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Mutational Analysis of *TARDBP* in Neurodegenerative Diseases

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Abstract

Neurodegenerative diseases are often characterized by the presence of aggregates of misfolded proteins. TDP-43 is a major component of these aggregates in Amyotrophic Lateral Sclerosis (ALS), but has also been observed in Alzheimer's (AD) and Parkinson's Diseases (PD). In addition, mutations in the *TARDBP* gene, encoding TDP-43, have been found to be a significant cause of familial ALS (FALS). All mutations, except for one, have been found in exon 6. To confirm this observation in ALS and to investigate whether *TARDBP* may play a role in the pathogenesis of AD and PD, we screened for mutations in exon 6 of the *TARDBP* gene in three cohorts composed of 376 AD, 463 PD (18% familial PD) and 376 ALS patients (50% FALS). We found mutations in ~7% of FALS and ~0.5% of sporadic ALS (SALS) patients, including two novel mutations, p.N352T and p.G384R. In contrast, we did not find *TARDBP* mutations in our cohort of AD and PD patients. These results suggest that mutations in *TARDBP* are not a significant cause of AD and PD.

1. Introduction

Neurodegenerative diseases are often characterized by the presence of intracellular or extracellular protein aggregates in the central nervous system. An evolving molecular classification of these disorders is based upon the biochemical nature of the proteins forming the inclusions. The presence of tau-negative, ubiquitinated inclusions (UBIs) in the perikaryon and proximal axons of surviving motor neurons is the neuropathological hallmark of amyotrophic lateral sclerosis (ALS) and indicates a failure of the proteasome to recycle damaged proteins (Leigh, et al., 1991). UBIs are also observed in cortical neurons of

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patients with tau-negative and ubiquitin-positive frontotemporal lobar dementia (FTLD-U), the most common neuropathological presentation of FTLN (Forman, et al., 2006).

TDP-43 is a protein of 43 kDa (414 residues) encoded by the *TARDBP* gene that has been identified as the major component of UBIs in brain tissues from ALS and FTLN-U patients (Neumann, et al., 2006). Its involvement in both disorders is consistent with the view that these two neurodegenerative conditions share common molecular pathways. TDP-43 belongs to the family of heterogeneous nuclear ribonucleoproteins (hnRNPs) and has been identified as a transcriptional repressor (Ou, et al., 1995) and as promoting exon skipping (Buratti, et al., 2001). Interestingly, mutations in the *TARDBP* gene have been described as major cause of familial ALS (FALS) and have also been identified in sporadic ALS cases (SALS) and FTLN with associated motor neuron disease (Benajiba, et al., 2009, Kabashi, et al., 2008, Sreedharan, et al., 2008, Van Deerlin, et al., 2008). With only one exception, the mutations have been found in exon 6.

By contrast with *TARDBP* gene mutations, which have been described only in ALS and FTLN-U, TDP-43 positive inclusions have been observed in diverse neurodegenerative disorders including not only FTD and ALS, but also Alzheimer's disease (AD), alpha-synucleinopathies (such as Parkinson's disease and dementia with Lewy bodies), and tauopathies (corticobasal degeneration). In autopsy studies, it is estimated that as many as 20% of AD (Amador-Ortiz, et al., 2007) and ~7% of Parkinson's disease (PD) (Nakashima-Yasuda, et al., 2007) cases reveal evidence of TDP-43 immunoreactivity in the central nervous system. These findings raise the hypothesis that primary germline *TARDBP* mutations may be present in neurodegenerative diseases other than ALS and FTLN-U. Although no *TARDBP* mutations have been identified so far in those populations (Kabashi, et al., 2009, Rutherford, et al., 2008, Van Deerlin, et al., 2008), it is possible that the cohorts studied were underpowered. To investigate this hypothesis, we screened the *TARDBP* gene for exon 6 mutations in two cohorts of AD and PD patients. To better assess the role of *TARDBP* in the pathogenesis of these disorders, we also screened a set of FALS and SALS patients.

