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MAPT gene duplications are not a cause of frontotemporal lobar degeneration

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Abstract

Recurrent deletions of the 17q21.31 region encompassing the microtubule-associated protein tau (*MAPT*) gene have recently been described in patients with mental retardation. This region is flanked by segmental duplications that make it prone to inversions, deletions and duplications. Since gain-of-function mutations of the *MAPT* gene cause frontotemporal lobar degeneration (FTLD) characterized by deposition of tau protein, we hypothesize that *MAPT* duplication affecting gene dosage could also lead to disease. Gene dosage alterations have already been found to be involved in the etiology of neurodegenerative disorders caused by protein or peptide accumulation, such as Alzheimer's and Parkinson's diseases. To determine whether *MAPT* gene copy number variation is involved in FTLD, 70 patients with clinical diagnosis of FTLD and no *MAPT* mutation (including 12 patients with pathologically proven tau-positive FTLD) were screened by using multiplex ligation probe amplification (MLPA) with specific oligonucleotide probes. No copy number variation in the *MAPT* gene was observed in cases. Although our study was limited by the relatively small number of patients, it does not support the theory that chromosomal rearrangements in this region are a cause of FTLD.

Keywords: Frontotemporal lobar degeneration; *MAPT*; Segmental duplication; Copy number variation; Tau

Following the 1998 consensus criteria for frontotemporal lobar degeneration (FTLD) diagnosis, three distinct clinical syndromes have been defined: frontotemporal dementia (FTD), semantic dementia (SD) and progressive nonfluent aphasia (PNFA) [13]. Definitive diagnosis of FTLD requires pathological examination, which reveals tau-positive inclusions in approximately half of the cases [5,8]. Mutations in different genes have been reported to cause FTLD, although only mutations in the gene of the microtubule-associated protein tau (*MAPT*) are associated with tau deposits in the neuropathological examination [12]. Over-expression of human tau in glia and neurons of mice recapitulates key features of human FTLD with accumulation of abnormal tau aggregates with increasing age. Recently, tau-negative FTLD has been found to be caused by null mutations in the progranulin gene (*PGRN*) [1,3]. However, a significant proportion of both sporadic and familial cases do not present *MAPT* or *PGRN* gene mutations, which suggests that other genes and/or mutational mechanisms, such as *MAPT* duplication, could be involved in the etiology of FTLD. Consequently, gene dosage alterations have already been implicated in the etiology of neurodegenerative disorders caused by protein or peptide accumulation: (1) α-sinuclein gene duplication or triplication has been found in some cases of Parkinson's disease [21]; and (2) amyloid precursor protein (*APP*) gene duplication has been reported as a cause of Alzheimer's disease [16].

The 17q21.31 region encompassing the *MAPT* gene has a complex genomic architecture and is flanked by three highly homologous blocks of segmental duplications or low-copy repeats [3] (Fig. 1). Segmental duplications are hotspots for chromosomal rearrangements such as deletions, duplications

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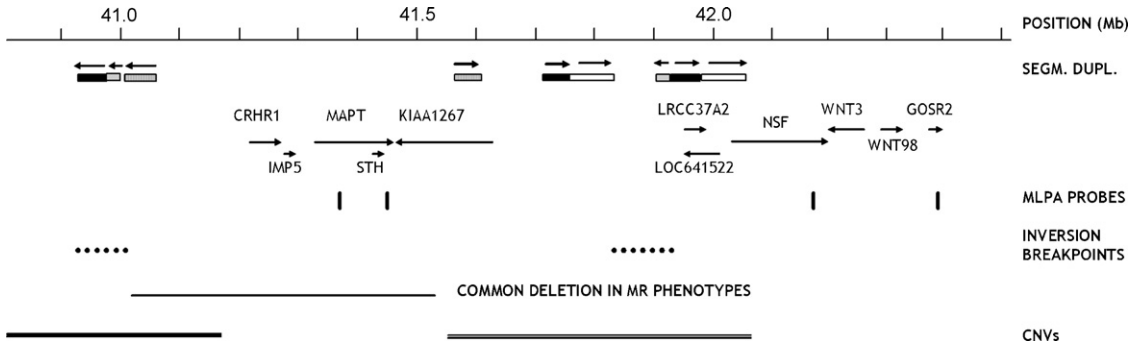

