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Sex difference in pathology and memory decline in rTg4510 mouse model of tauopathy

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Abstract

Abnormal phosphorylation of tau protein is a common event in many neurodegenerative disorders, including Alzheimer's disease and other tauopathies. We investigated the relationship between hyperphosphorylated tau in brain extracts and mnemonic functions in rTg4510 mouse model of tauopathy. We report that rTg4510 mice showed rapid deterioration in spatial learning and memory, which paralleled a significant increase of hyperphosphorylated tau in the brain between 3 and 5.5 months of age. At 5.5 months, rTg4510 females showed significantly higher levels of hyperphosphorylated tau than males, with no evidence of differential tau transgene expression between the sexes. The increased levels of hyperphosphorylated tau in females were associated with more severe impairment in spatial learning and memory as compared to transgenic males. We also showed that within studied age range, the decrease in memory performance was accompanied by other behavioral disturbances in the water maze related to search strategy, like thigmotaxic swim and cue response. These findings suggest that the onset of abnormal tau biochemistry and coincident cognitive deficits in the rTg4510 mouse model is sex-dependent with females being affected earlier and more aggressively than males.

Keywords: Tau; Transgenic mice; Neuronal loss; Memory impairment; Sex difference

1. Introduction

A group of neurodegenerative diseases, including Alzheimer's disease (AD), collectively termed tauopathies (Iqbal et al., 2005; Lee et al., 2001), is characterised by age-progressing dementia, profound neuronal loss and brain atrophy (Cotman and Su, 1996; Davies et al., 2005; Terry, 2006) with coinciding intra-neuronal accumulation of aggregated hyperphosphorylated tau protein in a form of neurofibrillary tangles (NFTs) (Dickson, 2003; Lee et al., 2001). Additionally to genetic mutations implicated in the onset of familial cases of AD (FAD) (Selkoe, 1997), there are other risk factors which can significantly affect the onset and the course of the disease (Erten-Lyons et al., 2009; Rhodin and Thomas, 2001; Williams et al., 2006). Among the genetic factors, the ε4 allele of apolipoprotein E (*APOE*) was identified and documented to be associated with late-onset of AD (Arendt et al., 1997; Corder et al., 1995a,b). However, the distribution and prevalence of risk factors varies between sexes and age groups (Azad et al., 2007). The presence of APOE-ε4 appears to have greater deleterious effect on hippocampus pathology and memory in women than men (Fleisher et al., 2005), however this risk varies between ethnic groups (Farrer et al., 1997). The frequency of APOE-ε4 is also higher in progressive supranuclear palsy (PSP) which has concomitant AD pathology, and APOE-ε4 increases risk of dementia after neurotrauma, ischemia, or human immunodeficiency virus (HIV) infection (reviewed by Raber, 2004), however the frequency distribution related to gender has not been identified. Although, female sex has been also associated with increased risk of hypertension, hyperlipidemia, and diabetes (Azad et al., 2007), its linkage to dementias in other neurodegenerative diseases is yet to be established.

The discovery of over thirty mutations in microtubule associated protein tau (*MAPT*) (reviewed by Goedert and Jakes, 2005) in patients with fronto-temporal dementia with

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Parkinsonism linked to chromosome 17 (FTDP-17), an autosomal dominantly inherited tauopathy (Clark et al., 1998; Hutton et al., 1998), and the evidence that intra-neuronal deposition of tau is also a feature of dying neurons during normal aging (Braak and Braak, 1997) underscored the importance of abnormally modified tau in the neuronal death leading to cognitive deficits. Hyperphosphorylated tau with a characteristic abnormal gel mobility is a biochemical hallmark uniformly present in many tauopathies including AD, hippocampal tauopathy in cerebral aging, PSP, corticobasal degeneration (CBD), and FTDP-17 (reviewed by Buee et al., 2000; Sergeant et al., 2005). It has also been shown that NFTs were better correlated with the degree and duration of dementia in AD than amyloid beta (Aβ) plaques (Arriagada et al., 1992; Berg et al., 1993).

