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Brief communication

Genetic analysis of *SS18L1* in French amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron disease including about 15% of genetically determined forms. A de novo mutation in the *SS18L1* (also known as *CREST* or *KIAA0693*) gene encoding the calcium-responsive transactivator and/or neuronal chromatin remodeling complex subunit has recently been identified by exome sequencing of 47 sporadic ALS trios. This Q388stop mutation deleting the last 9 amino acids was shown to impair activity-dependent dendritic outgrowth. A missense mutation (c.369T>G, p.Ileu123Met) was also found in 1 of 62 ALS families previously screened for other ALS-related genes and not carrying any mutation. To confirm the contribution of *SS18L1* to ALS, we sequenced the 11 coding exons and exon-intron boundaries in 87 familial ALS (FALS). We identified 2 variants: the c.660_668del, p.Gln222_Ser224del in a patient devoid of mutation in any ALS related genes and the c.790G>A, p.Ala264Thr in a patient carrying a p.Arg96Leu variant in the *OPTN* gene. As these variants were not found in Single Nucleotide Polymorphism databases and were absent from 180 controls they could be new *SS18L1* mutations causing ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an age-dependent, degenerative disorder of motor neurons that typically develops in the sixth decade and is uniformly fatal, usually within 5 years. It is caused by the progressive loss of motor neurons in the spinal cord, the cortex, and the brainstem. Most ALS cases are sporadic, whereas ~10% of the cases have a positive family history of familial ALS (FALS). This prevalence increases to 17%–23% in prospective studies with active investigation of genealogies (Andersen and Al-Chalabi, 2011). In the past 20 years, the list of ALS-linked genes has constantly grown and now includes *C9ORF72*, *SOD1*, *TARDBP*, *FUS*, *ANG*, *VAPB*, *DAO*, *OPTN*, *UBQLN2*, *VCP*, *SQSTM1*, and *PFN1*, encoding proteins involved in multiple pathways including RNA metabolism, oxidative stress, cytoskeleton organization, vesicular trafficking, ubiquitination, and protein degradation. To date, one-third of FALS causing genes remain unidentified. All the mutated genes in FALS have also been found in sporadic ALS (Andersen and Al-Chalabi, 2011). A recent study assessed the contribution of de novo mutations in ALS by sequencing the exome of 47 trios including ALS patients with an early age at onset and their unaffected parents (Chesi et al., 2013). A de novo mutation in the synovial sarcoma translocation gene on chromosome 18-like 1 (*SS18L1* also known as *CREST* and *KIAA0693*) introducing a premature termination codon in the last exon, which escaped the nonsense-mediated messenger RNA decay system and deleted the last 9 amino acids of the protein (c.1162C>T, p.Gln388*, Q388stop), was identified in 1 trio (Chesi et al., 2013). *SS18L1* encodes the calcium-responsive transactivator (CREST), a chromatin regulator gene, which is involved in activity-induced dendrite outgrowth. CREST was also shown to interact with the ALS-linked protein FUS (Chesi et al., 2013). The deletion of the last 9 amino acids of the protein disrupted the histone acetylase CREB-binding protein

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(CBP)-binding domain and inhibited activity-dependent dendrite outgrowth. In the same study, a missense mutation (c.369T>G, p.Ileu123Met) was also found in 2 patients from 1 out of 62 families with ALS previously excluded for all the known ALS related genes (Chesi et al., 2013). We aimed to confirm the contribution of this gene in our population of French FALS patients.

2. Methods

The 11 coding exons and exon-intron boundaries of *SS18L1* (ensemble reference sequence: ENSG00000184402) were sequenced by Sanger technique in 87 French familial ALS patients devoid of mutation in *C9ORF72*, *SOD1*, *TARDBP*, *FUS*, *VAPB*, *ANG*, *UBQLN2*, *VCP*, or *PFN1* (Millecamps et al., 2010b, 2012a, 2012b). Our FALS patients had also systematically been screened for *DAO*, *OPTN*, and *SQSTM1* (Millecamps et al., 2010a, 2011; Teyssou et al., 2013) mutations. However, patients carrying variants in these 3 genes were included in the present study as the pathogenicity of these variants remains unclear. Mean age of onset of the analyzed series was 60 years (standard error of the mean 1, range 33–80 years) and mean disease duration was 48 months (SEM 6, range 6–260 months). The number of the initial FALS population was 293. Control samples were obtained from 180 age-matched Caucasian individuals of French background. Polymerase chain reaction conditions for amplification of these exons are described in Table 1.

