Neurobiology of Aging 37 (2016) 209.e9–209.e16

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Identification of mutations in Korean patients with amyotrophic lateral sclerosis using multigene panel testing

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ARTICLE INFO

Article history:
Received 9 March 2015
Received in revised form 25 August 2015
Accepted 19 September 2015
Available online 30 September 2015

Keywords:
Amyotrophic lateral sclerosis
ALS
Next-generation sequencing
NGS
Multigene panel
Mutation

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease involving motor neurons. Because a growing number of genes have been identified as the genetic etiology of ALS, simultaneous screening of mutations in multiple genes is likely to be more efficient than gene-by-gene testing. In this study, we performed a multigene panel testing by using targeted capture of 18 ALS-related genes followed by next-generation sequencing. Using this technique, we tried to identify mutations in 4 index patients with familial ALS and 148 sporadic ALS in Korean population and identified 4 known mutations in *SOD1*, *ALS2*, *MAPT*, and *SQSTM1* genes, respectively, and 28 variants of uncertain significance in 9 genes. Among the 28 variants of uncertain significance, 6 missense variants were found in highly conserved residues and were consistently predicted to be deleterious by in silico analyses. These results suggest that multigene panel testing is an effective approach for mutation screening in ALS-related genes. Moreover, the relatively low frequency of mutations in known ALS genes implies marked genetic heterogeneity at least in Korean patients with ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by rapidly progressive paralysis and death from respiratory failure, typically within 2–3 years of symptom onset (Rowland and Shneider, 2001). No effective cure for ALS has yet been developed. As in other neurodegenerative disorders, ~10% of ALS is classified as familial (fALS), whereas the remaining 90% of cases are considered sporadic (sALS), as they occur randomly throughout the community (Rowland and Shneider, 2001).

To date, a number of genetic loci and disease-causing mutations in several genes have been reported to be associated with typical ALS or atypical motor neuron diseases. Hexanucleotide repeat expansion in *C9orf72* is the most common cause of ALS in patients of European ancestry, accounting for about 40% of all fALS and about 7% of all sALS cases (Renton et al., 2014). *SOD1* mutations are another common cause of ALS, accounting for about 15%–20% of all fALS and 2%–4% of all sALS cases (Dion et al., 2009; Rosen, 1993). Along with *C9orf72* and *SOD1*, *TARDBP*, *FUS*, *OPTN*, *VCP*, *UBQLN2*, and *PFN1* are major genes underlying ALS. The genetic etiology of two-thirds of all fALS cases and about 10% of all sALS cases has now been identified (Renton et al., 2014).

Several genetic studies have been performed in Korean patients with ALS. Kwon et al. screened for mutations in multiple

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http://dx.doi.org/10.1016/j.neurobiolaging.2015.09.012

ALS-related genes, including *SOD1*, *FUS*, *TARDBP*, *ANG*, and *OPTN*, in 258 Korean patients. Eight of 9 (88.9%) patients with fALS and 7 of 249 (2.8%) patients with sALS carried mutations in *SOD1* or *FUS* (Kwon et al., 2012). Moreover, Kim et al. and Jang et al. demonstrated that mutations in *UBQLN2*, *SIGMAR1*, and *C9orf72* are rare in Korean patients with ALS (Jang et al., 2013; Kim et al., 2014). The genetic heterogeneity of ALS complicates the molecular diagnosis of ALS and necessitates a more efficient method for screening for mutations in ALS-related genes.

Recently, multigene panel testing combined with high-throughput sequencing technology has been successfully used to test for various conditions with genetic heterogeneity (Couthouis et al., 2014). In this study, we performed multigene panel testing to identify mutations in ALS-related genes.

2. Materials and methods

2.1. Subjects

All participating patients were recruited between December 2010 and May 2012 at the ALS Clinic in the Neurology Department, Hanyang University Hospital in Seoul, Korea. One hundred fifty-two patients with a clinical diagnosis of definite, probable, or probable with laboratory support ALS according to the revised El Escorial criteria (Brooks et al., 2000; Mitchell, 2000) were enrolled. All patients were of Korean descent. The following data were collected at the time of diagnosis and/or enrollment: patient demographics (age, sex, and family history of ALS), degree of diagnostic certainty, site of symptom onset, revised ALS Functional Rating Scale (ALSFRS-R) score (Cedarbaum et al., 1999) and calculated progression rate (ΔFS) (Kimura et al., 2006). In this study, fALS was defined as when 2 or more patients were present through third degree pedigree; all other patients were classified as sALS. The progression rates were calculated (ΔFS, [48 − ALSFRS-R at the time of diagnosis]/duration from onset to diagnosis) and divided into 3 groups: slow (lower tertile <0.66), intermediate (intertertile 0.66–1.00), and rapid (upper tertile >1.00). Clinical characteristics are summarized in Table 1. Among 152 patients enrolled in this study, 4 patients were fALS (2.6%) and remaining 148 patients were sALS (97.6%). The pedigrees of the 4 patients with fALS are shown in Fig. 1. This study was approved by the Institutional Review Boards of the College of Medicine, Hanyang University (#HYI-10-01-01) and Samsung Medical Center (#2013-04-131-001), and all participants gave written informed consent per the approved study protocol.

