

## SHORT REPORT

## The *tau* gene A0 polymorphism in progressive supranuclear palsy and related neurodegenerative diseases

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### Abstract

**Progressive supranuclear palsy is characterised pathologically by the deposition of neurofibrillary tangles consisting of tau protein. Patients with the disease have been reported to have a more frequent occurrence of one allele of an intronic polymorphism of the *tau* gene. Other diseases which may involve tau deposition include frontotemporal dementia and corticobasal degeneration. This polymorphism has been studied in a series of subjects with progressive supranuclear palsy, corticobasal degeneration, frontotemporal dementia, idiopathic Parkinson's disease, and normal controls to (1) confirm this association in a large series and (2) to investigate a possible role for this association in other disorders which involve tau deposition. The results confirm the finding of an overrepresentation of the A0 allele and the A0/A0 genotype in patients with progressive supranuclear palsy, in the largest series reported to date. The A0 allele was found in 91% of patients with progressive supranuclear palsy as opposed to 73% of controls ( $p < 0.001$ ) and the A0/A0 genotype was seen in 84% of patients as compared with 53% of controls ( $p < 0.01$ ). There was no significant difference between patients with Parkinson's disease, frontotemporal dementia, or corticobasal degeneration, and controls. The A0 allele may have a direct effect on tau isoform expression in progressive supranuclear palsy or it may be in linkage disequilibrium with an adjacent determinant of *tau* gene expression. The explanation for this difference between a predisposition factor to progressive supranuclear palsy and the other conditions may lie in the molecular pathology of these diseases.**

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Progressive supranuclear palsy is an extrapyramidal syndrome characterised by a vertical supranuclear gaze palsy and early postural

instability. Pathologically there is marked cell loss in the substantia nigra, globus pallidus, subthalamic nucleus, and brain stem nuclei,<sup>1</sup> accompanied by the deposition of neurofibrillary tangles consisting of tau protein. The ultrastructural appearance of the neurofibrillary tangles of progressive supranuclear palsy differs from those in Alzheimer's disease in appearing as predominantly straight filaments. The immunoelectrophoretic properties of the abnormal tau protein also differ; in progressive supranuclear palsy major bands are seen at 64 and 68 kDa while the Alzheimer's disease isoforms appear as a triplet of major bands with molecular weights of 58, 64, and 68 kDa.<sup>2</sup> Tau is expressed as six different isoforms in adult human brain varying in the presence of three or four microtubule binding domains and the presence or absence of an amino terminus insert.<sup>3</sup> Some other neurodegenerative conditions are characterised by tau deposition ("tauopathies") in different forms, including corticobasal degeneration, some forms of frontotemporal dementia, postencephalitic parkinsonism, and the parkinsonism-dementia complex of Guam.<sup>4</sup> The different molecular pathological characteristics of these diseases may relate to the differential expression of tau isoforms.<sup>5-7</sup>

In 1997 Conrad *et al* described the first evidence that there may be a genetic predisposition to sporadic progressive supranuclear palsy involving the *tau* gene.<sup>8</sup> They reported an association between the A0 allele of *tau* and the disease. The A0 allele is defined by the occurrence of 11 TG repeats in intron 11 of *tau*, alleles A1, A2, A3, and A4 defined by the presence of 12, 13, 14, or 15 TG repeats respectively. Both the A0 allele and the A0/A0 genotype were significantly overrepresented in patients with progressive supranuclear palsy compared with normal controls and patients with Alzheimer's disease.<sup>8</sup> Since this publication, this finding has been replicated in a further small North American series,<sup>9</sup> but there have been no further insights into the function of this polymorphism or reports of its role in other tauopathies. We have studied this polymorphism in a larger population of British patients with progressive supranuclear palsy and analysed this polymorphism in other con-

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Tau alleles and genotypes. Number in each group with percentages in parentheses