3. Results

We identified, at the heterozygote state, 2 variants. The c.660_668del and p.Gln222_Ser224del (in exon 6) in 1 affected man devoid of mutation in any of the previously reported ALS-related genes (Fig.1A). This patient had lower limb onset at the age of 77 years and died at 78 years after disease duration of 15 months. This patient had no cognitive impairment. The index case's brother had a similar phenotype: he started the disease at 80 years of age and died after 13 months disease course. Their parents had died at 72 years and 83 years from other medical causes (Fig. 1C). We also identified the c.790G>A, p.Ala264Thr in exon 7 (Fig. 1B), in a patient carrying the c.287G>T, p.Arg96Leu (R96L) variant in *OPTN*, we previously reported (Millecamps et al., 2011). This patient had lower limb onset at 56 years of age and 38-months disease duration. His mother died from ALS disease at the age of 76 years. For both families, the affected relatives were deceased so the segregation of these new *SS18L1* variants could not be established. Amino-acid residues affected by these modifications are relatively well conserved in mammals (Fig. 1D). As these variants were not found in the dbSNP short genetic variations, the 1000 genomes project, and the NHLBI ESP exome variant server databases and in our analysis of 180 control Caucasian individuals (360 chromosomes), they could be new *SS18L1* mutations. Thus in our genetic analysis, patients with variants in *SS18L1* account for 2.3% (2/87) of FALS patients devoid of mutation in main ALS related genes or 0.7% (2/293) of total FALS.

4. Discussion

The aim of this study was to confirm the contribution of the *SS18L1* gene to ALS in our population of French FALS patients. Mutations in this gene had previously been identified in a family with autosomal dominant transmission of the disease and in a family with a de novo mutational event.

We identified 2 variants in *SS18L1*. Taking into account that Chesi et al. (2013) identified 1 *SS18L1* mutation among 62 FALS of European descent the mutational frequency of this gene represents therefore, by now ~2% (3/149) of Caucasian FALS without known pathogenic mutation after pooling their study with ours (although it should be verified that both FALS populations had similar median of disease onset). Indeed, the late onset ALS presented by our index case and his brother contrasted with the early onset disease displayed by patients reported by Chesi et al. (Chesi et al., 2013). However, the variants we found were not in the same region of the protein. The first variant we identified is supposed to introduce an in-frame deletion of 3 amino acids (Gln, Gly, Ser) in the glycine rich domain of the protein. Interestingly, these residues belong to the predicted prion-like region of SS18L1, which is also present in other ALS-linked proteins that are prone to aggregate such as TDP-43 and FUS (Chesi et al., 2013; Gitler and Shorter, 2011).

The second variant is a substitution (c.790G>A, p.Ala264Thr). Two other Single Nucleotide Polymorphism are reported for the same amino acid (Ala264) in dbSNP short genetic variations database: the rs35943394 (that was found in 1 out of our 180 controls) and the rs369721048, which was not found in our control population. However, the variant we found is novel, absent from our control population, and not recorded in databases.

Interestingly, the patient with the c.790G>A, p.Ala264Thr variant in *SS18L1* also carried a variant in *OPTN* (c.287G>T, p.Arg96Leu), which is mutated in some ALS patients (Maruyama et al., 2010) and in adult onset primary open angle glaucoma (Rezaie et al., 2002). This novel R96L *OPTN* variant, which was not predicted to alter splicing and did not cause optineurin protein accumulation was not likely to be pathogenic and cause ALS disease (Millecamps et al., 2011). As we have now identified a variant in *SS18L1* (A264T) in this patient, we cannot rule out the possibility that co-occurrence of these 2 variants could contribute to the ALS phenotype. A growing number of genetic studies have similarly reported "double mutant" ALS patients with mutations concurrently affecting different ALS genes (Lattante et al., 2012;

Table 1
SS18L1 amplification conditions

Exons	Forward primer	Reverse primer	Annealing temperature	Fragment size (pb)	Specific notification
1	CGCCCAGCGCAGCCGGAGTATC	CAGGCGTGGGACGCGGGGAA	70	297	Solution Q (Qiagen) glycerol 5%
2	TAGATGTGTAGCGATGGCTG	GACTCTTGGGACGTATCTGG	65	239	
3	TCAGGCTGTCTTGTTCACACTTT	CTACCTGAGATGACATGCCCAC	65	271	
4	TGGCTGCTGATTACGAACATTGA	CTGTGGCTACCCCAGGAGGA	65	424	
5	ACTTGAAGGGAAATTGGGGTGT	AACGTGCATGTGTCAAGGGTCT	60	337	
6	GGGCGCAGGAGGTAGTTGGGT	AGGCTCCCCGAAGAGAAGCACA	65	334	
7	CCCAGTGAGCGAGCAGGTCCTCT	CCACACCCTCCTTGCCCTCCTT	65	261	
8	GCATGGTTTTGGGGAACACGG	AGGGACTCACTGAAGGCAGGCAC	65	345	
9	CAATGGATTTGGTTTTGGGA	GGTACTGAGCACGTACCCATGT	60	309	
10	AAGAAAGAGGTGTCCGTTTTG	GAAATCTTCATTATGTCTCGACAG	60	246	
11	TGGACGTCTGTCTTCCTTTCA	ACCAAAAGTATCACACCACCAA	60	300	