Table 1
Baseline characteristics of the 152 patients enrolled in this study

Variable	Total	fALS index	sALS	*p* value
Number of patients	152	4	148	
Male:female	77:75	4:0	73:75	
Age at onset, mean ± SD (y)	55.7 ± 5.8	46.7 ± 9.4	56.0 ± 11.2	0.103
ALSFRS-R at enrollment, mean ± SD	38.7 ± 5.8	38.0 ± 10.0	38.8 ± 5.7	0.890
ΔFS, mean ± SD	0.74 ± 0.59	0.79 ± 0.95	0.74 ± 0.59	0.875
Site of symptom onset, N				
Limb	99	3	96	
Bulbar	49	1	48	
Other[a]	4	0	4	

ΔFS = (48 − [ALSFRS-R at the time of diagnosis])/duration from onset to diagnosis.
Key: ALSFRS-R, a revised version of the Amyotrophic Lateral Sclerosis Functional Rating Scale; fALS, familial ALS; ΔFS, calculated progression rate; sALS, sporadic ALS; SD, standard deviation.
[a] Two patients with axial, 1 patient with respiratory and 1 patient with combined limb and bulbar involvement.

2.2. Genetic analysis

2.2.1. Gene selection for ALS panel

Candidate genes consisted of the following: (1) genes previously reported to contain disease-causing mutations in patients with ALS and (2) genes known to be associated with the ALS phenotype. Many of the physiological and neuropathological roles of ALS-causative genes overlap; moreover, several molecules implicated in the pathogenesis of ALS share similar cellular functions and potentially colocalize in intraneuronal aggregates. For example, RNA-binding proteins such as FUS and TAF15 have structural and functional similarities to TDP-43 (Couthouis et al., 2011; Lagier-Tourenne and Cleveland, 2009). Other molecules including VCP, UBQLN2, OPTN, FIG4, and SQSTM1 are involved in protein degradation. Furthermore, the TDP-43-positive cytoplasmic pathology that is observed in carriers of *TARDBP* mutation is also observed in carriers of mutations in *VCP*, *UBQLN2*, *SQSTM1*, *OPTN*, *VAPB*, and *C9orf72* (Cooper-Knock et al., 2012; Deng et al., 2011; Millecamps et al., 2010; Neumann et al., 2006; Renton et al., 2014; Teyssou et al., 2013). Candidate genes were further curated based on their expression pattern and functional significance (Table 2). In total, 18 genes comprising 254 exons were ultimately selected for the targeted sequencing panel: *SOD1*, *SETX*, *FUS*, *ANG*, *TARDBP*, *TAF15*, *VCP*, *UBQLN2*, *SQSTM1*, *SIGMAR1*, *ALS2*, *FIG4*, *VAPB*, *OPTN*, *DAO*, *MAPT*, *SPG11*, and *GRN*. The *C9orf72* was not included in this panel because of the nature of mutation (hexanucleotide repeat expansion), it was tested separately as described previously (Jang et al., 2013).

2.2.2. Multigene panel testing

Multigene panel testing of the 18 genes in the 4 patients with fALS and 148 patients with sALS was performed using a customized SureSelect Exome Enrichment kit (version 1.0, Agilent, Santa Clara, CA, USA) according to the manufacturer's protocols. The enriched DNA was paired-end sequenced on a HiSeq 2000 sequencing system (Illumina, San Diego, CA, USA). The Burrows-Wheeler Aligner was used to align sequence reads to the human reference genome (hg19), and variants were called using Sequence Alignment and/or Map Tools software. Based on the hypothesis that the mutations underlying this rare disease are not present in the general population, single nucleotide polymorphisms (SNPs) with allele frequencies >0.01 identified in the dbSNP139 database (National Center for Biotechnology Information, Bethesda, MD, USA), 1000 Genomes Project (1000 Genomes Project Consortium et al., 2012), or Exome Variant Server (NHLBI GO Exome Sequencing Project, Seattle, WA, USA) were filtered out. Next, synonymous changes were identified and further filtered from the variant list. Sanger sequencing was then performed with customized primers to the remaining variants in the patients with ALS. As an additional step, variants found in 622 ethnically-matched normal control subjects were also filtered out. The normal control data were obtained at the Korean Reference Genome Database (http://152.99.75.168/KRGDB/). The variants of this study were also compared with inhouse disease control exome data from 88 patients without ALS or frontotemporal dementia (FTD) (Jang et al., 2015).

2.2.3. Bioinformatics analysis

Several bioinformatics analyses were performed to identify the functional and structural significance of missense mutations or splice-site variants observed in patients. Orthologs and paralogs were identified by computational analysis based on DNA and amino acid sequence similarities using the evolutionary annotation database Evola (version 7.5, http://www.h-invitational.jp/evola_main/annotation.cgi). To determine whether nonsynonymous changes could cause disease, their functional consequences were predicted in silico using Sorting Intolerant From Tolerant software (SIFT,