2. Materials and methods

2.1 Study population

To determine whether *TARDBP* mutations are present in AD and PD, we obtained two cohorts of 376 AD patients and 463 PD patients, with IRB approval. All AD samples were diagnosed with definite AD according to the NINCDS-ADRDA criteria (McKhann, et al., 1984) and were obtained from the Massachusetts General Hospital Alzheimer Disease Research Center Tissue Repository. No information is available regarding the family history of the AD cases screened. PD samples were collected at the Coriell Institute for Biomedical Research Cell Repositories (panels NDPT005, NDPT015 and NDPT016) and at the Massachusetts General Hospital Institute for Neurodegenerative Diseases. The PD cohort included 82 patients (18%) with a positive family history for Parkinsonism. All the patients met the UK Brain Bank Criteria for idiopathic PD (Hughes, et al., 1992), and clinical records were reviewed by a neurologist with expertise in movement disorders. Additionally, we screened DNA from 397 ALS patients (208 FALS and 188 SALS) who had been diagnosed with ALS, according to the revised El Escorial criteria (Miller, et al., 1999). All FALS patients tested negative for mutations in the *SOD1* and *FUS/TLS* genes.

2.2 Molecular analysis

Mutational screening of exon 6 of *TARDBP* was performed by touchdown PCR using primers TDP43E×6F (agtaaacgacggccagtgatcagtggttaattcttcttg) and TDP43E×6R

(gcaggaaacagctatgacaaaaattgaattccaccattc). These primers anneal to adjacent intronic and 3'UTR regions of exon 6 and contain 5' tails encoding M13 forward and reverse. PCR-products were subsequently purified by incubation with Exonuclease I and Shrimp Alkaline Phosphatase, sequenced with M13 primers using the BigDyeTerminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA USA) and then resolved by capillary electrophoresis on an ABI 3730XL DNA Analyzer (Applied Biosystem). Sequence analysis was performed using the PHRED/PHRAP/Consed software suite (<http://www.phrap.org/>) and variations in the sequences were identified with the Polyphred v6.15 software (<http://droog.gs.washington.edu/polyphred/>).

3. Results

In our ALS cohort, the screening of exon 6 of the *TARDBP* gene revealed 10 different heterozygous missense mutations in 14 patients (Table 1 and Supplemental Table S1). Three of the mutations observed were novel (N352T, N378D and G384R), although the substitution of asparagine 352 with a serine has been previously described in a German kindred (Kuhnlein, et al., 2008). In contrast, the screening of both the PD and AD cohorts did not reveal the any mutations within the coding region of exon 6.

With the exception of G295R, which has been identified in a single SALS individual, all other mutations have been found exclusively in FALS cases. Thus, the frequency of *TARDBP* mutations in our cohort is more than ten-times higher in FALS than SALS. Our observation that *TARDBP* mutations account for ~6.3% of all *SOD1* and *FUS/TLS* negative FALS patients is consistent with the results of previous studies (Corrado, et al., 2009). On the contrary, we observed a lower-than-expected *TARDBP* mutational frequency in SALS (~0.5%) in comparison with other screenings that reported mutations in ~2% of sporadic cases (Corrado, et al., 2009, Daoud, et al., 2009, Van Deerlin, et al., 2008).

Three variants (I383V, G348C, and N352T) account for half of the *TARDBP* mutations identified in our cohort and are present in ~1% of FALS patients respectively. The majority of the 30 *TARDBP* mutations described so far have been identified in isolated kindred or sporadic cases. The most frequently observed mutation, A382T, has been reported in nine patients of French and Italian origin (Corrado, et al., 2009, Kabashi, et al., 2008). We did not detect A382T in our cohort, possibly due to a different ethnic background of our patients.

With the exception of I383V, all the detected mutations change residues that are conserved throughout evolution. Accordingly, they are predicted to have a deleterious effect on protein structure or function by at least one of the two following *in silico* software applications: SNAP (<http://cubic.bioc.columbia.edu/services/SNAP/>) and PMut (<http://mmb2.pcb.ub.es:8080/PMut/>). Although not fully conserved, we hypothesize that the substitution of isoleucine 383 with valine is pathogenic, since the mutation has been observed in four FALS cases (Rutherford, et al., 2008), including ours, but not in 4252 healthy controls from different populations (Corrado, et al., 2009, Kabashi, et al., 2008, Kuhnlein, et al., 2008, Sreedharan, et al., 2008, Van Deerlin, et al., 2008).

