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Screening of the *PFN1* gene in sporadic ALS and in FTD

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Abstract

Mutations in the profilin 1 (*PFN1*) gene, encoding a protein regulating filamentous actin growth through its binding to monomeric G-actin, have been recently identified in familial amyotrophic lateral sclerosis (ALS). Functional studies performed on ALS-associated PFN1 mutants demonstrated aggregation propensity, alterations in growth cone and cytoskeletal dynamics. Previous screening of *PFN1* gene in sporadic ALS (SALS) cases led to the identification of the p.E117G mutation, which is likely to represent a less pathogenic variant according to both frequency data in controls/cases and functional experiments. To determine the effective contribution of *PFN1* mutations in SALS, we analyzed a large cohort of 1168 Italian SALS patients and also included 203 FTD (Frontotemporal Dementia) cases given the great overlap between these two neurodegenerative diseases. We detected the p.E117G variant in 1 SALS and the novel synonymous change p.G15G in another patient, but none in a panel of 1512 controls. Our results suggest that *PFN1* mutations in sporadic ALS and in FTD are rare, at least in the Italian population.

Keywords

amyotrophic lateral sclerosis; frontotemporal dementia; profilin 1; mutation analysis

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disease mainly caused by the loss of upper and lower motor neurons, resulting in progressive muscle atrophy and paralysis. Although most ALS cases are sporadic (SALS), ~5% of them are

familial (FALS), usually with an autosomal-dominant inheritance pattern. Approximately 5% of all ALS cases exhibit signs of frontal lobe degeneration (FTD), supporting the increasing evidence of a pathophysiological and genetic link between these two disorders (Orr et al., 2011). Repeat expansions in *C9ORF72* gene and *SOD1* mutations represent the most frequent causes of FALS, accounting overall for 40–50% of cases (Ratti et al., 2012), while mutations in *TARDBP* and *FUS/TLS* genes are responsible each of ~5% of FALS (Andersen and Al-Chalabi, 2011). Recently, using an exome-sequencing approach on two large ALS families and direct sequencing on a large FALS cohort, we identified 4 different mutations within profilin 1 (*PFN1*) gene in 2.6% FALS cases (Wu et al., 2012). Remarkably, one mutation (p.M114T) was identified in an ALS family of Italian origin. Functional studies demonstrated that PFN1 mutants form insoluble ubiquitinated aggregates often containing TDP-43 protein and show defects in growth cones morphology with altered actin levels (Wu et al., 2012). Sequencing of this novel FALS-associated gene in sporadic cases resulted only in the identification of the less pathogenic variant p.E117G leaving open the issue about the relevance of *PFN1* in the genetics of SALS forms (Wu et al., 2012).

To further assess the frequency of *PFN1* mutations in SALS, we performed a mutational screening of a large cohort of 1168 SALS cases of Italian origin. An additional panel of 203 FTD cases was also included in the study, considering the recent evidence of an increasing genetic and neuropathological overlap between these two neurodegenerative disorders.

Methods

Genomic DNA was obtained from peripheral blood of 1168 SALS individuals and 203 FTD patients. The control group consisted of 1512 healthy subjects of Italian origin, of whom 380 samples previously analyzed (Wu et al., 2012). The entire coding region of *PFN1* (NCBI reference sequence NM_005022.3) was amplified by PCR and directly sequenced (Full methods and cohort description are available in Supplementary Data).

Results

We sequenced *PFN1* gene in 1168 SALS cases, of whom 49 individuals showed concomitant ALS and FTD, and in 203 FTD subjects, of whom 30 represented familial cases. We identified the previously described p.E117G variation in 1 SALS patient presenting with bulbar onset ALS at age 73 and carrying no mutations in other ALS-linked genes (Supplementary Data). No mutations were found in 1512 Italian controls, including 380 individuals already analyzed in our previous report (Wu et al., 2012) and additional 1132 subjects fully sequenced for *PFN1* coding region.

In addition, our analysis detected two synonymous variants, p.G15G and p.L112L, respectively. The p.G15G (c.45G>C) represents a novel variation and was identified in 1 SALS case, while the single nucleotide polymorphism p.L112L (c.334C>T; rs13204) was detected with a similar allele frequency in cases and controls (Table 1 in Supplementary Data).

Discussion

The recent identification of *PFN1* gene, encoding for a G-actin binding protein, as a novel causative gene in FALS highlighted the importance of cytoskeletal alterations in the pathogenesis of ALS. Our mutational study on a large cohort of Italian SALS identified only the p.E117G variant in 1/1168 patients, suggesting that *PFN1* mutations are rare in sporadic cases, at least in the Italian population. In the previous mutation analysis of *PFN1* gene in SALS cases, we had similarly observed the p.E117G variation in 2/816 SALS individuals.

This variant was also described in control subjects (3/7560), although with an allele frequency significantly different between cases and controls (Wu et al., 2012). In this study we did not found such mutation in our additional control group of 1132 Italian subjects. According to experimental observations p.E117G is likely to be a less pathogenic mutant by exhibiting only moderate aggregation propensity and no decrease in bound G-actin levels (Wu et al., 2012). However, in the etiology of sporadic diseases, it is well-known that multiple genetic variants of small effect in combination with environmental factors may contribute to increase the risk of developing the disease (Talbot, 2009).

Although we found no variants in our FTD cases, further genetic screenings on larger cohorts are needed to definitely assess the contribution of *PFN1* in the genetics of FTD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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