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Short Communication

FOXP2 Expression in Frontotemporal Lobar Degeneration-Tau

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Abstract. FOXP2 is altered in a variety of language disorders. We found reduced mRNA and protein expression of FOXP2 in frontal cortex area 8 in Pick's disease, and frontotemporal lobar degeneration-tau linked to *P301L* mutation presenting with language impairment in comparison with age-matched controls and cases with parkinsonian variant progressive supranuclear palsy. *Foxp2* mRNA and protein are also reduced with disease progression in the somatosensory cortex in transgenic mice bearing the *P301S* mutation in *MAPT* when compared with wild-type littermates. Our findings support the presence of FOXP2 expression abnormalities in sporadic and familial frontotemporal degeneration tauopathies.

Keywords: FOXP2, frontotemporal lobar degeneration, language, Pick's disease, *P301S* transgenic mice, tauopathy

INTRODUCTION

Frontotemporal dementia (FTD) represents a heterogeneous group of cognitive disorders that generally affect language and behavior, eventually leading to dementia. Clinically, several subgroups have been proposed including a behavioral variant, progressive non-fluent aphasia, semantic dementia, and logopenic aphasia. The pathological hallmark of the disease is frontotemporal lobar degeneration (FTLD) [1, 2]. Microtubule-associated protein tau gene (*MAPT*), located on chromosome 17, is causative of a subgroup of familial FTD categorized as fFTLD-tau [3, 4].

Forkhead box P2 gene (*FOXP2* in human, *Foxp2* in mouse) encodes a protein that belongs to the forkhead box family of transcription factors. The gene structure is almost the same in mice and chimpanzees, whereas orangutans show only minor changes in the secondary structure, while a minor change at position 325 occurs in humans [5]. Disruption of *FOXP2* leads to language impairment [6]. FOXP2 is abnormally regulated in several language disorders [7–12]. Certain *FOXP2* polymorphisms modulate phenotypical expression of FTD, leading to a decrease in verbal fluency and decreased cortical volume in selected areas such as the left inferior frontal cortex and Broca. *FOXP2* polymorphisms are risk factors for FTD [13]. Interestingly, adult heterozygous Foxp2 mice mutants emit abnormal ultrasonic vocalizations [14].

The present study was focused on learning whether *FOXP2* mRNA and protein expression are altered

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in the frontal cortex in human cases with sporadic FTLD-tau (sFTLD) and fFTLD-tau. Cases of sFTLD-tau including Pick's disease (PiD) and cases of fFTLD-tau are represented by those bearing the *P301L* mutation in *MAPT*. For comparative purposes, the frontal cortex of cases with progressive supranuclear palsy-parkinsonian variant (PSP-P) was included as tauopathy with little cortical impairment. To learn whether or not these changes are related to cell loss at terminal stages in human cases, *Foxp2* mRNA and protein expression were analyzed in the somatosensory cortex of *MAPT P301S* transgenic mice used as a model of fFTLD-tau at different ages [15, 16]. Interestingly, newborn transgenic mice exhibit abnormal ultrasonic vocalization [17], which was interpreted in the present context as a counterpart of language impairment in humans [18].

MATERIAL AND METHODS

Human brain tissue was obtained from the Institute of Neuropathology Brain Bank (HUB-ICO-IDIBELL Biobank, Barcelona, Spain) and Clinic Hospital-IDIBAPS Biobank (Barcelona, Spain) following the guidelines of Spanish legislation and of the local ethics committee. One hemisphere was immediately cut in coronal sections, 1 cm thick, and selected areas of the encephalon were rapidly dissected, frozen on metal plates over dry ice, placed in individual air-tight plastic bags, numbered with water-resistant ink, and stored at –80°C. The other hemisphere was fixed by immersion in 4% buffered formalin for 3 weeks for morphological study. Frontal cortex from 34 cases was used in the present study: 3 PiD, 11 PSP-P, 4 FTLD-tau (*P301L*), and 15 age-matched controls. Cases with associated pathologies (e.g., vascular diseases, synucleinopathies, TDP-43 pathies) were excluded from the present study.

