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SHORT REPORT

Genetic analysis of *SIGMAR1* as a cause of familial ALS with dementia

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron diseases (MND), while frontotemporal lobar degeneration (FTLD) is the second most common cause of early-onset dementia. Many ALS families segregating FTLD have been reported, particularly over the last decade. Recently, mutations in *TARDBP*, *FUS/TLS*, and *C9ORF72* have been identified in both ALS and FTLD patients, while mutations in *VCP*, a FTLD associated gene, have been found in ALS families. Distinct variants located in the 3′-untranslated region (UTR) of the *SIGMAR1* gene were previously reported in three unrelated FTLD or FTLD–MND families. We directly sequenced the coding and UTR regions of the *SIGMAR1* gene in a targeted cohort of 25 individual familial ALS cases of Caucasian origin with a history of cognitive impairments. This screening identified one variant in the 3′-UTR of the *SIGMAR1* gene in one ALS patient, but the same variant was also observed in 1 out of 380 control chromosomes. Subsequently, we screened the same samples for a *C9ORF72* repeat expansion: 52% of this cohort was found expanded, including the sample with the *SIGMAR1* 3′-UTR variant. Consequently, coding and noncoding variants located in the 3′-UTR region of the *SIGMAR1* gene are not the cause of FTLD–MND in our cohort, and more than half of this targeted cohort is genetically explained by C9ORF72 repeat expansions.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron diseases (MND), with an incidence of 1–2/100 000 person-years,[1] while frontotemporal lobar degeneration (FTLD) is the second most common cause of early-onset dementia with an incidence of 3–5/100 000 person-years.[2] Interestingly, up to 50% of ALS patients are deemed likely to develop some cognitive impairments.[3] In 2006, the TDP-43 protein was found to be an important constituent of aggregates in neurons of MND–FTLD patients,[4] which suggested a common pathological pathway for the two conditions. Mutations in the *TARDBP* gene encoding the TDP-43 protein,[5,6] and the *FUS/TLS* gene encoding the FUS protein[7,8] been identified in both ALS and FTLD patients, while mutations in ALS cases recently reported in *VCP*,[9] a gene in which mutations were previously associated with FTLD.[10] Furthermore, neurons of ALS and FTLD patients both display a nuclear clearing and cytoplasmic sequestration of normal cellular TDP-43 or FUS proteins,[11] which are encoded by genes that, when mutated, are estimated to account for up to 5–10% of all ALS cases.[6,7] Twelve families linked to a locus on chromosome 9p have been reported to have members with either MND or FTLD, while a few members displayed both phenotypes.[12–17] The causative pathogenic hexanucleotide repeat in the gene *C9ORF72* was recently identified by two different groups.[18,19] One of these reports found that this repeat expansion explains ∼3% of sporadic FTD, 11.7% of

familial FTD, 4.1% of sporadic ALS, and 23.5% of familial ALS cases.[18] The second report shows that the repeat expansion is detectable in about 50% of familial and 20% of sporadic Finnish cases, in more than one third of familial cases of wider European ancestry, and in more than 29% of the Finnish FTD population among which 36% have a family history.[19] In addition, recently, a variant in the 3′-untranslated region (UTR) of the *SIGMAR1* gene, encoding a receptor protein having an important role in various cellular functions, was reported to segregate in a FTLD–MND pedigree.[20] Two additional 3′-UTR variants were identified by the same group in two unrelated FTLD families thought to be linked to the chromosome 9p locus, but no segregation could be observed in these. Another group recently conducted homozygosity mapping in a large consanguineous family with six members affected with juvenile ALS. They identified a *SIGMAR1* missense mutation in all affected individuals.[21] Considering the finding of variants in the *SIGMAR1* gene and the recent publication of hexanucleotide repeat expansions in *C9ORF72* located in the same chromosomal region, we wanted to evaluate the potential contribution of *SIGMAR1* variants in ALS patients of European descents. We selected 25 individual familial ALS cases with a family history of cognitive impairments, and directly sequenced the entire coding and UTR regions of the *SIGMAR1* gene. We also evaluated this specific targeted cohort for *C9ORF72* repeat expansions.

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Received 27 December 2011; revised 15 May 2012; accepted 21 May 2012; published online 27 June 2012

MATERIALS AND METHODS

Standard Protocol Approval, Registration, and Patient Consents

Protocols were approved by the ethics committee on human experimentation of the Center Hospitalier de l'Université de Montréal. All patients gave written informed consent after which patient information and blood were collected.

Subjects

Clinical information from 260 familial ALS cases of Caucasian origin not mutated in *SOD1*, *TARDBP*, or *FUS* was analyzed; 25 families were found to have a history of cognitive impairments. One affected member from each family was selected for sequencing the entire coding and UTR regions of the *SIGMAR1* gene (NM_005866.2). In addition, 190 control participants were sequenced for one fragment encompassing the first 396 bp of the 3′-UTR, in which one novel substitution was identified in one familial ALS patient. Moreover, the same patients were assessed for the presence of expanded hexanucleotide repeat in *C9ORF72* (NM_001256054.1).

