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LETTER TO THE EDITOR

Mutations in *progranulin* explain atypical phenotypes with variants in *MAPT*

Stuart M. Pickering-Brown,[1,2] Matt Baker,[3] Jenny Gass,[3] Bradley F. Boeve,[4] Clement T. Loy,[5,6] William S. Brooks,[6,7] Ian R. A. Mackenzie,[8] Ralph N. Martins,[7] John B. J. Kwok,[5,7] Glenda M. Halliday,[6,7] Jillian Kril,[9] Peter R. Schofield,[5,6,7] David M. A. Mann[2] and Mike Hutton[3]

[1]Division of Regenerative Medicine, University of Manchester, Manchester, UK, [2]Clinical Neuroscience Research Group, Division of Medicine and Neuroscience, Greater Manchester Neurosciences Centre, University of Manchester, Hope Hospital, Salford, UK, [3]Department of Neuroscience, Mayo Clinic Jacksonville, Jacksonville, FL, [4]Department of Neurology, Mayo Clinic, Rochester, Rochester, MN, USA, [5]Garvan Institute of Medical Research, Darlinghurst, NSW 2010 Australia, [6]Prince of Wales Medical Research Institute, Barker Street, Randwick, Sydney 2031, Australia, [7]The University of New South Wales, Sydney 2052, Australia, [8]Department of Pathology and Laboratory Medicine University of British Columbia and Vancouver Coastal Health, Vancouver, British Columbia, Canada and [9]Disciplines of Medicine and Pathology, University of Sydney, Sydney, Australia

Correspondence to: Stuart M. Pickering-Brown, Division of Regenerative Medicine, University of Manchester, Stopford Building, Oxford Road, Manchester; M13 9PT, UK
E-mail: SPB@Manchester.ac.uk
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Mutations in *presenilin-1* (*PSEN1*) cause autosomal dominant Alzheimer's disease and mutations in *MAPT* cause the familial tauopathy Frontotemporal dementia linked to chromosome 17 (FTDP-17). However, there have been reports of mutations in *PSEN1* and *MAPT* associated with cases of FTD with ubiquitin-positive tau-negative inclusion pathology. Here, we demonstrate that the *MAPT* variants are almost certainly rare benign polymorphisms as all of these cases harbour mutations in *Progranulin* (*PGRN*). Mutations in *PGRN* were recently shown to cause ubiquitin-positive FTDP-17.

Keywords: frontotemporal dementia; FTLD-U; MAPT; Progranulin

Abbreviations: FTLD = frontotemporal lobar degeneration; NCI = neuronal cytoplasmic inclusions; NII = neuronal intranuclear inclusions; PGRN = progranulin; PSEN1 = presenilin-1; ub-ir = ubiquitin-immunoreactive

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Frontotemporal lobar degeneration (FTLD) is the second most common form of primary degenerative dementia after Alzheimer's disease (Neary *et al.*, 1998). FTLD is thought to represent up to 20% of cases of presenile dementia. Significantly, a large proportion of FTLD patients (35–50%) have a family history of dementia suggesting a large genetic contribution to the aetiology of this disease (Neary *et al.*, 1998). There have been seven chromosomal loci for FTLD identified to date on chromosomes 3, 9p (two loci), 9q, 17q21 (two loci) and 17q24 with only four genes (*MAPT*, *VCP*, *CHMP2b* and *PGRN*) identified to date.

Approximately 15–20% of familial FTLD result from mutations in the *MAPT* gene on chromosome 17q21, which encodes the microtubule associated protein tau (Hutton *et al.*, 1998). There have now been over 35 *MAPT* mutations reported in exons 1, 9, 10, 11, 12 and 13 in families worldwide. At autopsy, almost all of the reported cases with *MAPT* mutations have intraneuronal hyperphosphorylated tau based neuropathology and glial cell tau inclusions are observed in some families (Reed *et al.*, 2001).

Very recently we reported that mutations in a second gene on chromosome 17q21, called *Progranulin* (*PGRN*), cause autosomal dominant FTLD in a series of families that had previously been shown to lack mutations in *MAPT* (Baker *et al.*, 2006; Cruts *et al.*, 2006). Moreover, pathologically these families display tau-negative, ubiquitin-immunoreactive (ub-ir) neuronal cytoplasmic inclusions (NCI) and characteristic lentiform ub-ir neuronal intranuclear inclusions (NII) within affected regions of cerebral cortex and hippocampus. All *PGRN* mutations identified lead to the creation of null alleles with the majority introducing premature termination codons that invoke nonsense mediated RNA decay. This suggests that haploinsufficiency is the likely pathogenic mechanism in these cases.

