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TARDBP mutations are not a frequent cause of ALS in Finnish patients

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In previous studies 1-3 % of ALS patients have *TARDBP* mutations as the cause of the disease. *TARDBP* mutations have been reported in ALS patients in different populations but so far there are no studies on the frequency of *TARDBP* mutations in Finnish ALS patients. A cohort of 50 Finnish patients, 44 SALS and 6 FALS patients, were included in the study. Genomic DNA was extracted from venous blood or muscle tissue and a mutation analysis of *TARDBP* was performed. No definitely pathogenic mutations could be identified in *TARDBP* in our patient cohort. However, two previously unknown variations were found: one silent mutation in exon 2 and one relatively deep intronic single nucleotide insertion in intron 5. In addition, two previously known non-pathogenic polymorphisms in intron 5 were detected. The size of our cohort is obviously not large enough to conclusively exclude *TARDBP* mutations as a very rare cause of ALS in Finland. However, based on our results *TARDBP* mutations do not appear to be a frequent cause of familial or sporadic ALS in Finland.

Key words: Amyotrophic lateral sclerosis, mutation screening, *TARDBP*

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal neuromuscular disease characterized by progressive loss of motor neurons in the brain and spinal cord, leading to paralysis and death from respiratory failure typically within 3-5 years after symptom onset (1). The incidence is 2-3 per 100 000 person-years and prevalence 4-6 per 100 000 (2-4). Most cases are sporadic (SALS), but approximately 10 % are familial (FALS). Mutations in *SOD1* gene account for 15-20 % of FALS cases (5, 6), and to an even higher degree in Finnish ALS patients (7). Mutations in several other genes, including *TARDBP, ALS2, SETX, FUS, VAPB, ANG, DCTN1*, and *UBQLN2*, are described as rare causes of FALS (6, 8-16). Recently, Renton et al. (2011) reported a hexanucleotide repeat expansion within *C9orf72* gene as the cause of chromosome 9p21-linked ALS (17). In their data this expansion mutation accounted for 46 % of familial and 21 % of sporadic ALS in the Finnish population, and in one-third of FALS cases of wider European ancestry making it the most common genetic cause of ALS identified to date.

Transactive response DNA-binding protein 43 (TDP-43) is a nuclear protein composed of 414 amino acids, encoded by TAR-DNA-binding protein-gene (*TARDBP*) on chromosome 1p36.22 (18). *TARDBP* contains 5 coding and 2 non-coding exons (19). Mutations in *TARDBP* gene are found in 1-3% of ALS cases (20). All identified mutations seem to cluster in exon 6, except for one in exon 4. Missense mutations have been found in sporadic and familial *SOD1*-negative ALS (8, 21-24), and a frame-shift mutation that creates a premature stop codon (Y374X) has also been reported (25). TDP-43 is a protein expressed ubiquitously in the tissues of the human body. It has been identified as taking part in numerous cellular processes including regulating transcription and alternative splicing, as well as transport, translation and metabolism of RNA (26). Besides being primarily mutated, TDP-43 has been recognized in the inclusions of motor neurons also in other kinds of ALS, and is therefore considered a major disease protein in ALS (27, 28). Even though the neurotoxicity of TDP-43 aggregates has been established, the exact molecular mechanism of neurodegeneration remains elusive. Phosphorylation, truncation, mislocalization (26) and ubiquitination of the protein have been reported to contribute to the disease pathogenesis (16, 27, 28).

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TARDBP mutations have been reported in ALS patients in different populations but so far no studies on the frequency of *TARDBP* mutations in Finnish ALS patients have been performed.

Patients and methods

Subjects

A cohort of 50 ALS patients was included in the study. DNA was extracted from a blood sample in 19 patients and from a muscle tissue sample in 31 patients.