Gene screening

Six sets of primers were used for each sample to amplify the open-reading frame of the *SIGMAR1* gene (Table 1). The PCR products containing the exons included a minimum of 50 bp from each of the flanking introns. Primers were designed using the ExonPrimer software from the UCSC human genome browser website (http://genome.ucsc.edu/cgi-bin/hgGateway). Amplification was conducted by PCRs using the AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA) as per manufacturer's instructions. PCR products were amplified with the same procedure of direct sequencing in patients and controls, and were sequenced at the Genome Quebec Innovation Center (Montréal, Québec, Canada) using a 3730XL DNA analyzer (Montréal). Mutation survey or software (version 3.10) was used for mutation detection analyses (SoftGenetics, State College, PA, USA).

Hexanucleotide repeat analysis

We performed a repeat-primed PCR assay using the FastStart PCR Master Mix (Roche, Indianapolis, IN, USA) using the reported optimized conditions.[18,19] PCR products were analyzed on an ABI 3730 sequencer with GeneMapper software version 4.0 (Applied Biosystem, Carlsbad, CA, USA).

RESULTS AND DISCUSSION

Only one variant (c.672* 43G > T) located in the 3′ UTR of the *SIGMAR1* gene was identified in one patient. The substituted guanine is well conserved through different species. Other affected family members were not available for testing, but the variant was not present in six other unaffected family members. However, after testing 190 matched controls, the variant was identified in one control, suggesting that the substitution is not causative of ALS in the family.

No other unknown variants were identified in the 25 patients tested. Interestingly, after performing the repeat-primed method, we found that 13 patients actually carried a hexanucleotide repeat expansion in *C9ORF72*. This represents 52% of the total tested. The cause of the disease for the remaining 12 patients is still unexplained genetically.

The *SIGMAR1* gene was a positional candidate based on linkage in an Australian MND–FTLD family.[17] The c.672*51G > T 3′-UTR substitution in the *SIGMAR1* gene was identified in the linked family after the sequencing of about 200 candidate genes in the chromosome 9p candidate region. The nonpolymorphic nucleotide change segregated with the disease haplotype of this Australian pedigree. The variant was not identified in 1269 controls. In addition, c.672*26C > T and c.672*47G > A substitutions also located in the 3′-UTR of *SIGMAR1* were identified in different FTLD pedigrees of Australian and Polish origins, respectively. These variants were also absent in matched controls. A modification in *SIGMAR1* gene expression was found in the Australian-origin family, while a small expression difference was observed for the other two families, each with only one patient actually carrying the variant. It was also shown that modulating the expression of *SIGMAR1* influences the cellular localization of TDP-43 and FUS, and it was suggested that the 3′-UTR of *SIGMAR1* variant causes a pathogenic alteration of *TARDBP* and *FUS* expression levels.[20] Considering that the *C9ORF72* hexanucleotide repeat expansion was not reported when the *SIGMAR1* mutations were identified and that the genes are close to each other on chromosome 9p, it is possible that the *SIGMAR1* variants identified in the first report actually segregated with *C9ORF72* expansions. The finding of a 3′-UTR variant in our specifically targeted cohort permitted to test and confirms this hypothesis.

Further studies in different populations will help to evaluate the contribution of the sigma nonopioid intracellular receptor 1 protein in MND and/or FTLD, and future studies need to address the reported effects on expression levels of the different proteins involved. However, based on our study, it can be concluded that coding mutations or UTR variants in *SIGMAR1* are not a cause of ALS in our cohort of Caucasian ALS families with a history of cognitive impairments, but that repeat expansions in *C9ORF72* genetically explain a significant proportion of the same cohort.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Table 1 List of primers

Localization	Forward primer	Reverse primer	Size of fragment (bp)
5′-UTR-x1	5′-GCTCCGATT GGTCAGGG-3′	5′-ACGATCAGAC GAGAGAAGGC-3′	456
x2–x3	5′-GGCAGTACG CTGGTGAGC-3′	5′-GAGGAAGGG AACCATGAGG-3′	683
x4	5′-GCCCAGTGA GGTAGGGC-3′	5′-GCTCATACAGC AGGAACTCAG-3′	497
3′-UTR_1	5′-CCAGGACTTC CTCACCCTCTT-3′	5′-ATAATACCCTC CCCCATCCTT-3′	490
3′-UTR_2	5′-CATGGGAACA AATGAGACACA-3′	5′-CGCTGACTTC AAGCATTCTT-3′	478
3′-UTR_3	5′-TCAACCCAGC AGCAATTTGA-3′	5′-CCATGAATCAC ACAGCAAGAG-3′	580

Abbreviation: UTR, untranslated region.

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