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Neurobiology of Aging 33 (2012) 1843.e19–1843.e24

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Novel deletion mutations of *OPTN* in amyotrophic lateral sclerosis in Japanese

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Received 28 July, 2011; received in revised form 10 December 2011; accepted 28 December 2011

Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by selective motor neuron death in the brain and spinal cord. Many disease genes for ALS have been identified; however, each disease gene is responsible for very small fractions of ALS. Recently, mutations of the gene encoding optineurin (*OPTN*) are reported in familial and sporadic ALS. *OPTN* is also responsible for a small number of ALS, 3.8% of familial and 0.29% of sporadic ALS in Japanese. The low prevalence may be an underestimation due to incomplete screening of the mutation. To examine *OPTN* mutations more extensively, we screened the *OPTN* deletions using a quantitative PCR system. We examined 710 Japanese ALS subjects who had previously been found to have no *OPTN* mutations by a screening using a PCR-direct sequence strategy. We identified 3 kinds of deletions in 5 patients; one was homozygous, and the remaining were heterozygous. All deletions occurred due to the Alu-mediated recombination and are expected to result in null alleles. Our results suggest that the *OPTN* deletion mutation in ALS is not infrequent and the prevalence of the *OPTN* mutation in Japanese sporadic ALS is considerably high.

Keywords: Amyotrophic lateral sclerosis; Optineurin; Deletion; Alu-mediated recombination

1. Introduction

Optineurin is a 577 amino acids multifunctional protein involved in NF-κB regulation, vesicular trafficking, immune response and transcription regulation (Chalasani et al., 2009). Optineurin is encoded by *OPTN*, whose mutations cause primary open-angle glaucoma (POAG); 3 disease-causing mutations (E50K, 691_692insAG, and R545Q) and one nonsynonymous substitution (M98K) have been described (Rezaie et al., 2002).

Recently, Maruyama et al. (2010) preformed a homozygosity mapping in consanguineous families with amyotrophic lateral sclerosis (ALS), and identified *OPTN* as a causative gene for ALS. They examined a total of 689 Japanese ALS subjects (92 familial ALS (fALS), and 597 sporadic ALS (sALS)), and found 3 types of *OPTN* mutations, a homozygous deletion of an exon; a homozygous nonsense mutation (p.Q398X) and a heterozygous missense mutation. We also examined *OPTN* mutations in Japanese ALS and found 2 kinds of missense mutations in one fALS and 2 sALS patients (Iida et al., 2012). Taken together, the results of the 2 reports show that the *OPTN* mutation is found in 3.8% of fALS and 0.29% of sALS (Iida et al.,

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10.1016/j.neurobiolaging.2011.12.037

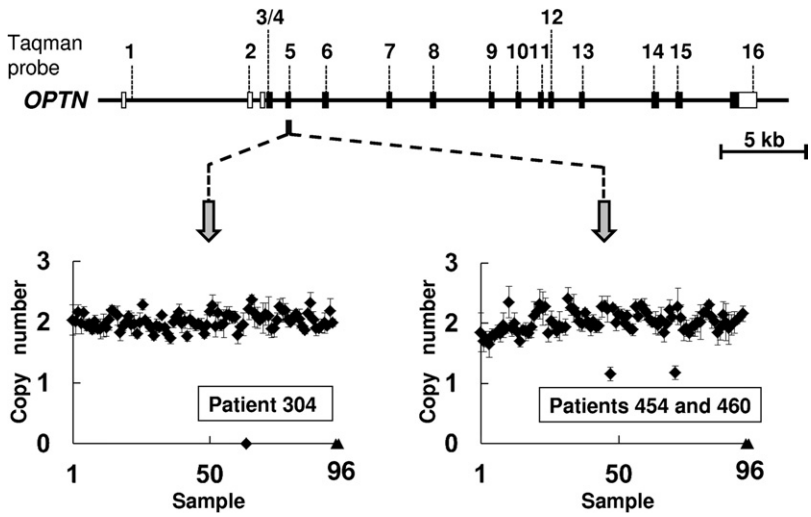

Fig. 1. TaqMan qPCR assay for detection of *OPTN* deletions. Top: Genomic structure of the *OPTN* gene and the position of the TaqMan probes for the assay. Open rectangles indicated the 5′- and 3′-untranslated regions, and filled rectangles the coding regions. Bottom: Scatter-plot analysis views of the assay for the exon 5 probe that indicate homozygous (left) and heterozygous (right) deletions.

2012). The low prevalence is quite similar to other ALS disease genes. In ALS genes other than *OPTN, SOD1* is the most prevalent in the Japanese. Its mutational frequency in sALS is 1.6% (7/439) (Akimoto et al., 2011). *TARDBP* is the second most prevalent ALS gene in the Japanese, and its mutational frequencies in fALS and sALS are 3.0% and 0.29%, respectively (Iida et al., 2010). However, these figures may be an underestimation due to incomplete screening of the mutation, because the mutation screenings in both studies were based on a PCR-direct sequence method, in which it is easy to overlook heterozygous deletions. Notably, Maruyama et al. (2010) reported a deletion mutation.

To examine *OPTN* mutations more extensively, we screened the *OPTN* deletion by quantitative PCR covering *OPTN* exons. We have identified 5 deletions in 710 ALS subjects who had previously been examined for *OPTN* mutations by a PCR-direct sequence method with negative results.

2. Methods

A total of 710 Japanese ALS patients (685 were sporadic and 25 familial) were examined in this study. All DNA samples were obtained from the Biobank Japan project (Nakamura, 2007). Their clinical information was described previously (Iida et al., 2012). They had previously been examined for *OPTN* mutations by a PCR-direct sequence method and were found to have no mutations (Iida et al., 2012). The control subjects consisted of a general population recruited through several medical institutions in Japan as previously described (Nakajima et al., 2010). Written informed consent was obtained from all the subjects. The ethical committees at the participating institutions approved this project.

To examine the copy number of *OPTN*, we employed a quantitative PCR (qPCR) method as previously described (Hosono et al., 2009). A total of 15 TaqMan probes for the qPCR covering all of 16 *OPTN* exons were designed with the aid of Primer Express software v2.0 (Applied Biosystems) (Fig. 1, top). All but 2 probes were designed in the exons. Probe 1 was designed not in exon 1 but in intron 1 because of the high GC content of exon 1. Probe 3/4 that should cover exons 3 and 4 was designed in exon 4, because the 2 exons were very near (only 96 bp apart). The RNase P gene was used as a reference gene (Applied Biosystems). All assays were performed with TaqMan Universal PCR Master Mix (Applied Biosystems) according to the recommended protocol.

The breakpoint sequences of patients were determined by direct sequencing of PCR amplicons using primer sets described in Supplementary Table 1. The 3730xl DNA analyzer (Applied Biosystems) was used according to a standard protocol. The breakpoints were determined by comparing the sequences of the patients with reference sequences. Repeat sequences were examined by the RepeatMasker program (www.repeatmasker.org/).

3. Results

We screened the copy number abnormalities of *OPTN* using a system composed by 15 sets of qPCR assays based on the TaqMan platform. We found 3 kinds of deletions in 5 among 710 ALS patients (Table 1): one was known (Maruyama et al. 2010) and homozygous; 4 were novel and heterozygous. All deletions were detected in sporadic ALSs and were not detected in 470 unrelated Japanese controls. All deletion breakpoints were in Alu repeats, suggesting that they occurred due to the Alu-mediated recombination. The