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Genetic analysis of amyotrophic lateral sclerosis in the Slovenian population

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a complex fatal neurodegenerative disease characterized by progressive degeneration and loss of upper motor neurons in the cerebral cortex and lower motor neurons in brainstem and spinal cord. We established the frequencies of mutations in 4 major ALS-associated genes, *SOD1*, *TARDBP*, *FUS*, and *C9ORF72* in a representative cohort of 85 Slovenian patients with sporadic form of ALS. Pathogenic massive hexanucleotide repeat expansion mutation in *C9ORF72* was detected in 5.9% of patients and was the most common cause of the disease. In the remaining 3 genes, we identified 4 changes in 3 patients, p.Val14Met in *SOD1*, silent mutation p.Arg522Arg in *FUS*, and p.Gly93Cys in *SOD1* together with a novel synonymous variant c.990A>G (p.Leu330Leu) in *TARDBP* gene, respectively. This study represents the first genetic screening of major causative genes for ALS in a cohort of sporadic ALS patients from Slovenia and is according to our knowledge the first such study in Slavic population. Overall, we genetically characterized 8.2% sporadic ALS patients.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disease characterized by progressive degeneration and loss of upper motor neurons in the cerebral cortex and lower motor neurons in brainstem and spinal cord. The disease usually occurs around the age of 60 years and presents with symptoms like muscular weakness, atrophy, and later on paralysis which lead to death due to respiratory failure within 2–5 years from onset (Rowland and Shneider, 2001). The disease starts in the muscles of upper and lower limbs in approximately two-thirds of all patients (spinal onset) and in one-third of patients in those required for swallowing or speech (bulbar onset) (Wijesekera and Leigh, 2009).

ALS is mainly a sporadic disease (SALS) but occurs also as familial form (FALS), usually dominantly inherited in about 10% of patients. It is classified as rare disease with 1–2 per 100,000 new cases per year (Johnston et al., 2006). The prevalence is about 4–8 per 100,000 per year (Chio et al., 2013). Many genes have been linked to ALS as causative for the disease, among which *SOD1* (Rosen, 1993), *FUS* (Kwiatkowski et al., 2009; Vance et al., 2009), *TARDBP* (Kabashi et al., 2008; Sreedharan et al., 2008; Van Deerlin et al., 2008), and *C9ORF72* (DeJesus-Hernandez et al., 2011; Renton et al., 2011) are represented in the highest frequencies.

We established the frequencies of mutations in these 4 major genes in 85 Slovenian patients with sporadic form of ALS. This study represents the first genetic analysis of ALS in this small 2 million Central European population of Slavic origin and thus contributes additional insight into genetics of ALS.

2. Methods

2.1. Patients

Blood samples of 85 Slovenian patients clinically diagnosed with ALS were collected at the Institute of Clinical Neurophysiology,

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Table 1
Clinical data of Slovenian ALS patients with detected genetic changes

Gene	Nucleotide change	AA change	Frequency (%)	Gender	Onset; age of onset; disease duration; associated symptom	Reference
SOD1	c.43G>A	p.Val14Met	2.3	F	Spinal; 67 y; 4+ y	Deng et al., 1995
SOD1	c.280G>T	p.Gly93Cys		F	Spinal; 51 y; 5+ y	Rosen, 1993
TARDBP	c.990A>G	p.Leu330Leu	1.2			This study
FUS	c.1566G>A	p.Arg522Arg	1.2	M	Spinal; 84 y; 1 y	Ticozzi et al., 2009
C9ORF72	Expansion GGGGCC		5.9	M	Spinal; 52 y; 0.5 y	Renton et al., 2011
				F	Spinal; 60 y; 6+ yrs	
				M	Spinal; 61 y; 2+ yrs; FTD	
				F	Bulbar; 55 y; 2 y	
				M	Spinal; 70 y; 1 y	

The symbol + indicates disease in progress.
Key: ALS, amyotrophic lateral sclerosis; F, female; FTD, frontotemporal dementia; M, male.

University Medical Centre Ljubljana, Slovenia. Among patients 46 were males and 39 were females (M/F ratio was 1.2), and the mean age of onset was 63 years (ranging from 37 to 89 years). The average disease duration of 37 deceased patients was 3 years (range 0.5–5 years). Sixty-four of 85 patients (75%) had spinal and 21 of 85 patients (25%) had bulbar onset form of the disease. None of the patients were related. Five patients (5.9%) had associated symptoms of frontotemporal dementia (FTD), 2 patients (2.4%) had some associated symptoms of Alzheimer's disease, and 2 patients (2.4%) had associated signs of parkinsonism.

We obtained approval for this study from National Medical Ethics Committee of Republic of Slovenia and a written informed consent from all participants.

2.2. Molecular methods and analysis

DNA was isolated from blood samples using QIAamp Blood Midi Kit (Qiagen, Germany) following their protocol.