	Control	PD	PSP	FTD	CBD
n	75	50	53	32	13
Alleles:					
n	150	100	106	64	26
A0	109 (72.7)	75 (75.0)	96 (90.6)	51 (79.7)	20 (76.9)
A1	12 (8.0)	6 (6.0)	4 (3.8)	1 (1.6)	0 (0)
A2	2 (1.3)	1 (1.0)	0 (0)	2 (3.1)	0 (0)
A3	27 (18.0)	18 (18.0)	6 (5.7)	9 (14.1)	6 (23.1)
A4	0 (0)	0 (0)	0 (0)	1 (1.6)	0 (0)
Genotypes:					
n	75	50	53	32	13
A0/A0	40 (53)	29 (58.0)	44 (84.2)	20 (62.5)	8 (61.5)
A0/A1	9 (12)	4 (8.0)	4 (7.0)	1 (3.1)	0 (0)
A0/A2	0 (0)	1 (2.0)	0 (0)	2 (6.3)	0 (0)
A0/A3	20 (27.0)	12 (24.0)	4 (7.0)	8 (25.0)	4 (30.8)
A1/A3	3 (4.0)	2 (4.0)	0 (0)	0 (0)	0 (0)
A2/A2	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
A3/A3	2 (2.7)	2 (4.0)	1 (1.9)	0 (0)	1 (7.7)
A3/A4	0 (0)	0 (0)	0 (0)	1 (3.1)	0 (0)

PD=Parkinson's disease; PSP=progressive supranuclear palsy; FTD=frontotemporal dementia; CBD=corticobasal degeneration.

ditions which involve tau deposition—frontotemporal dementia, and corticobasal degeneration.

### Methods

Subjects with progressive supranuclear palsy met a modified NINDS diagnostic criteria for clinically probable or clinically possible progressive supranuclear palsy.<sup>10</sup> Although prominent early postural instability was used as a positive inclusion criteria, falls in the first symptomatic year were not considered mandatory and diagnostic exclusion criteria were applied as available from case notes. Pathological confirmation of the diagnosis of progressive supranuclear palsy was made following standardised criteria and these pathologically diagnosed cases included 12 cases with atypical clinical presentations or incomplete clinical details.<sup>11</sup> Corticobasal degeneration was diagnosed in patients with non-levodopa responsive parkinsonism and evidence of parietal cortical dysfunction, such as an alien limb phenomenon, cortical sensory loss, or cortical myoclonus and on pathological grounds in one case. Frontotemporal dementia was diagnosed using the Lund/Manchester criteria.<sup>12</sup>

DNA was extracted from frozen brain, paraffin fixed brain, and white blood cells with a phenol-chloroform extraction, following proteinase K incubation. The polymerase chain reaction (PCR) primers and conditions described by Conrad *et al* were used to amplify the region of interest (Genbank Accession No L77209).<sup>8</sup> One hundred nanograms genomic DNA was denatured at 95°C for 5 minutes and cycled with 5 pmol fluorescent labelled forward primer and 5 pmol unlabelled reverse primer for 26 cycles with denaturing, annealing, and extension temperatures of 95, 58, and 72°C, for 30, 30, and 15 seconds, respectively, with 1.25 units of Taq polymerase (Promega) in standard buffer containing 1.5 mM MgCl<sub>2</sub>. A final extension step at 72°C for 5 minutes was used. The products were analysed using an ABI 377 DNA sequencer and compared with a standard size control. DNA from a representative sample which was homozygous for the smallest allele identified in our series, was sequenced using a dye terminator reaction kit

(Perkin-Elmer) and ABI 373 DNA sequencer. It was confirmed that this smallest allele contained 11 TG repeats, and was designated A0, following the classification of Conrad *et al*.<sup>8</sup>

The project was approved by the ethics committee of the National Hospital, Queen Square. The subjects were unrelated white Europeans.

To test the proposed effect of the A0 polymorphism the frequencies of the alleles and genotypes were calculated in the control, progressive supranuclear palsy, Parkinson's disease, frontotemporal dementia, and corticobasal degeneration groups and comparisons were made of the A0 allele compared with all other alleles, and A0 homozygotes compared with heterozygotes and other genotypes using a  $\chi^2$  test.

