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A novel deletion in progranulin gene is associated with FTDP-17 and CBS☆

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Abstract

In the last decade familial frontotemporal dementia (FFTD) has emerged as a distinct clinical disease entity characterized by clinical and genetic heterogeneity.

Here, we provide an extensive clinical and genetic characterization of two Italian pedigrees presenting with FFTD (FAM047: 5 patients, 5 unaffected; FAM071: 4 patients, 11 unaffected). Genetic analysis showed a conclusive linkage (LOD score for D17S791/D17S951: 4.173) to chromosome 17 and defined a candidate region containing *MAPT* and *PGRN* genes. Recombination analysis assigned two different disease haplotypes to FAM047 and FAM071. In affected subjects belonging to both families, we identified a novel 4 bp deletion mutation in exon 7 of *PGRN* gene (Leu271LeufsX10) associated with a variable clinical presentation ranging from FTDP-17 to corticobasal syndrome. The age-related penetrance was gender dependent.

Both mutations in *MAPT* and *PGRN* genes are associated with highly variable clinical phenotypes. Despite the profound differences in the biological functions of the encoded proteins, it is not possible to define a clinical phenotype distinguishing the disease caused by mutations in *MAPT* and *PGRN* genes.

Keywords: Frontotemporal dementia; Corticobasal syndrome; Linkage analysis; *PGRN*; Mutation; Genotype–phenotype correlation

1. Introduction

Frontotemporal dementia (FTD; MIM 600274) is a heterogeneous condition characterized at onset by prominent behavioral disturbances, affective disorders, impairment in language, and poor executive function (Neary et al., 1998; The Lund and Manchester Groups, 1994).

Linkage analyses of large pedigrees indicated that loci at chromosomes 3, 9, 16 and 17 are associated with familial FTD (FFTD) (Brown et al., 1995; Hosler et al., 2000; Rademakers et al., 2002; Ruddy et al., 2003). Extrapyramidal features are variably present in FTD patients: a number of families in which FTD is inherited as an autosomal dominant trait have been shown to be linked to chromosome 17q21 and to present a symptomatology that has led investigators to name the disease "Frontotemporal Dementia and Parkinsonism linked to chromosome 17" (FTDP-17) (Foster et al., 1997). A proportion of FTDP-17 were demonstrated to be caused by mutations in the *MAPT* gene (MIM 157140) located on chromosome 17 and encoding the microtubule-associated tau protein (Hutton et al., 1998; Ingram and Spillantini, 2002; Poorkaj et al., 1998; Spillantini et al., 1998; Wszolek et al., 2005). Missense and 5′ splice-site mutations in the *MAPT* gene seem to be responsible for between 2.5 and 14% of all familial FTD cases, although some studies

☆ Accession numbers and URLs for data presented herein are as follows: Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/; GDB Human Genome Database, http://gdbwww.gdb.org/.

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suggest higher proportion depending on the analyzed population (Rademakers et al., 2004; Signorini et al., 2004). Neuropathological studies demonstrated that FTD patients carrying *MAPT* mutations present neuronal and glial tau deposition (Morris et al., 2001). Interestingly, families where the disease is linked to the region on chromosome 17 but with no pathogenic *MAPT* mutations, lack tau pathology and show ubiquitin-positive inclusions (Froelich et al., 1997; Lendon et al., 1998; Mackenzie et al., 2006; Rademakers et al., 2002; Rosso et al., 2001; van der Zee et al., 2006). The absence of *MAPT* mutations in many familial cases suggested that FFTD could be caused by mutations in a different gene.

It has been recently demonstrated that tau negative ubiquitin-positive FFTD is caused by mutations in progranulin gene (*PGRN*, MIM 138945) located on chromosome 17q21 (Baker et al., 2006; Cruts et al., 2006; Gass et al., 2006). Progranulin is a 593 amino acid secreted glycoprotein composed of 7.5 tandem repeats of highly conserved motifs of 12 cysteines, which can be proteolytically cleaved to form a family of 6-kDa peptides called granulins (Zhu et al., 2002); progranulin is a growth factor involved in the regulation of multiple processes including tumorigenesis, wound repair, development and inflammation (Bateman and Bennett, 1998; Bhandari et al., 1992; Diaz-Cueto et al., 2000; He and Bateman, 2003).

