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Negative results

Clinical and genetic analysis of *MAPT*, *GRN*, and *C9orf72* genes in Korean patients with frontotemporal dementia

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ABSTRACT

The hexanucleotide repeat expansion (GGGGCC) in chromosome 9 open-reading frame 72 (*C9orf72*) and mutations in the microtubule-associated protein tau (*MAPT*) and progranulin (*GRN*) genes are known to be associated with the main causes of familial or sporadic amyotrophic lateral sclerosis and frontotemporal dementia (FTD) in Western populations. These genetic abnormalities have rarely been studied in Asian FTD populations. We investigated the frequencies of mutations in *MAPT* and *GRN* and the *C9orf72* abnormal expansion in 75 Korean FTD patients. Two novel missense variants of unknown significance in the *MAPT* and *GRN* were detected in each gene. However, neither abnormal *C9orf72* expansion nor pathogenic *MAPT* or *GRN* mutation was found. Our findings indicate that *MAPT*, *GRN*, and *C9orf72* mutations are rare causes of FTD in Korean patients.

1. Introduction

Frontotemporal dementia (FTD) is a presenile dementia syndrome characterized by impairment in behavior, language, and cognition associated with focal degeneration of the frontal and anterior temporal lobes. FTD consists of 3 subtypes: behavioral variant FTD (bvFTD) presenting with abnormal frontal behavioral symptoms and 2 language variants, semantic dementia (SD) with semantic anomia and progressive nonfluent aphasia (PNFA) with nonfluent and agrammatic speech (Neary et al., 1998). Up to 15% of

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patients with FTD have comorbid motor neuron disease (Lomen-Hoerth et al., 2002).

Nearly 40% of patients with FTD have a positive family history of dementia, and about 10% have an autosomal dominant pattern of inheritance (Rohrer et al., 2009). Seven genes have so far been recognized to cause familial FTD, including microtubule-associated protein tau (*MAPT*) (Hutton et al., 1998), progranulin (*GRN*) (Baker et al., 2006), valosin-containing protein (*VCP*) (Watts et al., 2004), chromatin-modifying protein 2B (*CHMP2B*) (Skibinski et al., 2005), TAR DNA-binding protein (*TARDBP*) (Benajiba et al., 2009), fused in sarcoma (*FUS*) (Van Langenhove et al., 2010), and chromosome 9 open-reading frame 72 (*C9orf72*) (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Of these, mutations in the *MAPT* and *GRN* genes are found in approximately 50% of the familial FTD cases and a recently identified *C9orf72* hexanucleotide repeat expansion accounts for about 10%–30% of familial FTD (Seelaar et al., 2011; van Swieten and Grossman, 2012). In contrast, 4 other genes, *VCP, CHMP2B, TARDBP*, and *FUS*, are implicated in <5% of all FTD cases.

Even though it is generally known that FTD has a strong genetic component, there may be geographic and ethnic differences in its prevalence. Most studies about familial FTD have been reported in North America and Europe, whereas the limited number of studies from Asia have suggested a much lower incidence of familial FTD (Ikeda et al., 2004; Kang et al., 2010; Ren et al., 2012). So far, only 1 Korean familial FTD has been reported (Kim et al., 2011).

Thus, in this study, we screened *MAPT, GRN*, and *C9orf72* hexanucleotide repeat expansions, which are considered to be the main genetic causes of FTD, in the Korean FTD population to investigate whether there are any differences in mutation frequencies between Western and Asian populations.

2. Methods

2.1. Patients

Between October 2012 and May 2013, patients were prospectively recruited from 11 neurology clinics across Korea. All the patients enrolled in this study met the research criteria for FTD proposed by Knopman et al. (2008) and were subclassified into bvFTD, SD, and PNFA. Patients who had clinical and electrophysiological evidence of motor neuron disease (MND) were also enrolled as FTD-MND, regardless of the clinical subtype of FTD. This study has been conducted as part of the Clinical Research Center for Dementia of South Korea–Frontotemporal dementia registry study. Thus, all participants were registered in the Clinical Research Center for Dementia of South Korea–Frontotemporal dementia registry. The institutional review boards at all participating centers approved this study, and informed consent was obtained from patients and caregivers.