There have been reports of two mutations in *MAPT* and a mutation in *presenilin-1* (*PSEN1*) with clinical diagnoses of frontotemporal dementia (FTD) in which autopsies have revealed unexpectedly tau-negative pathology. This is in marked contrast to the tauopathy and Alzheimer's disease-type plaque and tangle neuropathologies normally associated with mutations in *MAPT* and *PSEN1*, respectively. These findings seemed to imply that specific mutations in these genes might result in a somewhat different pathogenic mechanism leading to alternative pathologies.

We have recently demonstrated that a variation at codon 352 of *PSEN1* (PSEN1ins352) in a patient with clinical FTD (Amtul *et al.*, 2002). Other members of this family were unavailable to demonstrate segregation. This case has recently come to autopsy and interestingly this revealed ub-ir NCI and NII pathology. Subsequent sequence analysis of *PGRN* in this case revealed *PGRN* IVS1 + 1G > A splice donor site mutation in intron 1 (Fig. 1) (Boeve *et al.*, 2006).

We previously reported a *MAPT* IVS10 + 29 variant that segregated with disease in a small Australian FTD family with a maximum LOD score of 1.02 and tau-negative neuropathology (Stanford *et al.*, 2003). Assays of *MAPT* exon 10 alternative splicing suggested that this variant caused increased production of tau protein isoforms with three microtubule binding repeats (3R tau) (Stanford *et al.*, 2003). However, the *MAPT* IVS10 + 29 mutation was also found at low frequency in controls (D'souza *et al.*, 1999; Stanford *et al.*, 2003) questioning the pathogenic nature of the variant, although the possibility remained that it was a genetic risk factor for FTLD. We have also reported a tau-negative neuropathology in a British individual with a A239T mutation in *MAPT* (Pickering-Brown *et al.*, 2002). This mutation was absent in over 900 control individuals, however, the lack of other family members precluded segregation analysis of this mutation. In light of the aforementioned PS-1/PGRN finding, we then questioned whether cases with variants in *MAPT* associated with tau-negative FTD also had ub-ir NCI and NII pathology. Re-evaluation of these two *MAPT* + 29 and A239T cases, using a sensitive automated ubiquitin immunostaining protocol shown to detect ub-ir pathology in other FTLD patients thought to display a tau- and ubiquitin-negative histology (known as dementia lacking distinctive histology) (Mackenzie *et al.*, 2006), indeed revealed NCI and NII to be present in both instances (Fig. 1A and B).

Given the proximity of *PGRN* and *MAPT* (1.7 Mb) on chromosome 17 it is possible that these rare variants in *MAPT* might have been inherited with pathogenic mutations in *PGRN* effectively acting as a linked marker. Consistent with this hypothesis, sequence analysis of *PGRN* revealed that both the *MAPT* IVS10 + 29 and *MAPT* A239T cases also carried probable null mutations in *PGRN* (Fig. 2). The *MAPT* IVS10 + 29 case was found to have a *PGRN* Ex11 + 64C > T change that created a premature stop codon (Arg493X). This particular mutation is the most common mutation we have found to date with it being detected in nine separately ascertained cases (Gass *et al.*, 2006). The *MAPT* A239T case contained a partial deletion of *PGRN* exon 11. The deletion starts 16 bp upstream from the exon and extends into the exon for 177 bp (IVS10-16_Ex11 + 177del194 bp). As the splice acceptor site is destroyed, we predict the effect of this deletion would be to splice out exon 11, resulting in an in-frame deletion of exon 11, thereby creating a shorter *PGRN* transcript and protein. Alternatively, it is possible that a frameshift occurs due to the presence of a cryptic splice acceptor site within the undeleted part of exon 11. However, in the absence of a source of mRNA, we were unable to determine the precise effect of this deletion.

