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TDP-43 A315T Mutation in Familial Motor Neuron Disease

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Abstract

To identify novel causes of familial neurodegenerative diseases, we extended our previous studies of TAR DNA-binding protein 43 (TDP-43) proteinopathies to investigate *TDP-43* as a candidate gene in familial cases of motor neuron disease. Sequencing of the *TDP-43* gene led to the identification of a novel missense mutation, Ala-315-Thr, which segregates with all affected members of an autosomal dominant motor neuron disease family. The mutation was not found in 1,505 healthy control subjects. The discovery of a missense mutation in *TDP-43* in a family with dominantly inherited motor neuron disease provides evidence of a direct link between altered TDP-43 function and neurodegeneration.

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Motor neuron disease (MND) is a neurodegenerative disorder involving the loss of upper and/or lower motor neurons, and it is characterized clinically by progressive weakness and death within a few years of onset; the most common clinical MND phenotype is amyotrophic lateral sclerosis (ALS). Recently, TAR DNA-binding protein 43 (TDP-43) was identified as the major pathological protein of the motor neuron inclusions found in sporadic MND and also in frontotemporal lobar degeneration with ubiquitin-immunoreactive, tau-negative inclusions (FTLD-U), which can be associated with MND, but not in familial MND with *Cu/Zn superoxide dismutase-1 (SOD1)* mutation.[1-4]

Although largely sporadic, about 10% of MND cases are familial, and of these about 20% have mutations in the *SOD1* gene.[5] Evidence suggests that *SOD1* mutations cause MND by a toxic gain of function.[6] The recent discovery that pathological TDP-43 inclusions are present in sporadic/non-*SOD1* cases of MND, but absent from *SOD1* cases and *SOD1* transgenic mice, suggests that the sporadic form of the disease may have a different underlying pathophysiology. Therefore, new genetic insights into MND are needed to further the understanding of disease pathogenesis and to develop animal models representative of the sporadic form of the disease.

Familial forms of neurodegenerative diseases can carry pathogenic mutations in the genes encoding the proteins present in the inclusions characterizing the disorder, for example, amyloid precursor protein in Alzheimer's disease,[7] α-synuclein in Parkinson's disease,[8] and tau in FTLD with tauopathy.[9] Given that TDP-43-positive inclusions are present in most cases of sporadic and familial ALS, FTLD-MND, and FTLD-U, this gene is a strong biological candidate gene for familial forms of these disorders.

TDP-43 protein structure is evolutionarily conserved and consists of two RNA recognition motifs and a glycine-rich domain[10] (Fig, A). TDP-43 can bind DNA and RNA, is involved in exon skipping of cystic fibrosis transmembrane conductance regulator (CFTR) gene, and binds to human immunodeficiency virus type 1 TAR DNA sequence motifs.[11-13]

Methods

In families of European descent, we have undertaken mutation analysis of the *TDP-43* gene in 8 families with MND/ALS with an autosomal dominant pattern of inheritance and no mutation within the *SOD1* gene, 5 families with familial FTLD-MND, and 25 families with FTLD-U.[14] No sporadic cases of MND, FTLD-MND, or FTLD-U were screened. In brief, high-molecular-weight DNA was extracted from whole blood, serum, or brain tissue according to standard procedures. DNA from serum was whole-genome amplified using the REPLI-g Midi Kit (Qiagen, Valencia, CA) before genetic analysis. DNA from a single affected individual from each family was used for sequencing of TDP-43. All the exons and the intronexon boundaries of *TDP-43* gene were amplified using gene-specific intronic primers. Direct sequencing of the amplified fragments was performed using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Wellesley, MA) and standard protocols. For most of the fragments, the primers used for sequencing were the same as those used for polymerase chain reaction amplification (primer sequences available on request). Reactions were run on an ABI3130, and mutation analysis was performed using

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Sequencher software v4.6 (Gene Codes Corporation, Ann Arbor, MI). Positive calls for sequence variants were made only if the variant was observed in both forward and reverse sequence reads. Where possible, sequence variants were tested for segregation with the disease and screened in a set of 1,505 unrelated ethnically matched control subjects.

Results

This analysis led to the identification of a novel missense mutation, Ala-315-Thr (c.1077 G>A), within exon 6. In TDP-43, this alanine residue is highly conserved through the evolutionary spectrum from *Homo sapiens* to *Xenopus tropicalis*, supporting its likely functional importance (see Fig, B). The A315T mutation segregated with all affected members of an autosomal dominant MND family (additional noncoding sequence variants were also identified in cases with FTLD-U, FTLD-MND, and MND; see Supplementary Table 1 and Figs, C, D). This mutation was absent from a large series of ethnically matched elderly control subjects (n = 1,505).

