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Comprehensive targeted next-generation sequencing in Japanese familial amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by loss of motor neurons. We have recently identified *SOD1* and *FUS* mutations as the most common causes in a consecutive series of 111 familial ALS pedigrees in Japan. To reveal possible genetic causes for the remaining 51 patients with familial ALS (45 pedigrees), we performed targeted next-generation sequencing of 35 known ALS/motor neuron diseases-related genes. Known variants in *ANG*, *OPTN*, *SETX*, and *TARDBP* were identified in 6 patients. A novel likely pathogenic homozygous variant in *ALS2* was identified in 1 patient. In addition, 18 patients harbored 1–3 novel variants of uncertain significance, whereas hexanucleotide repeat expansions in *C9ORF72* were not detected using repeat-primed polymerase chain reaction. Collectively, in our Japanese cohort, the frequencies of *SOD1*, *FUS*, *SETX*, *TARDBP*, *ANG*, and *OPTN* variants were 32%, 11%, 2%, 2%, 1%, and 1%, respectively. These findings indicate considerable differences in the genetic variations associated with familial ALS across populations. Further genetic analyses and functional studies of novel variants are warranted.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive motor neuron disease (MND) characterized by the degeneration of upper and lower motor neurons, which leads to muscle wasting and weakness, bulbar palsy, and eventually death within 2–3 years after the onset of the first symptoms (Rowland and Shneider, 2001). Approximately, 5%–10% of ALS patients have a familial history, and these cases are classified as familial ALS (Renton et al., 2014), whereas the remaining cases of ALS are considered to be sporadic.

In 1993, *SOD1* mutations were first recognized as a cause of familial ALS (Aoki et al., 1993; Deng et al., 1993; Rosen et al., 1993). Thereafter, more than 20 genes that are causative for familial ALS have been identified, including *TARDBP* (Sreedharan et al., 2008), *FUS* (Kwiatkowski et al., 2009; Vance et al., 2009), *OPTN* (Maruyama et al., 2010), *VCP* (Johnson et al., 2010), *UBQLN2* (Deng et al., 2011), and *C9ORF72* (DeJesus-Hernandez et al., 2011; Renton et al., 2011). In a recent Japanese study, known pathogenic variants were identified in 48.7% (19/39) individuals with familial ALS and 3.0% (14/469) individuals with sporadic ALS (Nakamura et al., 2016), mainly using next-generation sequencing of 28 ALS-linked genes. Nevertheless, there is a substantial fraction of individuals with familial ALS in whom genetic variations have not been identified, thus suggesting that there is considerable heterogeneity in the genetic spectrum underlying the disease. Moreover, a lower MND phenotype is linked to a subset of the aforementioned genes, and ALS phenotypes may be closely mimicked by MNDs such as spinal muscular atrophy and hereditary motor neuropathy. Thus, it remains to be clarified whether MNDs-related genes (rather than ALS) affect the pathology of familial ALS.

We have recently identified *SOD1* and *FUS* mutations as being the most common causes of ALS in a consecutive series of 111 familial ALS pedigrees in Japan (Akiyama et al., 2016). However, it is uncertain how other ALS-linked genes contribute to the disease in the remaining Japanese patients with familial ALS. To reveal the unidentified causative genetic factors, we conducted targeted next-generation sequencing of ALS/MNDs-related genes.

2. Materials and methods

2.1. Standard protocol approval, registration, and patient consent

This study was approved by the Ethics Committee of the Tohoku University School of Medicine, and we obtained informed consent from all individuals before their inclusion in the study.

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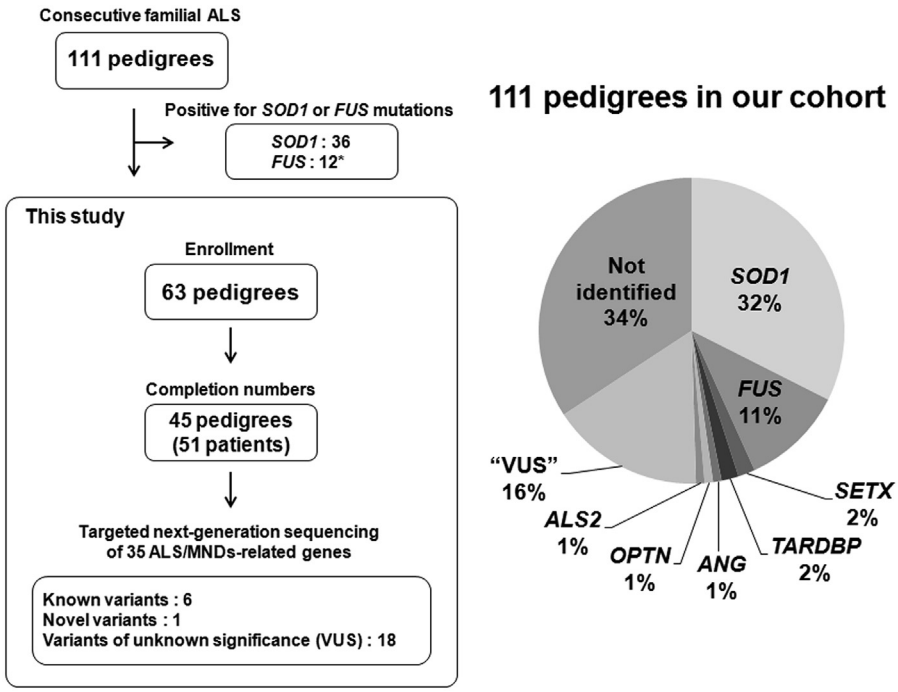