Male heterozygous transgenic mice expressing human *P301S* tau (line PS19) and wild-type (WT) littermates were killed by cervical dislocation at 1, 3, 6. and 10 months of age. Five animals per age (1 to 10 months) and genotype were used in this study. The generation of mice expressing the human mutated form *P301S* tau has already been described [15]. All animal procedures were carried out following the guidelines of the European Communities Council Directive 2010/63/EU and with the approval of the local ethical committee of the University of Barcelona. The brain was rapidly removed from the skull; the left hemisphere was dissected into selected brain regions which were frozen and stored at –80ºC until use for biochemical studies. The right hemisphere was fixed in 4% buffered paraformaldehyde for histological study.

Human frontal cortex area 8 and murine somatosensory cortex samples were processed for qRT-PCR and western blotting as detailed elsewhere [16]. TaqMan RT-qPCR assay for *Foxp2* (Mm00475030_m1) and *FOXP2* (Hs00362818_m1) was performed in duplicate on cDNA samples in 384-well optical plates using an ABI Prism 7900 Sequence Detection system (Applied Biosystems, Life Technologies, Waltham, MA, USA). Parallel assays for each sample were carried out using probes for X-prolyl aminopeptidase (aminopeptidase P) 1 (*Xpnpep1*) (Mm00460040_m1) to normalize mouse samples and β-glucuronidase (β-*GUS*) (Hs00939627_m1) in human cases. TaqMan PCR data were captured using the Sequence Detection Software (SDS version 1.9, Applied Biosystems). Assays were performed without cDNA samples to verify the degree of contamination. Results in the frontal cortex in human cases were analyzed with 1-way analysis of variance, followed by Dunnett's *post hoc*, while in murine samples 2-way analysis of variance with genotype and age as between factors was followed by Tukey *post hoc* or Student *t*-test when required. The significance level was set at $p < 0.05$ for all experiments. Values were expressed as the mean values ± standard error of the mean (SEM).

For western blotting, the membranes were first incubated with the rabbit polyclonal anti-FOXP2 antibody (ab16045, Abcam, Cambridge, UK) used at a dilution of 1:1,000, followed by the secondary antibody (1:2,000, Dako, Carpinteria, CA, USA), and then revealed with a chemiluminescence reagent (ECL, Amersham, GE Healthcare, Buckinghamshire, UK). β-actin (1:30,000, Sigma-Aldrich, St Louis, MO) was blotted in parallel in each membrane to normalize protein loading. Densitometry of bands in western blots was analyzed with the software TotaLab (TL100 v.2006b).

RESULTS

FOXP2 mRNA and protein expression in the frontal cortex in PiD, PSP-P, and FTLD-tau (P301L)

A significant decrease in *FOXP2* mRNA was found in PiD and FTLD-tau (*P301L*) ($p < 0.01$ and $p < 0.05$, respectively) when compared with controls. In con-

trast, no difference in *FOXP2* mRNA levels was observed in frontal cortex area 8 in PSP-P (Fig. 1A). Protein levels of FOXP2 were analyzed in the same cases with western blotting. Three group bands of approximately 140 kDa, 85 kDa, and 70 kDa were detected in the immunoblots. The band of 140 kDa was a single band, whereas the band of 85 kDa was in fact a doublet, while the band of 75 kDa was composed of three packet bands in human cases. The triplet of 70 kDa was expressed in control and PSP-P cases, whereas only the upper band of the triplet was preserved in PiD and FTLD-tau. The doublet of 85kDa was only seen in control and PSP-P. The band of 140 kDa was strong in control and PSP-P, weak in PiD, and absent in FTLD-tau (*P301L*). Densitometric analysis showed significantly decreased FOXP2 expression in PiD and FTLD-tau (*P301L*) ($p < 0.001$) when compared with control (Fig. 1B).