The identification of pathogenic tau mutations enabled the creation of mouse models of tauopathies which confirmed that the accumulation of aggregated hyperphosphorylated tau protein is directly implicated in neurodegeneration and dementia (reviewed by Eriksen and Janus, 2006). The overexpression of mutated human tau genes in these models led to the development of functional motor (Lewis et al., 2000), or cognitive (Santa Cruz et al., 2005; Schindowski et al., 2006) deficits, depending on part of the central nervous system targeted by transgenic tau expression. In our study we used the rTg4510 repressible model of tauopathy, which over-expresses human P301L mutant tau linked to FTDP-17 specifically in the cortex, limbic system, and basal ganglia (Santa Cruz et al., 2005). The model is characterised by spatial memory deficits and the formation of a distinct 64 kDa abnormally hyperphosphorylated 4R0N isoform of tau and increase in NFTs, with a rapidly progressing neuronal loss in the hippocampus by 5.5 months of age (Ramsden et al., 2005; Santa Cruz et al., 2005; Spires et al., 2006). While investigating the possibility of circumstantial effects on the behavioral phenotype of this model, we identified a profound difference between the susceptibility of females and males of rTg4510 mice to tau pathology and corresponding behavioral phenotypes. Sex-dependent differentiation in spatial memory has been noted before in more complex transgenic mouse models co-expressing both *APP* and *tau* genes. Female transgenic APP/tau mice showed worse spatial learning than males (Ribé et al., 2005). Separately, in a second model, APP/tau females showed enhanced neurofibrillary pathology in the limbic system and olfactory cortex as compared to males (Lewis et al., 2001). Also, recent studies using a 3 × Tg mouse model (co-expressing mutant *APP*, *PS1* and *tau* transgenes) showed that females had significantly exacerbated Aβ pathology over males, but with no gender differences in tau pathology (Hirata-Fukae et al., 2008). In our study, we characterised spatial reference memory of 5.5-month-old rTg4510 mice since this memory system is highly dependent on intact hippocampus (Eichenbaum, 1996; Morris, 1984; Morris et al., 1982; Squire, 1992). The significant degree of neuronal loss in hippocampus already manifest in the 5.5-month-old rTg4510 (Spires et al., 2006) provides an attractive experimental time frame to focus on differences in memory function specifically due to tau pathology.

We demonstrate for the first time a direct interaction between sex and tau pathology. The sex-dependent onset of the development of tau pathology and compromised mnemonic function in the rTg4510 mice may present an attractive model to study factors external to the CNS modulating the onset and progression of dementia.

2. Methods

2.1. Mice

The generation of rTg4510 mice was described previously (Santa Cruz et al., 2005). Briefly, a human tau cDNA lacking exons 2 + 3 but containing exon 10 with the P301L mutation (4R0N tau_{P301L}) was placed downstream of a tetracycline-operon-responder (TRE) construct. To activate the transgene, the responder has to be co-expressed with an activator construct, consisted of the tetracycline conditional gene expression system (tet-off, tTA) (Gossen and Bujard, 1995). The tTA activator system was placed downstream of Ca^{2+}-calmodulin kinase II promoter (CaMKII) thus restricting the expression of TRE mainly to forebrain structures (Mayford et al., 1996). The tau transgene responder was expressed in the FVB/N (Charles River) mouse strain, while the tTA activator system was maintained on 129 S6 (Taconic) mouse strain. Both congenic parental mouse strains were hemizygous with reference to tau and tTA transgenes, and their F1 progeny carried both responder and activator transgenes (tTA/tau, henceforth called rTg4510), necessary for the expression of the tau transgene, along with littermates carrying single transgenes (tau responder or tTA activator, respectively) and non-transgenic mice. The FVB/129 F1 genetic background of rTg4510 mice and their littermates is suitable for behavioral testing (Banbury Conference on Genetic Background in Mice, 1997; Crawley and Paylor, 1997), being unaffected by recessive traits of parental strains such as retinal degeneration present in FVB strain or partial development of corpus callosum in 129 strain (Wahlsten et al., 2005). The analysis of the frequency of all the genotypes at the time of weaning (21 + 2 days of age; $N = 120$) revealed close to predicted (25%) distribution of pups within each genotype (29% tTA/tau, 25% tau, 21% tTA, 25% non-Tg; $\chi^2(3) = 1.7$, NS). Mice were genotyped by the analysis of tail DNA using tau cDNA-specific primers to exon 1 and exon 5, and primers specific to tetracycline trans-activator against an internal control (T cell receptor). The rTg4510 mice over-express the tau transgene about 13 times over the endogenous mouse tau, and this higher expression level of tau likely results in the observed early onset of tau pathology in the mouse brains, since the line with lower, 7-fold over-expression of the transgene exhibited accumulation of hyperphosphorylated tau by 14 months and NFTs inclusions at 20 months (Santa Cruz et al., 2005). Since our initial characterisation of the model