Here, we report linkage data, according to which the disease locus is mapping to chromosome 17q21 in two Italian pedigrees affected by familial frontotemporal dementia: the two candidate genes (*MAPT* and *PGRN*) located in this locus were analyzed.

2. Materials and methods

2.1. Subjects

Patients underwent clinical and neurological examination at the Memory Clinic of the IRCCS "Centro San Giovanni di Dio-Fatebenefratelli" in Brescia, Italy.

Clinical diagnosis of patients affected by FTD, corticobasal syndrome (CBS) and Alzheimer disease (AD) was made according to international guidelines (Boeve et al., 2003; Litvan et al., 1997; McKhann et al., 1984; The Lund and Manchester Groups, 1994). The family history was determined by collection of the Family History Questionnaire (modified from Family History Questionnaire, J.H. Growdon). Starting from nuclear families, two large pedigrees were drawn (FAM047 and FAM071).

The enrollment of family members has been then guided to obtain maximum LOD scores using FastSLink v2.51, based on SLINK (Weeks et al., 1990).

Blood samples were collected from patients and unaffected at-risk family members after obtaining informed consent, as approved by the local ethical committee. DNA was isolated according to standard procedures.

2.2. Mutation analysis

The presence of mutations in *MAPT* (MIM 157140) and *PGRN* (MIM 138945) genes was investigated by direct sequencing of exonic and flanking intronic regions (Baker et al., 2006; Binetti et al., 2003; Cruts et al., 2006). Mutations validation was performed screening 120 aged control individuals sequencing the relevant exons. *MAPT* haplotypes were reconstructed as previously described (Ghidoni et al., 2006). Sequence reactions were separated on DNA sequencer ABI Prism Genetic Analyzer 3100 and analyzed using Sequence Navigator Software (Applied Biosystems, Monza, Italy).

The presence of large deletions or duplications in *MAPT* gene exon 10 was investigated by real-time quantitative PCR TaqMan assay on demand (Applied Biosystems, Monza, Italy).

2.3. Microsatellites analysis

A total of 25 DNA samples of members of families FAM047 and FAM071 were included in the genetic analysis: 9 patients (5 from FAM047 and 4 from FAM071) and 16 consanguineous families members (5 from FAM047 and 11 from FAM071). Microsatellite markers were chosen from the genomic loci on chromosomes 3 (D3S1595, D3S1598, D3S1603, D3S1271), 9 (D9S301, D9S1867, D9S167) and 17 (D17S798, D17S1787, D17S951, D17S791, D17S931, D17S806, D17S787) previously described to be linked to FTD (Brown et al., 1995; Hosler et al., 2000; Rademakers et al., 2002). Primer sequences were obtained from the GDB Human Genome Database. PCR products were analyzed using ABI GeneScan 3.7 (Applied Biosystems) and allele was assigned by GeneMapper software.

2.4. Simulation and linkage analysis

For linkage analysis we selected a dominant disease model with age-dependent penetrances ranging from 0.22 to 0.98 in different age classes, with a phenocopy rate increasing from 0.0025 to 0.23. Penetrances were calculated from the age of onset curve of the combined dataset (Ott, 1999). Phenotype unknown (=0) was assigned to pedigrees members younger than 50 years and to subjects presenting cognitive and/or behavioral symptoms, however not completely fulfilling the clinical criteria for a diagnosis of FTD. Liability class 1 and penetrance 0.80 were assigned to spouses. A number of six alleles of equal frequency and a dominant disease model have been used for the simulation: under these conditions we obtained a simulated maximal LOD score of 5.246 for the combined dataset (2.072 for FAM047 and 3.474 for FAM071).

Two-point linkage analysis was performed using the FastLink v4.1—Two-Point Parametric Linkage Analysis program (Schaffer, 1996). Multipoint linkage analysis was carried out by SimWalk v2.91—Multipoint Linkage Parametric Analysis (Sobel and Lange, 1996).