For mutation detection in genes *SOD1*, *FUS* and *TARDBP* coding regions and flanking intronic regions of interest were amplified with polymerase chain reaction (all 5 exons of *SOD1* gene, exons 6, 14 and 15 of *FUS* gene and exon 6 of *TARDBP* gene) and the products were run on 2% agarose gel electrophoresis. Purification was performed with Diffinity RapidTip (Diffinity Genomics, USA) followed by Sanger sequencing on ABI310 Genetic Analyzer (Applied Biosystems, USA).

Specific 2-step protocol for the detection of hexanucleotide repeat expansion mutation (*HREM*) in *C9ORF72* gene was followed as previously described (DeJesus-Hernandez et al., 2011; Renton et al., 2011; Smith et al., 2013). Fragment length analysis was performed on ABI310 Genetic Analyzer (Applied Biosystems), and peaks were visualized by Gene Scan 3.7 software. Repeat expansions are seen as sawtooth pattern with a 6-base pair periodicity.

2.3. Bioinformatic analysis

Nucleotide sequences for each patient were evaluated using Ensembl online tool (http://www.ensembl.org/index.html). To determine potential new genetic change, we used ALS Online Genetics Database (http://alsod.iop.kcl.ac.uk/) (Abel et al., 2012), dbSNP (http://www.ncbi.nlm.nih.gov/project/SNP), Exome Variant Server (http://evs.gs.washington.edu/EVS/), and 1000 Genomes Database (www.1000genomes.org/). For evaluation of potential functional effect of novel genetic variant, we used in silico prediction tools ESEfinder 3.0 software (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home) and NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/).

3. Results

Screening of 85 Slovenian ALS patients for mutations in genes *SOD1*, *FUS*, and *TARDBP* revealed 4 different variations in the coding DNA sequence in 3 patients. In *SOD1* gene, 2 mutations (p.Val14Met and p.Gly93Cys) were detected. Sequencing of *FUS* and *TARDBP* genes revealed 2 synonymous substitutions, 1 in *FUS* (c.1566G>A, p.Arg522Arg) and 1 in *TARDBP* (c.990A>G, p.Leu330Leu). The *TARDBP* substitution is new not previously described variant, and we detected it together with *SOD1* gene mutation p.Gly93Cys in 1 patient. Screening of the first intron of *C9ORF72* revealed *HREM* in 5 of 85 patients (5.9%) (see Table 1).

4. Discussion

This study represents the first genetic screening in a representative cohort of Slovenian patients with ALS. Presumably, all analyzed patients had sporadic forms of ALS. Namely, in any of the cases the criteria for possible, probable, or definite FALS were not fulfilled (Byrne et al., 2011).

The most common detected disease causing change was pathogenic *HREM* of *C9ORF72* gene. Five of 85 ALS patients (5.9%) had this genetic determinant. The frequency of the pathologically expanded repeats in Slovenian population, which belongs geographically to Central European populations is thus consistent with carrier frequency of sporadic cases, for which progressive decrease from Northern to Southern Europe ranging from 8% to 4.7%, respectively, was recently reported (Fogh et al., 2014). Although for our neighboring Italian population the frequency of pathologic expansion in sporadic ALS is lower (about 5%) in North Italy (Bertolin et al., 2014; Ratti et al., 2012) and higher (6.5%) in some isolated Southern regions, like island Sardinia (Borghero et al., 2014).

The ratio of bulbar versus spinal onset of the disease did not differ in our cases with *HREM* in *C9ORF72* and those without this mutation (20% vs. 25%), respectively; although for C9ORF72-linked ALS, the reported onset of the disease is more commonly bulbar (40% vs. 25%) (Chio et al., 2012). This discrepancy is most likely due to a relatively small sample of our ALS cases. Pathologic expansion did not affect the average age of onset in our cohort but decreased the survival and increased the appearance of FTD, what is in concordance with other studies (Byrne et al., 2012).

Sanger sequencing of *SOD1*, *TARDBP*, and *FUS* genes identified 4 changes in 3 patients. In *SOD1* gene, 2 mutations were found, p.Val14Met and p.Gly93Cys. A patient with a pathogenic p.Gly93Cys mutation in *SOD1* gene (Rosen, 1993) also has a new previously undescribed nucleotide change c.990 A>G in *TARDBP* gene (p.Leu330Leu). This synonymous substitution (genomic coordinates chr1:11082457) is apparently a very rare variant because it is absent from publically available databases such as dbSNP and 1000 Genomes and is also not present in 6503 individuals (4300 Caucasians and 2203 Africans) included in a large database of exomes from the exome variant server. *In silico* analysis with the ESEfinder 3.0 software (Smith et al., 2006) showed that *TARDBP* c.990 A>G may disrupt exonic splicing enhancer motif.