4. Discussion

Our mutational screening failed to identify *TARDBP* exon 6 mutations within a cohort of 376 AD and 463 PD patients. However, within ALS samples we identified seven previously described *TARDBP* mutations and three novel mutations, N352T, N378D and G384R, all affecting residues conserved throughout evolution and predicted to be pathogenic by *in silico* analysis. The *TARDBP* mutations represent ~6.3% of our FALS cohort. Since *SOD1* and *FUS/TLS* mutations are observed in ~25% of ALS pedigrees, we estimate a mutational

frequency for *TARDBP* of 4.7%. These findings are consistent with previous reports (Corrado, et al., 2009), and substantiate the observation that *TARDBP* mutations represent the second most frequent cause of FALS after *SOD1*. In the SALS cohort, we observed a G295R mutation in a single patient, for a mutational frequency of ~0.5%. Our observations thus differ from other studies that report *TARDBP* mutations in ~2% of all SALS cases (Corrado, et al., 2009, Daoud, et al., 2009, Van Deerlin, et al., 2008). The discrepancy may reflect the differing ethnic backgrounds among the patient cohorts studied to date. Alternatively, it may reflect a statistically insignificant variant; because *TARDBP* variants are much less frequent in SALS than FALS, larger cohorts are likely required to correctly assess their mutational frequency in SALS.

Although TDP-43 positive inclusions have been described in neurons of patients affected with AD, PD and other neurodegenerative diseases, *TARDBP* mutations have been observed only in ALS with or without associated FTLT. Three previous reports could not find mutations in 46 AD (Rutherford, et al., 2008), 125 PD (Kabashi, et al., 2009), and 41 patients with neurodegenerative diseases other than ALS or FTLT-U (Van Deerlin, et al., 2008). Given the low mutational frequency of *TARDBP* in sporadic cases, we attempted to validate these results in two larger AD and PD cohorts. Given that we also failed to find exon 6 mutations in our cohort, it seems unlikely that *TARDBP* mutations in exon 6 are involved in the pathogenesis of AD and PD. Our rationale in screening only exon 6 of the *TARDBP* gene relies on the observation that almost all the ALS-associated mutations are clustered in the C-terminal region of the gene. Moreover, in neuronal inclusions TDP-43 is abnormally cleaved to generate 25kDa C-terminal fragments (Neumann, et al., 2006), suggesting that the region encoded by exon 6 is crucial for the formation of aggregates. We cannot, however, exclude that mutations in other regions of the gene may be associated to AD and PD pathogenesis. Also, since the frequency of TDP-43 inclusions in idiopathic PD is ~7%, it is possible that our PD cohort, while considerably larger than those previously studied, is still underpowered to detect *TARDBP* mutations. Further studies are necessary to determine if TDP-43 is an innocent bystander that is co-precipitated in aggregates or, more interestingly, if the protein is actively involved in the pathogenic pathways leading to AD and PD. Supporting the latter hypothesis is the evidence that the presence of a TDP-43 abnormal immunoreactivity is associated with a modified AD phenotype characterized by a greater cognitive impairment and a more pronounced hippocampal atrophy (Josephs, et al., 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TARDBP mutations within Familial and Sporadic ALS.

Table 1

Nucleotide Change	Amino acid Mutation	FALS		SALS	
		N	%	n	%
883G>C	G295R	0	0	1	0.5
943G>A	A315T	1	0.5	0	0
1035C>A	N345K	1	0.5	0	0
1042G>T	G348C	2	1.0	0	0
1055A>G	N352T	2	1.0	0	0
1132A>G	N378D	1	0.5	0	0
1135T>C	S379P	1	0.5	0	0
1147G>A	I383V	3	1.4	0	0
1150G>C	G384R	1	0.5	0	0
1168A>G	N390D	1	0.5	0	0
Total		13	6.3	1	0.5