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p62/SQSTM1 analysis in frontotemporal lobar degeneration

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ABSTRACT

Mutations in the gene p62/SQSTM1 have been reported as a relatively rare cause of frontotemporal lobar degeneration (FTLD). To establish whether this was the case for cases of FTLD from the United Kingdom, we sequenced the sequenced the entire open reading frame of this gene in a large cohort of patients. We identified 3 novel mutations in p62/SQSTM1 in 4 patients. One of these was a premature stop codon that removed the last 101 amino acids of the protein that presumably has a negative effect on protein function. Another mutation was also found in a case with a repeat expansion mutation in C9orf72 confirmed by Southern blot. These findings confirm a role of p62/SQSTM1 as a cause of FTLD.

1. Introduction

Frontotemporal lobar degeneration (FTLD) is now recognized as the second most common form of early-onset dementia. The 3 most common clinical syndromes associated with FTLD are behavioral variant frontotemporal dementia, semantic dementia, and progressive nonfluent aphasia (Neary et al., 1998). Neuronal pathologic inclusions associated with FTLD are composed of the protein TDP-43, tau, or occasionally FUS (Mackenzie et al., 2010). FTLD can also co-occur with amyotrophic lateral sclerosis (ALS) and sometimes parkinsonism.

Up to 40% of patients with FTLD report a family history of similar disease, indicating that genetics plays a major defining role in the etiology of this disease. In recent years, there has been much progress in understanding genetic causes of FTLD. Until recently, the main known genetic risks for familial FTLD were associated with mutations in MAPT (Hutton et al., 1998; Spillantini et al., 1998) and progranulin (Baker et al., 2006; Cruts et al., 2006). However, it is now known that the most common genetic cause of FTLD is a hexanucleotide repeat expansion in a noncoding region of the gene, C9orf72 (DeJesus-Hernandez et al., 2011; Renton et al., 2011). This particular mutation similarly is a major cause of ALS demonstrating that these 2 conditions are part of a disease spectrum. This had long been considered to be so given that about 50% of cases of FTLD, and around 85% of cases of ALS, demonstrate TDP-43-positive pathologic inclusions in their brains.

Likewise, ALS can be inherited and numerous genes causing autosomal dominant disease have been identified, including SOD1, TARDBP, and FUS in addition to C9orf72 (Conte et al., 2012). Recently, mutations in the gene p62/sequestosome1 (p62/SQSTM1) have been claimed to be associated with ALS and FTLD (Chen et al., 2013; Hirano et al., 2013; Kwok et al., 2013; Le Ber et al., 2013; Rubino et al., 2012; Shimizu et al., 2013; Teyssou et al., 2013). p62 is a ubiquitin-binding protein involved in protein homeostasis and is also present in the TDP-43 pathologic neuronal inclusions in both FTLD and ALS. In addition, mutations in p62/SQSTM1 are major causes of Paget disease of bone (Michou et al., 2006). How certain mutations in p62/SQSTM1 can lead to neurodegeneration, whereas other lead to Paget disease in the absence of any apparent neuronal dysfunction, is currently unclear. Given the shared etiology between FTLD and ALS, we sequenced the whole coding open reading frame of p62 in a large cohort of FTLD patients from the North West of England to establish to what extent mutations in p62/sequestosome-1 can also be a cause of FTLD.

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Table 1
A list of the known variants detected in our cohort with their relative frequency in our patient population.

Exon	RS number	Base/AA change	MAF in local controls
5	rs11548633	AAG: Lys to GAG: Glu	0.00027
6	rs4797	AGG to AGA; silent Arg	0.477
6	rs4935	GAC to GAT; silent Asp	0.49
6	rs145001811	CAC to CAT; silent His	0.00134
6	rs55793208	GAG: Glu to GAC: Asp	0.0174
6	rs56092424	TCC to TCT; silent Ser	0.01474
7	rs146164139	TCG to TCA; silent Ser	0.0068
8	rs104893941	CCG: Pro to CTG: Leu	0.00131
8	rs143511494	GGC: Gly to AGC: Ser	0.00131

Key: AA, amino acid; MAF, minor allele frequency.

2. Methods

2.1. Patients

The study group comprised 465 consecutive patients, 42% men and 58% women, referred to the Cerebral Function Unit, University of Manchester, who were diagnosed with one of the clinical syndromes associated with FTLD (Neary et al., 1998; Rascovsky et al., 2011). All patients had undergone clinical and neuropsychological assessments within the Cerebral Function Unit, and in 1 case the diagnosis had been pathologically confirmed at postmortem. Patients' mean age at onset of symptoms was 61 years (30–82 years). A positive family history of dementia in a first degree relative was recorded in 40% of cases. Controls, usually spouses of affected patients (n = 525), were neurologically normal and had a mean age of 59 years (36–79 years) at time of sampling.