The phenotype of the four affected family members with *TDP-43* A315T mutation involved a slowly progressive lower motor neuron degeneration syndrome with respiratory involvement, with only minimal involvement of upper motor or bulbar neurons and absence of dementia (Table). Brain autopsy in this kindred remains to be undertaken. Similar clinical phenotypes have been reported in sporadic MND and in kindreds with *SOD1* mutations.[5,6]

Discussion

The *TDP-43* mutation in familial MND reported here supplements other familial neurodegenerative conditions that affect predominantly lower motor neurons including mutations in the vesicle-associated membrane protein-associated protein B (VAPB), dynactin (DCTN1), alsin (ALS2), immunoglobulin μ-*binding protein 2 (IGHMBP2)*, and *glycyl-tRNA synthetase (GARS)* genes, and other mutations in juvenile MND, although some of these mutations have been identified in MNDs and hereditary motor neuropathies with variable clinical phenotypes.[6]

These data have important implications for both sporadic and familial forms of MND and FTLD-U, which are linked by a common molecular pathology: TDP-43 proteinopathy. The discovery of a missense mutation in *TDP-43* in a family with dominantly inherited MND provides evidence of a direct link between TDP-43 function and neurodegeneration, and should facilitate the generation of in vitro and transgenic models to enable the further elucidation of mechanisms in the pathogenesis of the TDP-43 proteinopathies. In addition, they may generate novel targets for therapeutic intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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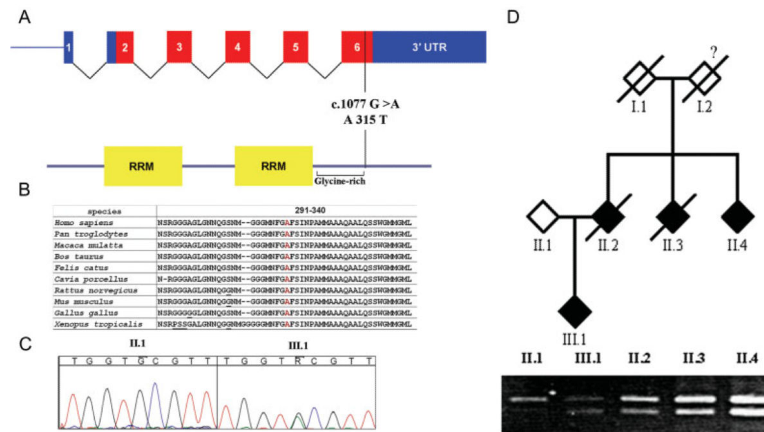

Fig.

TDP-43 missense mutation A315T within a highly conserved region of exon 6 segregates with all affected members of an autosomal dominant motor neuron disease (MND) family. (A) TAR DNA-binding protein 43 (TDP-43) genomic structure, position of missense mutation, and location of amino acid change adjacent to glycine-rich domain. (B) TDP-43 protein (291-340 amino acids) displays high similarity between species. Residues underlined indicate differences when compared with humans. TDP-43 A315T location is indicated in red. (C) Chromatogram of exon 6 displays a base-pair change (c.1077 G>A) compared with family control. (D) Pedigree of family displays segregation of the mutation with disease (open symbols denote unaffected; closed symbols denote affected with mutation; diagonal line denotes deceased). Rsa*I* restriction digest was used to screen family members and 1,505 control subjects. Direct sequencing was also performed on all family members in this study to verify the mutation.

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Table

Clinical Features of the Family with Motor Neuron Disease with *TDP-43* A315T Mutation

Subject No.	Age at Onset/Death (yr)	Clinical Findings					Electrophysiology	
		Mental Status	**Cranial Nerves**	**Respiratory Involvement**	**Site of Onset**	**Disease Course**	**Nerve Conductions (age performed [yr])**	**Electromyography**
II-2	72/79	Normal	Normal	Yes	Left lower extremity	Progressive, asymmetric lower motor neuron loss in legs before arms, distal before proximal; brisk reflexes; death from respiratory weakness	Normal SNAP amplitudes, normal sensory and motor velocities (72)	Fibs/PSW in legs, thoracic paraspinous muscles; reduced recruitment; occasional large motor units; fasciculations throughout
II-3	64/74	Normal	Normal	Yes	Left lower extremity	Progressive asymmetric lower motor neuron loss in legs before arms, distal and proximal; brisk reflexes; death from respiratory weakness	Normal SNAP amplitudes, normal sensory and motor velocities (68)	Fibs/PSW in legs and arms; reduced recruitment; occasional large motor units; fasciculations throughout
II-4	83	Normal	Normal	Yes	Right lower extremity	Progressive asymmetric lower motor neuron loss, distal and proximal, legs before arms; brisk reflexes	Not available	Not available
III-1	48	Normal	Normal	No	Right upper extremity	Progressive asymmetric lower motor neuron loss, distal before proximal, arms before legs	Normal SNAP amplitudes, normal sensory and motor velocities (49)	Fibs/PSW in arms; fasciculations in arms/legs

TDP-43 = TAR DNA-binding protein 43; SNAP = sensory nerve action potential; Fibs = fibrillations; PSW = positive sharp waves.