Of the patients, 24 were male and 26 female with a mean age at onset of symptoms of 62.4 years. The diagnosis of ALS, based on El Escorial criteria (29), was made between 2004 and 2011. Upper motor neuron (UMN) signs were evaluated clinically and based on findings divided in to probable and definite UMN signs (30) by neurologist. Neuropsychological assessment was performed when a patient was suspected to have cognitive decline. Six patients had FALS and 44 SALS. In all FALS cases and 17 SALS cases *SOD1* D90A mutation was excluded before their inclusion in the present study. A muscle biopsy was obtained from all patients as part of the routine diagnostic evaluation. Medical history and demographic data were collected from patient documents.

All the patients gave their written informed consent. The study was approved by the Institutional Review Board of Tampere University Hospital and performed in accord with the Helsinki declaration.

Mutation screening

Genomic DNA was extracted from venous blood or muscle tissue using Archive Pure DNA Blood Kit (5 Prime, Hamburg, Germany). The coding region of *TARDBP*, i.e. exons 2-6 were amplified by polymerase chain reaction (PCR Master Mix, Fermentas, St. Leon-Rot, Germany). Primer sequences were designed to include the entire exons and exon-intron borders with Primer3 version 0.4.0 (31). PCR products were sequenced using BigDye Terminator v3.1 kit on ABI3130xl automatic DNA sequencer system (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed using Chromas 1.45 (Griffith University, Southport, Queensland, Australia) and MACAW 2.0.5 (National Center for Biotechnology Information, Bethesda, MD, USA) software.

Results

DNA extracted from venous blood or from muscle tissue was used to screen for mutations in *TARDBP* gene in the 50 ALS patients. Clinical information from the 44 SALS and 6 FALS patients is summarized in Table 1. At the time of the analysis of the data, 12 patients were alive, 35 deceased, and of three patients there was no current information available. One of the patients was mechanically ventilated. The age of symptom onset ranged from 41.0 to 78.75 years and the age at the point of ALS-diagnosis from 42.25 to 79.25 years. The disease duration ranged from 0.75 to16.5 years for all patients and for the

Table 1. Clinical characteristics and TARDBP mutations in the Finnish ALS patients.

	Number of patients	Mean age at onset of symptoms, yrs	Mean age at diagnosis, yrs	Mean disease duration, yrs [a]	Mean disease duration, deceased, yrs	Upper motor neuron signs, probable[b] / definite[c]
All patients	50	62.4	64.4	3.3	2.5	9 / 41
Male	24	61.7	65.1	3.6	2.9	6 / 18
Female	26	62.0	63.6	3.1	2.7	3 / 23
FALS	6	60.5	62.2	4.5	3.0	0 / 6
SALS	44	62.4	64.7	3.2	2.5	9 / 35
Spinal onset	37	60.3	62.3	3.5	2.6	8 / 29
Bulbar onset	13	68.3	70.1	2.9	2.4	1 / 12
Identified variants in *TARDB*						
c.163C > A R55R	1					
c.715-75_715-74insT	2					
c.714+68_714+69insG	46					
c.715-126delG	42					

[a] From the onset of symptoms to exitus/ last follow-up; [b] Probable upper motor neuron signs: disproportionately active reflexes in weak and atrophic muscles; [c] Definite upper motor neuron signs: Hoffmann sign, Babinski sign, pathologic muscle stretch reflexes or spasticity.

35 deceased patients from 0.75 to 6.5 years. Nine patients had probable and 41 definite UMN signs at the time of diagnosis. Frontotemporal dementia was diagnosed in three patients, nonspecific dementia in one and mild cognitive decline in three patients.

No definitely pathogenic mutations could be identified in the genetic analysis of *TARDBP*. We identified a previously unknown silent mutation in exon 2 (c.163C > A R55R), and a deep intronic single nucleotide insertion in intron 5 (c.715-75_715-74insT). Neither of these is recorded in the international polymorphism database, dbSNP (32). According to the prediction program Human Splicing Finder version 2.4.1 the effect of these two new mutations on splicing cannot be excluded (33). To estimate the pathogenicity of these two previously unknown variations we screened 101 Finnish normal population controls for them. None of the control samples harboured the mutation c.163C > A (p.R55R) whereas, two control samples were heterozygous for intronic c.715-75_715-74insT. Two previously known common polymorphisms (c.714+68_714+69insG and c.715-126delG) in intron 5 were detected as well. The majority of patients 41/50 had both of these polymorphisms. Forty-six patients showed the common insertion polymorphism, c.714+68_714+69insG. Thirty-seven of these were homozygous and 9 heterozygous. The common deletion polymorphism, c.715-126delG, was found in 42 patients (33 homozygous and 9 heterozygous). Thus, these two known polymorphisms in intron 5 constitute the major allele in the Finnish population (Table 1).