2.2. Immunohistochemistry

This was performed as described previously (Mann et al., 2013).

2.3. p62/SQSTM1 sequencing

All exons of p62/SQSTM1 were amplified as previously described (Le Ber et al., 2013). Sequence analysis was performed using an ABI3730.

2.4. Genotyping analysis

Single-nucleotide polymorphisms were genotyped using the appropriate Taqman assay (ABI) and genotyped using ABI7900HTS. Southern blotting was performed as described in Mann et al. (2013).

3. Results

By sequencing the entire open read frame of p62/SQSMT1 in our FTLD cohort identified a range of previously known SNPs (Table 1). In addition, we identified 3 novel mutations in 4 patients with FTLD (Table 2). These mutations were absent from dbSNP, 1000 Genomes Project, and 525 local control samples, the latter genotyped using taqman. Two apparently unrelated patients shared the same R183C mutation. The patient with the G351A mutation, with a SIFT score of 0.01, also had a confirmed repeat expansion in C9orf72 with approximately 2500 repeats (Fig. 1). This case came to autopsy and had an unremarkable FTLD-TDP type B pathology. In addition, this case also exhibited dipeptide repeat (DPR) inclusions that were positive for all 5 DPR antibodies(Mann et al., 2013), which are normally associated with a C9orf72 expansion (Fig. 2). The other mutation (W321X) introduced a premature stop codon that removes the final 101 amino acids from the protein, including the

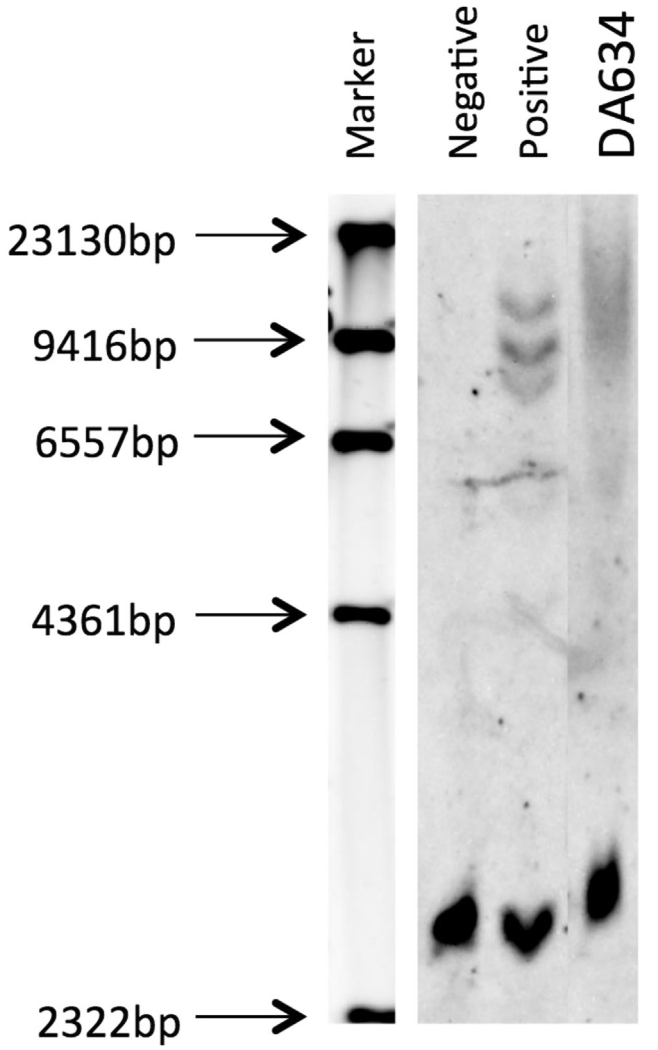

Fig. 1. Southern blot confirming a repeat expansion in C9orf72 in case DA634. The figure represents ladder, negative control, lymphoblastoid cell line positive control; and case DA634.

Table 2
Summary of clinical features of patients with novel variants in p62.

Case	Sex	Age at onset (y)	Diagnosis	Family history	Genetic variation	Sift value
DA275	M	65	SD	AD brother	R183C	0.03
DA266	M	68	CBS	Father	R183C	0.03
DA443	F		bvFTD	Schizophrenia father and brother	W321X	Loss of last 101 AA
DA634	F	52	bvFTD	bvFTD mother and sister	G351A	0.01

Key: AD, Alzheimer's disease; bvFTD, behavioral variant frontotemporal dementia; CBS, corticobasal syndrome; SD, semantic dementia.