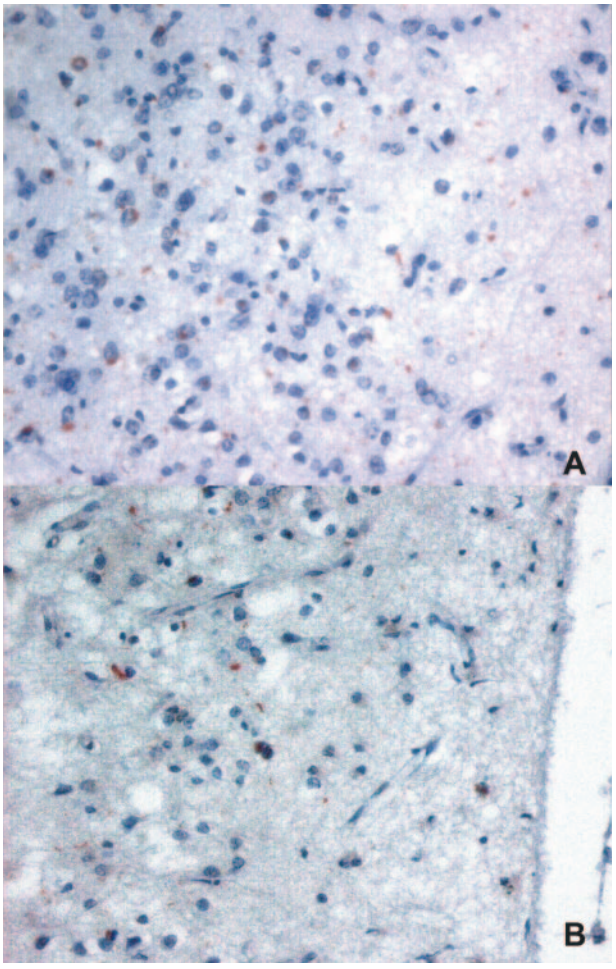

Fig. 1 Representative cortical ubiquitin-positive neuronal inclusions in the MAPT IVS10 + 29 (**A**) and MAPT A239T (**B**).

The identification of probable null mutations in *PGRN* in combination with FTLD-U pathology with ub-ir NCI and NII indicates that the variants in *MAPT* reported in these cases (Pickering-Brown *et al.*, 2002; Stanford *et al.*, 2003), like that in *PSEN1* (Amtul *et al.*, 2002), are almost certainly rare benign polymorphisms, and are not responsible for disease. This has important consequences since this finding also questions the pathogenic significance of *PSEN1* mutations that cause complete loss of function and

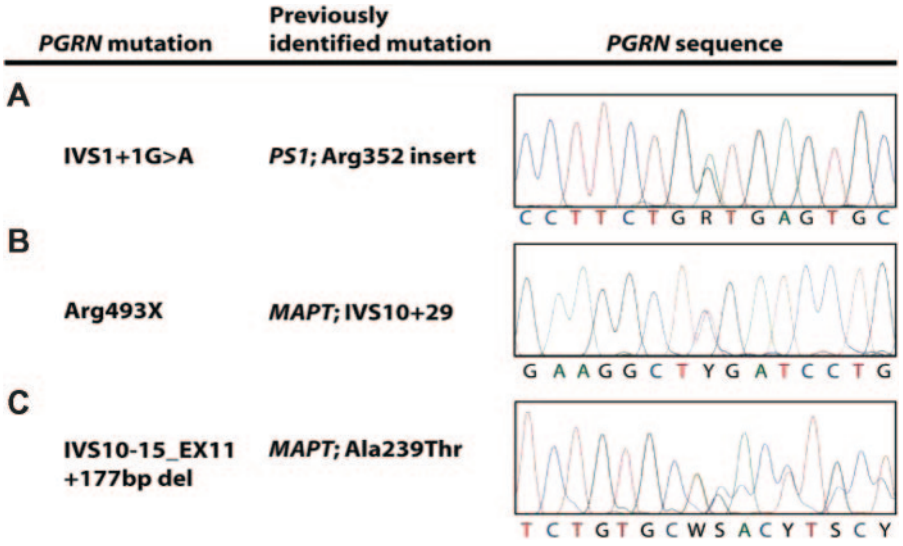

Fig. 2 (**A**–**C**) Sequence chromatographs demonstrating PGRN mutations in FTLD cases with previously identified variants in MAPT and PSEN1. PCR conditions and DNA sequence analysis performed as described previously (Baker *et al.*, 2006).

mutations in *MAPT* that increase the production of 3R tau. Collectively, the identification of *PGRN* mutations in cases previously thought to have mutations in *MAPT* and *PSEN1* demonstrates that caution should be taken when interpreting the pathogenic significance of rare variants that cannot be confirmed by other methods especially when these are associated with an atypical clinical or pathological phenotype. A bias to detect rare variants can occur when disease genes are fully sequenced in disease cases, but only the frequency of specific variants is assessed in controls. The data presented here suggest full gene sequencing in controls is required to avoid this bias.

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