Discussion

In this study we screened a cohort of 50 SALS and FALS patients for mutations in *TARDBP* gene, but we did not find any definitely pathogenic mutations. A previously unknown heterozygous silent mutation in exon 2 was identified in one patient. Silent mutations are likely to be insignificant. However, activation of cryptic splice sites is possible with silent mutations in coding sequences. Such an event cannot be excluded in our patient as the mutation in exon 2 was not detected in any of the 101 control samples. Two patients showed a heterozygous single nucleotide insertion mutation in intron 5. This mutation was another one not found in the database. However, the nucleotide insertion lies relatively deep in the intron and because it was found in two control samples it is unlikely to be pathogenic.

The incidence and prevalence of ALS in Finland are among the highest in the world outside Western Pacific (1, 34). Increasing incidence of ALS in Sweden and Norway has also been reported in the last few decades. These Nordic countries appear to show higher incidence than most other European countries. Explanations for this observed increase in incidence have ranged from aging population to improved diagnostics and better neurologic services. However, these factors alone could not account for the entire increase in incidence, and the real cause remains unclear (35, 36). Many studies have addressed the role of environment as contributory factor in ALS disease process and also as an explanation for increased incidence but none of the risk factors have been reported consistently (35-37). Genetic background could be more important aspect in Finland since the frequency of the new *C9orf72* gene mutation was reported to be higher in a Finnish cohort than in other similar European studies (17, 38).

The D90A allele of *SOD1* occurs with increased frequency in Finland and northern Sweden but it accounts for only a proportion of the high incidence of ALS in Finland (7, 39). A second and even more important cause of ALS in the Finnish population has been associated with chromosome 9p21 (40). Recently a hexanucleotide (GGGGCC) repeat expansion within *C9orf72* gene was identified as the cause of choromosome 9p21-linked ALS (17). This repeat expansion mutation is common in Finland: it was identified in 46.0% of FALS and 21.1% of SALS cases in Finnish population. In populations of wider European ancestry 38.1 % of FALS patients carried the same hexanucleotide mutation on a common haplotype background. Together with the *SOD1* D90A mutation, this repeat expansion explains 87% of familial ALS in Finland (17). Apart from these two causes of ALS, no other gene mutations have yet been identified in Finnish ALS patients.

A subgroup of frontotemporal dementias (FTD) is characterized by prominent TDP-43 pathology presenting another TDP-43 proteinopathy (41). Evidence shows that frontal executive deficits are found in half of ALS patients and obvious FTD in smaller group of patients (42). Our study was not designed to evaluate possible cognitive decline or behavioral symptoms in our patients, therefore, all patients were not routinely assessed by neuropsychologist. However, three patients were diagnosed with FTD and three patients with milder symptoms. Patients with ALS and FTD should be further screened for *C9orf72* gene (43).

In previous studies, the frequency of *TARDBP* mutations in ALS patients has been reported at 1-3 % (20). Studies on different populations show some variation of frequencies of *TARDBP* mutations in ALS patients. The occurrence was 0.5% in SALS and 0.6% in non-*SOD1* FALS patients in an English population (8) and 6.5% in non-*SOD1* FALS cases in a German cohort (44). Therefore, the size of our cohort may not be large enough to exclude *TARDBP* mutations as a very rare cause of ALS in Finland. We did not identify any definitely pathogenic mutations; however, pathogenicity of the silent mutation

is not excluded, although unlikely. In any case, based on our results *TARDBP* mutations do not appear to be a frequent cause of familial or sporadic ALS in Finland.

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