### Results

Seventy five controls, 53 patients with progressive supranuclear palsy (including 25 pathological cases), 50 patients with pathologically confirmed Parkinson's disease, 32 patients with frontotemporal dementia, and 13 patients with corticobasal degeneration were studied (table). The A0 allele was overrepresented in the progressive supranuclear palsy population compared with the control population (91% *v* 73%,  $\chi^2 = 14.3$ , *df*=1, *p*<0.001) and the A0/A0 genotype was also significantly more common (84% *v* 53%,  $\chi^2 = 12.3$ , *df*=2, *p*<0.01). This effect was less in the group of patients with pathologically diagnosed progressive supranuclear palsy (A0/A0 genotype frequency 72%) and this was largely accounted for by the clinically atypical/incompletely documented group (A0/A0 frequency in clinically typical group 85%; clinically atypical 66%). The other tauopathies studied however, showed no association effect for either the A0 allele ( $\chi^2 < 2$ , *df*=1, *p*>0.1) or the A0/A0 genotype ( $\chi^2 < 1$ , *df*=2, *p*>0.5) in all comparisons. The odds ratio for the effect of A0/A0 genotype on the development of progressive supranuclear palsy is 4.3 (95% confidence interval 3.6–10.1). However, as this is a rare disease (1.4/100 000),<sup>13</sup> the positive predictive value of A0/A0 genotype status in the general population for its development is <0.1%.

### Discussion

This study has confirmed an association between the tau A0 allele and A0/A0 genotype and the development of progressive supranuclear palsy in the largest series reported to date. Genetic association studies may produce positive results due to (1) a direct functional effect of the polymorphism on disease development; (2) linkage disequilibrium between the polymorphism and an adjacent functionally important genomic area, and (3) bias introduced by population stratification. Our study, in confirming the results of Conrad *et al* and Higgins *et al* in smaller North American series, suggests that the predisposition effect of the A0 allele to the development of progressive supranuclear palsy is not due to population stratification.<sup>8,9</sup> This study shows a progressive supranuclear palsy versus control prevalence of the A0/A0 genotype of 84% versus 53%, which is similar

to the other studies.<sup>8,9</sup> This polymorphism predisposition is the first aetiological factor in the development of the disease that has been demonstrated, with studies investigating an effect of apolipoprotein E genotype and an association with an  $\alpha$ -synuclein gene marker having been negative.<sup>9,14,15</sup>

A possible functional role for this intronic region or closely linked areas is suggested by its location. The polymorphism lies 5' of the alternatively spliced exon 10 which codes for the second tau microtubule binding repeat domain. Inclusion of exon 10 results in four microtubule binding repeat domain isoforms of the tau gene.<sup>3</sup> The use of exon specific tau antibodies and postmortem tissue based reverse transcriptase PCR techniques is leading to a classification of tauopathies based on the isoforms of tau expressed. In progressive supranuclear palsy, neurofibrillary tangles consist of four repeat tau,<sup>16</sup> whereas in Pick's disease three repeat tau seems to predominate.<sup>5</sup>

The importance of tau isoform expression in the pathology of neurodegenerative disease has recently been highlighted by the investigation of chromosome 17 linked dementia.<sup>6,7,17</sup> In this condition it has been shown that, in addition to coding mutations, mutations which disrupt a stem loop structure at the tau exon 10 intron-exon junction cause a switch to predominant expression of four repeat tau which is associated with the development of neurofibrillary tangles and neuronal death.<sup>7</sup> It is possible that homozygosity for the A0 allele or an adjacent linked functional area also increases the level of transcription of four repeat tau which in conjunction with other genetic or environmental factors leads to the pathological deposition of four repeat tau in patients with progressive supranuclear palsy. The lower frequency of A0/A0 homozygosity in the clinically atypical cases may relate to altered tau isoform expression in this disease variant.<sup>18</sup> The effect of environmental factors or other genetic factors is presumably large given the high frequency of the A0/A0 genotype in the normal population, illustrated by the low positive predictive value of A0/A0 homozygosity for developing the disease.

This study is the first to investigate the A0 polymorphism in frontotemporal dementia and corticobasal degeneration, in a small number of cases, and shows no association between this allele and the development of these diseases. Although corticobasal degeneration invariably involves tau deposition, the group of patients with clinically diagnosed frontotemporal dementia is likely to be pathologically heterogeneous and include some cases in which there is no tau deposition, in addition to some patients with the tau deposition of Pick's disease, corticobasal degeneration, and chromosome 17 linked dementia.<sup>12,19</sup> The lack of an association of the A0 allele with corticobasal degeneration and frontotemporal dementia may relate to

different pathological processes in these diseases, related to the presence of tau and its specific isoform expression.<sup>16</sup>

In conclusion, future research on the molecular aetiology of progressive supranuclear palsy should be directed to the possible functional influence of the A0 tau polymorphism and adjacent polymorphic areas of the tau gene on the differential expression of tau isoforms and to the additional factors which determine the development of neurofibrillary tangles and neuronal death.

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