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Analysis of hnRNPA1, A2/B1, and A3 genes in patients with amyotrophic lateral sclerosis

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Abstract

Mutations in the prion-like domain (PrLD) of *hnRNPA1* and *A2/B1* genes were recently identified in 2 families with inclusion body myopathy associated with Paget disease of bone, frontotemporal dementia (FTD), and amyotrophic lateral sclerosis, and in ALS patients. These mutations were shown to increase the propensity of hnRNPA1 and A2/B1 proteins, which are TDP-43–binding

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.05.025.

partners, to self-aggregate. hnRNPA3 protein contains a similar PrLD and was recently described in the p62-positive/TDP-43–negative inclusions in affected tissues of *C9orf72*-mutated ALS/FTD patients. We screened *hnRNPA1*, *A2/B1*, and *A3* genes in a cohort of 113 familial ALS (FALS) individuals without mutations in other known ALS-causative genes. We extended our analysis to 108 FALS with mutations in other ALS-associated genes and to 622 sporadic cases by screening specifically the PrLDs of *hnRNPA1*, *A2/B1*, and *A3*. We failed to find variants in each cohort. Our results suggest that mutations in *hnRNPA1*, *A2/B1*, and *A3* genes are a rare finding in ALS.

Keywords

ALS; Genetics; hnRNP; Prion-like domain; IBMPFD

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal neurodegenerative disorder affecting mainly the motor system. Degeneration of upper and/or lower motor neurons in ALS leads to a progressive and severe muscular weakness with paralysis and death generally occurring within 2–3 years after disease onset because of respiratory failure. Familial forms represent 5%–10% of cases, and several causative genes have been identified so far accounting for more than 50% of all inherited forms. Among these, the most frequently mutated genes are *C9orf72*, *SOD1*, *TARDBP*, and *FUS*.

Recently, mutations in *hnRNPA1* (p.D262V) and *hnRNPA2/B1* (p.D290V) genes have been reported in 2 families with inclusion body myopathy associated with Paget disease of bone, fronto-temporal dementia (FTD), and ALS, for which the term “multisystem proteinopathy” has been proposed. Interestingly, 2 different mutations in *hnRNPA1* gene were also identified in 1/212 FALS (p.D262N) and in 1/305 sporadic ALS (SALS) (p.N267S) cases (Kim et al., 2013). All the mutations fall within the prion-like domain (PrLD) and were shown to make the 2 mutant hnRNP proteins more prionogenic by deregulating and accelerating nucleation and polymerization processes. The mutant proteins also altered the dynamics of stress granules formation, hence negatively influencing RNA metabolism. Interestingly, hnRNPA1, hnRNPA2/B1, and hnRNPA3 proteins have been recently described as interacting factors of *C9orf72* hexanucleotide RNA repeats by pull-down assays (Mori et al., 2013). In contrast with hnRNPA1 and A2/B1, hnRNPA3 protein, which also contains a PrLD, was found not only to bind to *C9orf72* RNA repeats but also to colocalize with p62-positive/TDP-43–negative inclusions in affected tissues of FTD/ALS patients with *C9orf72* repeat expansions.

In this study, we performed a mutational analysis of *hnRNPA1*, *A2/B1*, and *A3* genes in ALS. We screened FALS patients negative for mutations in other ALS-associated genes and extended the analysis specifically to the PrLD-coding regions of an additional cohort of FALS and SALS individuals.

2. Methods

Genetic analysis of *hnRNPA1*, *A2/B1*, and *A3* genes was performed by Sanger sequencing (for cohort description, full methods, and in silico analysis, see Supplementary data).

3. Results

A cohort of 113 FALS individuals with no mutations in other known ALS-associated genes were collected by the Italian SLAGEN Consortium and analyzed for mutations in *hnRNPA1*, *hnRNPA2/B1*, and *hnRNPA3* genes. No variants were identified in the entire coding regions of these 3 genes except for a novel intronic variant in *hnRNPA2/B1* (c.695–14insT). This variant was not predicted to alter *hnRNPA2/B1* splicing by in silico analysis. As the previously identified mutations in *hnRNPA1* and *A2/B1* genes all mapped within their PrLDs (Supplementary Fig. 1), we screened an additional panel of 108 FALS, with known mutations in other ALS-associated genes, and 622 SALS specifically for the PrLD-encoding exons of *hnRNPA1*, *A2/B1*, and *A3* genes. No mutations were found in either cohort.

4. Discussion

The recent identification of variants in the PrLDs of *hnRNPA1* and *A2/B1* genes in individuals with multisystem proteinopathy prompted us to analyze a cohort of ALS patients of Italian descent. hnRNPA1 and A2/B1 proteins are well-known interacting partners of TDP-43, a protein forming pathological aggregates in ALS and in a subset of FTD. The physiological interaction of hnRNPA1 and A2/B1 with TDP-43 has been extensively characterized and shown to occur through the PrLDs of both hnRNP and TDP-43 proteins. Moreover, in disease conditions, such physiological interaction is perturbed leading to the formation of TDP-43 abnormal aggregates (Budini et al., 2012). As mutations in *TARDBP*, the gene encoding TDP-43, account for a subset of FALS cases, it is noteworthy that also mutations in genes coding for TDP-43–binding proteins were identified. As these mutations in hnRNP PrLDs triggered aggregate formation, other PrLD-containing genes could represent candidates for ALS and other neurodegenerative disorders characterized by the formation of distinctive pathologic inclusions. Among these, *hnRNPA3* is a good candidate as it belongs to the same RNA-binding protein family as *A1* and *A2/B1* and was recently identified to form pathological aggregates in FTD/ALS cases with *C9orf72* mutations possibly by binding to GGGGCC RNA repeats (Mori et al., 2013). Notwithstanding the previously mentioned premises, our genetic screening of a large cohort of 221 FALS and 622 SALS individuals revealed no pathological variants in *hnRNPA1*, *A2/B1*, and *A3* genes, suggesting that mutations in their PrLDs are a rare finding in ALS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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