Fig. 1. Transcriptional map and genomic architecture of the 17q21.31 region. Segmental duplications are depicted as rectangles with different designs indicating shared homology, while the horizontal arrows show their relative orientation. Short vertical lines show the target sequences for the MLPA probes used. Dotted lines illustrate the breakpoints responsible for the inversion polymorphism [3]. The solid line represents the common deleted region detected in mental retardation (MR) phenotypes [7,20]. Double lines depict genomic regions reported as copy number variants (CNVs) in phenotypically normal individuals [14].

or inversions mediated by non-allelic homologous recombination (NAHR) [4]. These rearrangements can lead to disease or genomic variation including large-scale copy-number variants (CNVs) and structural polymorphisms [14]. To date, both inversions and deletions of the 17q21.31 region have been reported in different subjects. A 900 kb inversion is polymorphic in the population, showing different prevalence among different ethnic groups and undergoing positive selection in the Icelandic population [3,22]. *De novo* deletions of the 17q21.31 region including the *MAPT* and *CRHR1* genes have been identified in several patients with developmental delay, hypotonia and learning disability [7,18,20,23]. The discovery of heterozygous polymorphic inversions in the transmitting parents of patients with deletions suggests that deletions are mediated by NAHR and can be facilitated by the inversion. Finally, genomic CNVs surrounding the flanking segmental duplications that may include the *MAPT* gene (Fig. 1) have been found in a significant proportion of controls and HapMap individuals by using array CGH technology [9,14,19].

Since the reciprocal rearrangement causing a duplication of the *MAPT* gene would have a similar frequency to the reported deletion in mentally retarded patients, we hypothesized that duplications could lead to sporadic or familial tau-positive FTLD through an increase in tau protein. To evaluate this hypothesis, we performed multiplex ligation probe amplification (MLPA) [17] analysis in 70 FTLD patients not carrying *MAPT* or *PGRN* mutations. Coding exons 1 and 9–13 of the *MAPT* gene and exons 0–12 of the *PGRN* gene were screened by direct sequencing as previously described [1,3,15]. Fifty-eight patients fulfilling clinical criteria of FTLD (40 FTD, 10 PNFA and 8 SD) were selected from the Hospital Clínic de Barcelona and Hospital Universitari de Bellvitge. Thirty-five of these patients were men and 23 women. Their mean age at onset of disease was 60 ± 9 years (range 39–75). The remaining 12 patients had pathologically proven tau-positive FTLD and were selected from the University of Barcelona-Hospital Clínic Barcelona Brain Bank [11]. Seven of them were men and five women. Their mean age at onset of disease was 64 ± 6 years (range 52–70). Positive family history of disease was considered in 20 cases with at least one first-degree relative affected by a clinical picture suggestive of dementia. To rule out polymorphic variation at the analysed loci in the normal population, we also screened 310 healthy age-matched control subjects. The study was approved by the local Ethics Committee and all participants gave written informed consent for genetic testing before inclusion.

All samples were subjected to MLPA with specific oligonucleotide probes for *MAPT* gene sequences along with control probes for genes located nearby and on different chromosomes (Table 1). In order to detect possible partial duplications in addition to whole gene duplications two different probes for the *MAPT* gene were used. PCR amplification of the different amplicons was performed in a single assay using 150 ngr of genomic DNA. The resulting fragments were separated by capillary electrophoresis in a sequencer (ABI Prism 3100, ABI). Samples were analyzed by visual examination of the peak profiles and by exporting the peak heights to an Excel sheet with data calculation as previously described [17]. A decrease of 35–55% in the relative peak height of a probe is indicative of deletion while an increase of 30–55% in relative peak height indicates an increase in copy number from two to three in a diploid genome. No copy number variation (deletion or duplication) was detected by MLPA in the 70 FTLD patients or in the healthy control group (310 subjects). A DNA sample from a subject with known regional trisomy due to iso-17q used as positive control for duplication consistently revealed a 40% increase in *MAPT* relative peak height (Table 2).

Therefore, we did not find *MAPT* gene dosage alterations in our cohort of patients with FTLD, including a subgroup with tau-positive inclusions as shown by their pathological examinations. Previous studies did not find *MAPT* rearrangements in cases of tau-negative ubiquitin-positive FTLD linked to chromosome 17 (FTDU-17), although it is now known that most of these cases are caused by *PGRN* gene mutations [3,2,6,10]. The absence of rearrangements in the 310 healthy controls studied also indicates that the *MAPT* gene is not part of the common CNV previously described by BAC CGH-arrays [9,14,19]. Thus, *MAPT* duplication could still be pathogenic or cause susceptibility to disease. Given the instability in the *MAPT* genomic region and the importance of the tau protein in neurodegeneration, somatic rearrangement might also be involved in sporadic dementias.