Fig. 1. Sequencing strategy (left) and results (right) for 111 familial ALS pedigrees. The circular chart represents the proportion of familial ALS cases identified according to each gene evaluated in our Japanese cohort. *SOD1* mutations in 36 patients (unpublished data) and **FUS* mutations in 12 patients (Akiyama et al., 2016) were primarily identified through Sanger sequencing. The causative genes of the remaining 63 pedigrees (57%) were not identified. We conducted targeted next-generation sequencing of 35 ALS/MNDs-related genes in 51 analyzable patients (45 pedigrees) by using a next-generation sequencer. *SETX*, *TARDBP*, *ANG*, and *OPTN* were identified in 2%, 2%, 1%, and 1% of patients, respectively. A novel likely pathogenic variant of *ALS2* was identified. In addition, 18 patients (16%) harbored variants of uncertain significance (VUS) in ALS/MNDs-related genes. In this study, a genetic cause of ALS in 26 patients (20 families) was not found. Abbreviations: ALS, amyotrophic lateral sclerosis; MNDs, motor neuron diseases.

2.2. Subjects

We recruited 111 pedigrees with familial ALS from 1991 to 2016 at the Department of Neurology, Tohoku University Hospital, Japan. All patients were of Japanese ancestry and exhibited a positive familial history of ALS, MNDs, or similar symptoms among first to sixth degree relatives. Each patient fulfilled the diagnostic criteria for ALS as outlined by the revised El Escorial criteria. Three families presented with lower MND and a family history of lower MND. To identify the genetic causes of Japanese familial ALS, we performed Sanger sequencing of all exons of *SOD1* and *FUS*, as previously described (Akiyama et al., 2016). This study included a total of 51 patients, belonging to 45 apparently unrelated pedigrees, whose DNA samples were available with informed consents for further genetic analysis (Fig. 1).

2.3. Targeted next-generation sequencing

We selected 35 ALS-related or other MNDs-related genes for inclusion in the screening panel (Supplementary Table 1). Specifically, 22 of the genes (*ALS2*, *ANG*, *ATXN2*, *C9ORF72*, *CHMP2B*, *DAO*, *DCTN1*, *FIG4*, *FUS*, *NEFH*, *OPTN*, *PFN1*, *PRPH*, *SETX*, *SIGMAR1*, *SOD1*, *SPG11*, *TAF15*, *TARDBP*, *UBQLN2*, *VAPB,* and *VCP*) are known causative genes in ALS. The other 13 genes (*ATP7A*, *BSCL2*, *GARS*, *HSPB1*, *HSPB3*, *HSPB8*, *IGHMBP2*, *PLEKHG5*, *SMN1*, *ATL1*, *SPAST*, *TRPV4,* and *VRK1*) are known causative genes in other MNDs distinct from ALS, such as spinal muscular atrophy, distal hereditary motor neuronopathy, or spastic paraplegia. Thus, we describe these 13 genes as "other MNDs-related genes" in this study. The panel included several customized regions covering the 100 bp flanking regions of the variant sites in noncoding regions that have been reported to be related to ALS or other MNDs (Supplementary Table 2).

The targeted regions were designed to include coding exons with intronic 50 bp flanking sites and 3′ and 5′ untranslated regions (UTRs) by using the SureDesign system (Agilent Technologies, Santa Clara, CA, USA). The regional source of coding exons was extracted from the RefSeq, CCDS, Ensemble, Gencode, or VEGA databases. In all the targeted regions, 269,860 base pairs were expected to be analyzable from 8661 amplicons, and the target coverage was expected to be 97.68%.

DNA extraction from peripheral blood samples from patients was performed using standard protocols. A spleen sample from 1 patient was acquired in the same manner. We performed whole-genome amplification by using an Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Vienna, Austria) for the sample from patient ID 21 before preparing libraries.

The sequencing libraries were prepared from genomic DNA by using a HaloPlex target enrichment system (Agilent Technologies). Targeted libraries were sequenced using the MiSeq platform according to the manufacturer's instructions (Illumina, San Diego, CA, USA). The Burrows-Wheeler Aligner was used to align the paired 151-bp reads to the human genome (UCSC Genome Browser hg19). Identification of single nucleotide variant and indel calling were performed with the Genome Analysis Toolkit v1.6. Depth of coverage analyses were performed using Genome Analysis Toolkit v2.6. ANNOVAR was used for annotation against the RefSeq database and the Single Nucleotide Polymorphism databases (dbSNP142 (http://www.ncbi.nlm.nih.gov/projects/SNP/)).

2.4. Variant analysis

According to the applied filtering strategy (Supplementary Table 3), we extracted the following variants with the exception of the customized regions: variants located in coding regions