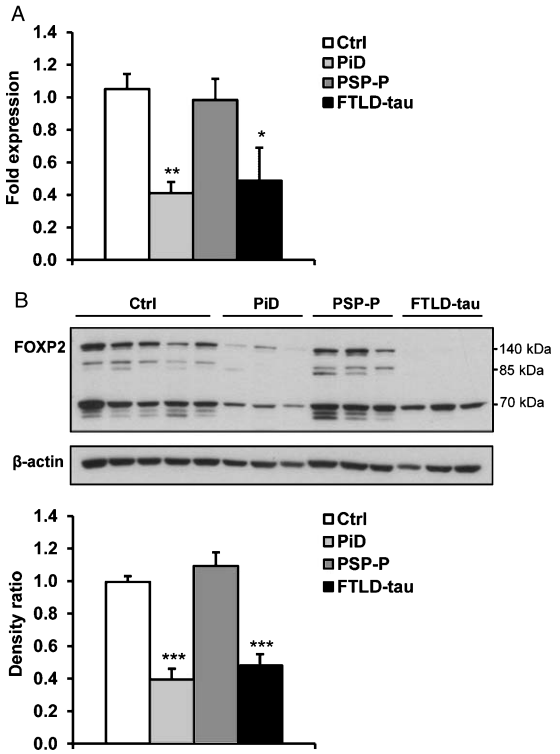

Fig. 1. A) *FOXP2* mRNA and protein levels in frontal cortex 8 in three Pick's disease (PiD) cases, three progressive supranuclear palsy-parkinsonian variant (PSP-P) cases, and three frontotemporal lobar degeneration-tau linked to *P301L* mutation (FTLD-tau) cases, in comparison with fifteen control (Ctrl) cases. *FOXP2* mRNA expression is significantly decreased in PiD and FTLD-tau when compared with controls. Data in graphs (columns and corresponding bars) represent mean values ± SEM of all cases analyzed. B) Western blotting of FOXP2 reveals several bands in human cases, one band of 140 kDa, a doublet of about 85 kDa, and a triplet of about 70 kDa. Western blotting of FOXP2 shows a significant reduction in PiD and FTLD-tau versus Ctrl. Data in graphs (columns and corresponding bars) represent mean values ± SEM of all cases analyzed by western blotting which includes three PiD, three PSP-P, three FTLD-tau, and five controls). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with controls (Dunnett's *post hoc*).

Foxp2 mRNA and protein expression in somatosensory cortex of P301S transgenic mice

Foxp2 mRNA significantly increased with age in WT mice aged 3 and 6 months when compared with mice aged 1 month ($p < 0.05$), whereas no modifications occurred in *P301S* transgenic mice with age. However, the expression of *Foxp2* mRNA was significantly decreased in *P301S* transgenic mice aged 6 months when compared with WT littermates ($p < 0.05$) (Fig. 2A). No differences in *Foxp2* mRNA were observed in mice aged 10 months. FOXP2 immunoreactivity in mice was represented by three bands of 140 kDa, 85 kDa, and 70 kDa. No doublets or triplets were observed in mice, in contrast to human cases. This may reflect species differences in FOXP2, although modifications related to postmortem delay cannot be ruled out in human cases. FOXP2 protein levels were significantly decreased in *P301S* transgenic mice aged 6 months and 10 months when compared with corresponding WT littermates ($p < 0.01$ and $p < 0.05$, respectively). Moreover, a significant reduction was also noted in *P301S* transgenic mice aged 10 months when compared with 3-month-old transgenic mice ($p < 0.01$) (Fig. 2B).

DISCUSSION

The present study shows decreased *FOXP2* mRNA and protein expression in the frontal cortex in PiD, a paradigm of sFTLD-tau, and in FTLD cases bearing the *P301L* mutation in MAPT [2–4, 19]. In contrast, no modifications in the expression levels of *FOXP2* mRNA and protein were found in the frontal cortex in PSP-P, a tauopathy with little cortical involvement and no language disturbances [20]. This observation suggests that FOXP2 associates with sporadic and familial FTLD-tau but not necessarily with other diseases with abnormal hyper-phosphorylated tau deposits such as PSP-P.