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit following the manufacturer's instructions (Promega, Madison, WI). All coding exons and their flanking introns of the *MAPT* and *GRN* genes were amplified using primer sets designed by the authors (available on request). The polymerase chain reaction was performed with a thermal cycler (model 9700; Applied Biosystems, Foster City, CA) as follows: 32 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds. After treatment of the amplicon (5 μL) with 10 U shrimp alkaline phosphatase and 2 U exonuclease I (USB Corp, Cleveland, OH), direct sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) on an ABI Prism 3730*xl* genetic analyzer (Applied Biosystems). The obtained sequences were analyzed using Sequencher software (version 4.10.1; Gene Codes, Ann Arbor, MI) and compared with the reference sequences for *MAPT* (NM_016835.4) or *GRN* (NM_002087.2) genes. To describe sequence variations, we followed the guidelines of the Human Genome Nomenclature Committee that "A" of the ATG translation start site was numbered +1 for the DNA sequence and the first methionine was numbered +1 for the protein sequence. Any novel variants were tested on 700 control chromosomes by sequencing. The *C9orf72* repeat expansion was tested using a 2-step polymerase chain reaction protocol, as described previously (Jang et al., 2013). First, the number of hexanucleotide repeats was determined in all patients using the genotyping primers (chr9:27563580F and chr9:27563465R; DeJesus-Hernandez et al., 2011). Then patients found to have a homozygous pattern were further analyzed using the repeat-primed polymerase chain reaction method (Renton et al., 2011). Two samples from the NINDS repository (ND06769 and ND08544) were purchased from Coriell Cell Repositories (Camden, NJ) and tested for the purpose of quality control.

2.3. Bioinformatics analysis

The functional consequences of the missense variants of unknown significance were predicted in silico using PolyPhen-2 (Adzhubei et al., 2010) and SIFT (Kumar et al., 2009) software. In PolyPhen-2, a mutation is classified as "probably damaging" if it has *q* probabilistic score >0.15; remaining variants are classified as benign. Using SIFT, scores ranging from 0 to 1 are obtained to represent the normalized probability that a particular amino acid substitution will be tolerated. SIFT predicts that substitutions with scores less than 0.05 are deleterious.

3. Results

3.1. Clinical findings

A total of 75 patients (42 men and 33 women) with FTD consisted of 22 bvFTD, 40 SD, 11 PNFA, and 2 FTD-MND patients. Two patients with FTD-MND showed abnormal behaviors as presenting symptoms. The mean age was 68.0 ± 9.2 years, and mean age at onset was 63.3 ± 9.7 years. The mean duration of follow-up was 5.0 ± 3.6 years. Detailed demographic data of the 4 subtypes are summarized in Table 1. A history of dementia in first-degree relatives (parents and siblings) was found in 6.7% (5 of 75) of patients with FTD.

3.2. Genetic findings

Sequencing of *MAPT* and *GRN* genes identified no known pathogenic mutation but only 2 novel missense variants of unknown significance in each gene: 1 was a heterozygous *MAPT* variant (c.530A > T; p.Asp177Val) and the other was a *GRN* variant (c.1138C > G; p.Gln380Glu). The *MAPT* p.Asp177Val variant was predicted to be possibly damaging by PolyPhen-2 but tolerated by SIFT, whereas the *GRN* p.Gln380Glu variant was predicted to be benign and tolerated by PolyPhen-2 and SIFT analysis, respectively (Table 2). However, neither variant was found in 700 control chromosomes. None of the patients had the *C9orf72* repeat expansion (Table 3), whereas the expected sawtooth pattern with a 6-bp periodicity was observed in the 2 samples from the NINDS repository (data not shown). The most common allele was 2 hexanucleotide repeats (105 of 150; 70%), followed by 6 (19 of 150; 12.7%) and 8 repeats (12 of 150; 8%).

4. Discussion

In our FTD population, no patient with the *C9orf72* repeat expansion was detected. Because the *C9orf72* hexanucleotide