Sequence analysis of tau 3′untranslated region and saitohin gene in sporadic progressive supranuclear palsy

M Ezquerra, J Campedelacreu, E Muñoz, R Oliva, E Tolosa

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Background: The extended tau H1 haplotype has previously been described in association with progressive supranuclear palsy (PSP). Recently, a new gene called saitohin (STH), nested within an intron of tau, has been discovered. The Q7R polymorphism of STH appears to be related to late onset Alzheimer’s disease.

Objectives: To search for genetic changes in the 3′untranslated region (3′UTR) of tau and adjacent sequence LOC147077, and in the coding region of STH in PSP patients.

Methods: The study included 57 PSP patients and 83 healthy controls. The genetic analysis of each region was performed through sequencing. The Q7R polymorphism was studied through restriction enzyme and electrophoresis analysis.

Results: No mutations were found in the regions analysed. The QQ genotype of the STH polymorphism was over-represented in participants with PSP (91.5%) compared with control subjects (47%) (p<0.00001). This genotype co-segregated with the H1/H1 haplotype in our PSP cases.

Conclusions: Our results do not support a major role for the tau 3′UTR in PSP genetics. The QQ genotype of STH confers susceptibility for PSP and is in linkage disequilibrium with the H1/H1 haplotype.

Progressive supranuclear palsy (PSP) is a parkinsonian syndrome accompanied by supranuclear gaze palsy, pseudobulbar signs, axial dystonia, postural instability, frontal dementia, and a poor response to levodopa. In typical PSP, aberrant forms of the microtubule associated protein tau precipitate in subcortical neurons and glial cells, leading to neurofibrillary tangles (NFTs). The NFTs and other protein tau isoforms are produced by hyperphosphorylated tau species.2 The Alzheimer’s disease, or corticobasal ganglionic degeneration, leading to NFTs and other non-codifying regions of tau could be responsible for PSP.

So far only four mutations in tau have been described in cases of atypical PSP: the R406W missense mutation, the S305S silent mutation, the homozygous delN296 mutation, and the missense R5L mutation. Many studies have failed to identify a causative mutation after analysing the entire coding and promoter regions of tau in typical PSP patients. Thus, these data suggest that a separate gene or other non-codifying regions of tau could be responsible for PSP.

Recently, a gene called saitohin (STH) was discovered in the intron between exons 9 and 10 of tau. STH expression is similar to tau, and a polymorphism in this gene appears to be associated with late onset Alzheimer’s disease.20 In this work, we have analysed the tau 3′untranslated region (3′UTR), the adjacent locus LOC147077 (NEDO human cDNA sequencing project, unpublished), and STH in order to search for mutations or new polymorphisms in typical PSP patients.

METHODS

Subjects

From the neurology service of our hospital we recruited 57 unrelated subjects (26 male, 31 female), who met the clinical diagnostic criteria for probable PSP. 61 63 67 83 healthy controls (34 male, 49 female) were recruited from among patients’ spouses, and healthy volunteers. Informed consent was previously obtained from all participants. The mean age of patients at the onset of PSP was 70 (5.5) years, and the mean age of the controls was 68.9 (7.5) years.

Genetic analysis

Genomic DNA was extracted from peripheral blood using standard procedures. STH amplification, sequencing assay, and Q7R polymorphism detection were performed as previously described 20. The tau promoter polymorphism was also genotyped as previously described.

Three pairs of primers were designed in order to amplify different overlapping fragments of the whole tau 3′UTR using the DNAstar software. The sequences of the designed forward and reverse tau primers were: 5′-ATCTCAGCAATGTCTCCTCAC-3′ and 5′-GGCTTCTCTCCACACTCC-3′ for fragment 1 (annealing 57°C); 5′-CAGTTGCGATGGGACGCAAAG-3′ and 5′-CCAGCCGCTCAAGACATAAG-3′ for fragment 2 (annealing 62°C); and 5′-TCGATGATGACCTCCTTAGAAA-3′ and 5′-GTACCTTCCTGAACCAAACC-3′ for fragment 3 (annealing 57°C). For the amplification of the LOC147077 sequence the primers were 5′-GGTTGCTTCCGCTTGTG-3′ and 5′-AGTCCTAATCCGTTGCTTCA-3′ (annealing 56°C). The PCR mix was constituted in a total volume of 25 μl and consisted of: 1 μl of each primer (30 pmol/μl); 4 μl of

Abbreviations: PSP, progressive supranuclear palsy; UTR, untranslated region; NFT, neurofibrillary tangle; FTD, frontotemporal dementia
Statistical analysis
The genotypic and allelic distribution of the STH polymorphism was analysed with a χ² test. All analyses were performed using computer software SPSS 10.0 for Windows (SPSS, Chicago, USA).

RESULTS
Sequencing of the tau 3’UTR, the adjacent locus LOC147077, and the STH coding region showed no mutations in three subjects with sporadic PSP. Analysis of the STH Q7R polymorphism allowed us to identify three different genotypes in patients and controls (fig 1). The frequencies revealed that the QQ genotype was present in 91.2% of patients and in only 47% of controls (p < 0.000001).

The analysis of the tau promoter G(–221)C polymorphism in participants with PSP** showed that the CC genotype cosegregated completely with the QQ genotype.

DISCUSSION
Many polymorphisms, in or near tau, have been described as associated with PSP and leading to an extended H1 haplotype.¹ ¹¹ This association might be due to linkage disequilibrium between these polymorphisms and a hypothetical adjacent functional mutation responsible for the disease. The half-lives and subcellular localisation of specific mRNAs may depend on specific sequences in 3’UTR.²³ Therefore, alterations in this region could potentially be responsible for some pathological processes. However, we did not find any genetic changes after sequencing this region and adjacent locus LOC147077 in three typical cases of PSP.

We found that the QQ genotype of the STH polymorphism was over-represented in our patients with PSP. This genotype is segregated with the H1 haplotype because it is in complete disequilibrium with the CC genotype of tau promoter G(–221)C polymorphism, which in turn segregates with this haplotype as we previously described.¹³ After sequencing the entire coding region of STH, we could not demonstrate additional mutations. Therefore, the STH polymorphism, which gives rise to an amino acid change, could behave as a risk factor for PSP. The exact function of STH is still unknown; however, the fact that STH and tau have very similar expression patterns suggests that these two proteins could function together in physiological or pathological processes.²⁰ Interestingly, the QQ genotype is the opposite of the genotype associated with Alzheimer’s disease (RR).²⁰ As STH nests in the intron between exons 9 and 10 of tau, we cannot rule out the possibility that STH polymorphism, through the regulation of exon 10 alternative splicing, may explain the different expression of tau isoforms in PSP and Alzheimer’s disease.

In conclusion, our results do not support a major pathogenic role of the tau 3’UTR in PSP genetics. The QQ genotype of the STH polymorphism may be considered a risk factor for PSP. Functional studies of STH could be very important in disclosing whether it plays a role in tau splicing or tau phosphorylation.

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REFERENCES

Table 1 Saithohin polymorphism analysis in subjects with progressive supranuclear palsy (PSP) and healthy controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Genotypes</th>
<th>Genes</th>
<th>Alleles</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>QQ (%)</td>
<td>QR (%)</td>
<td>RR (%)</td>
</tr>
<tr>
<td>PSP (n = 57)</td>
<td>52 (91.2)</td>
<td>5 (8.8)</td>
<td>0</td>
</tr>
<tr>
<td>Controls (n = 83)</td>
<td>39 (47)</td>
<td>40 (48.2)</td>
<td>4 (4.8%)</td>
</tr>
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</table>

n, number. *p < 0.000001 (χ² test).