific

Figure 2 Segregation of *DCTN1* variants



Genotypes are indicated beneath the pedigree member symbol; heterozygote mutation carriers are indicated with mt, noncarriers with wt.

populations in which they were identified, we genotyped ethnically matched patients with PD consisting of 359 samples from Norway (c.1288-10C>T), 543 from Tunisia (p.R785Q), and 119 from France (p.A1230T). No additional carriers were identified in the ethnically matched series, confirming that these novel *DCTN1* variants are rare.

For those variants located in close proximity to an exon-intron boundary and for which tissue was available, we assessed whether alternative mRNA transcripts were generated. Postmortem material was available for five patients presenting TDP-43-positive pathology: one patient with AD carrying a c.1288-3C>T variant and three with FTLD and one with AD with c.3529 + 5G>A. No alternatively spliced transcripts were identified.

DISCUSSION *DCTN1* genetic variability has been implicated in three major neurodegenerative phenotypes: parkinsonism, MND, and ALS/FTD. The present study describes a comprehensive sequence analysis of 286 patients and the identification of 36 novel *DCTN1* variants. Furthermore, all the identified coding variants, novel noncoding variants located within 25 bases from an exon-intron boundary, and those reported pathogenic for MND, ALS, FTD, and Perry syndrome were analyzed in three independent case–control series of PD, FTLD, and ALS. This analysis revealed that all the variants identified are very rare and do not associate with disease susceptibility. Nonsegregation within families confirmed the lack of involvement of *DCTN1* variants in disease development, although most pedigrees are small and segregation is therefore equivocal.

Of note, one of the originally reported pathogenic mutations for ALS (p.T1249I) was identified in three control individuals, five patients with PD, one patient with FTLD, and five patients with ALS, which in conjunction with lack of segregation in a large pedigree with a high incidence of PD further weakens the pathogenicity evidence of this variant (figure 2). All other reported mutations for MND (p.G59S), ALS/FTD (p.M571T, p.R785W, p.R1101K), and Perry syndrome (p.G71R/A/E, p.T72P, p.Q74P) were not identified in this study.[3,6,7,9]

To date, all proven pathogenic *DCTN1* mutations seem to cluster in the N-terminal cytoskeleton-associated protein-glycine-rich (CAP-Gly) domain of dynactin p150^{Glued}. Functionally, this domain has been shown to be involved in direct binding to microtubules, with the interaction mediated by the ^{67}GKNDG^{71} motif. The p.G59S mutation identified in one family with MND is located in the center of the CAP-Gly domain, whereas all Perry syndrome mutations are in (p.G71) or immediately adjacent to (p.T72 and p.Q74) the ^{67}GKNDG^{71} motif. Previous studies performed by our group and others demonstrate reduced binding affinity of mutant p150^{Glued} (CAP-Gly domain) for microtubules.[3,5,9]

Pathogenicity has not been established for any of the coding changes identified in other domains of the p150^{Glued} protein.[6,7] Variant p.R785W implicated in ALS was identified in a small pedigree with two affected and two unaffected carriers, p.M571T was observed in one familial ALS sample alone, and p.R1101K in a sib-pair with one carrier presenting ALS and the other FTD. In addition to this lack of segregation and replication, functional evidence does not support pathogenicity for these variants outside the CAP-Gly domain.[12] However, if confirmed pathogenic, it may reflect the biologic importance of p150^{Glued} and the dynactin complex. Mutations affecting the dimerization of the p150^{Glued} backbone, its interaction with other subunits of the dynactin complex, or a putative cargo might have detrimental effects on the cellular homeostasis and thus prove fatal for the cell.

Interestingly, the p150^{Glued} protein possesses a second microtubule binding domain (basic domain amino acids 116–145). In contrast to the CAP-Gly domain, which firmly attaches dynactin to microtubules, this second domain moves progressively along microtubules even in the absence of molecular motors. Due to this second domain, pathogenic mutations in the CAP-Gly domain may not completely ablate microtubule binding, but disrupt organized movement of the dynactin complex, leading to disease.[13] However, this scenario does not explain the selective vulnerability of different neuronal populations encountered in Perry syndrome, ALS, and FTD. A number of other protein interactions have also been mapped to the CAP-Gly domain, including CLIP170, α-tubulin, and end-binding (EB1) proteins.[14-16] Apart from reducing affinity for microtubule binding, it is conceivable that the disruption of the interaction with one of these or an as yet unknown binding partner could also be responsible for disease. Although it appears unlikely that common mutations in *DCTN1* play a major role in these neurodegenerative disorders, they nonetheless provide the first direct link between selective neuronal vulnerability observed in MND, FTD, and PD, cellular transport, and postmortem TDP-43 proteinopathy.

AUTHOR CONTRIBUTIONS

Statistical analysis was performed by C.V.-G.

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