It can be argued that decreased *FOXP2* mRNA and protein levels in PiD and FTLD-*P301L* are due, at least in part, to the severe neuronal cell loss found in the frontal cortex at terminal stages of the disease. Transgenic mice bearing the *P301S* mutation

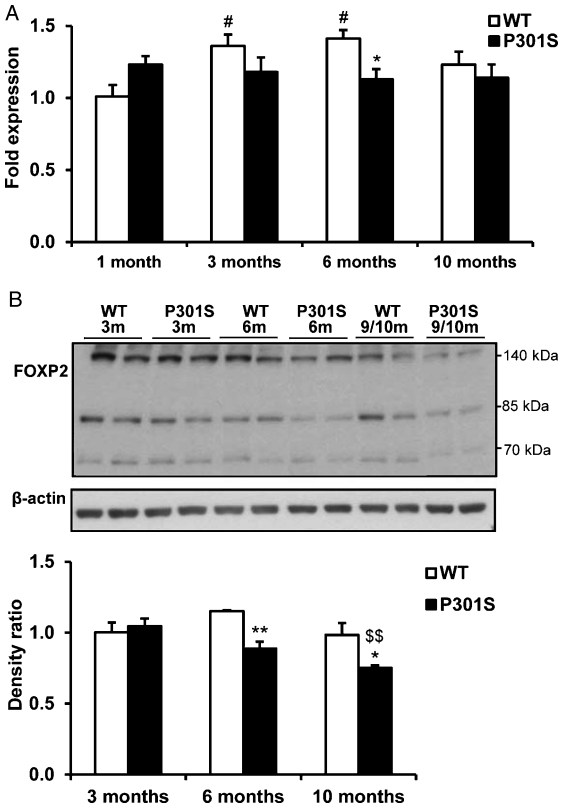

Fig. 2. Foxp2 mRNA and protein expression in *P301S* transgenic mice (P301S) and wild-type littermates (WT) at different ages. A) *Foxp2* mRNA expression increases at 3 and 6 months, but not at month 10 compared with 1 month in WT, and not in P301S transgenic mice. *Foxp2* mRNA is reduced in *P301S* mice at the age of 6 months. B) Western blotting reveals three bands in the cortex, one band of 140 kDa, another of 85 kDa, and a third of 75 kDa. Two representative cases per age and genotype were run in parallel FOXP2 protein, as revealed by western blotting, is significantly reduced at the ages of 6 and 10 months in *P301S* when compared with WT mice. FOXP2 protein is also significantly reduced at the age of 10 months when compared with 3 months in *P301S* mice. Data in graphs (columns and corresponding bars) represent mean values ± SEM of all cases analyzed (five animals per age and genotype). $^{*}p < 0.05$ and $^{**}p < 0.01$, P301S compared with WT (t-Student). $^{\#}p < 0.05$, WT aged 3 and 6 months compared with WT aged 1 month (Tukey's *post hoc* test). $^{\$\$}p < 0.01$, P301S aged 10 months compared with P301S aged 3 months (Tukey's *post hoc* test).

in Mapt aged up to 10 months do not have neuron loss in the somatosensory cortex [15, 16]. Yet *Foxp2* mRNA is significantly reduced in the somatosensory cortex in *P301S* transgenic mice at 6 months, as is FOXP2 protein at 6 months and 10 months when compared with corresponding WT littermates. Therefore, reduced FOXP2 expression in the frontal cortex occurs in PiD, human FTLD-*P301L*, and transgenic mice bearing the *P301S* mutation in *Mapt* independently of the presence or absence of neuron loss. Together, these observations point to the likelihood that FOXP2 underlies language disturbances in PiD and fFTLD-tau.

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