A New Mutation of the \( \tau \) Gene, G303V, in Early-Onset Familial Progressive Supranuclear Palsy

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Background: Progressive supranuclear palsy (PSP) is a clinicopathological syndrome related to \( \tau \) deposits and in linkage disequilibrium with \( \tau \) polymorphisms. Some rare familial PSP cases have been related to \( \tau \) gene mutations.

Objective: To present the clinical, pathological, and molecular data of one family with early-onset autosomal dominant PSP.

Design: We performed clinical examinations, quantitative neurological tests, positron emission tomographic scans with fluorodopa F 18 and raclopride C 11, analysis of \( \tau \) mutations, neuropathological examinations, and protein analyses on brain specimens.

Results: Three family members had PSP confirmed by pathological features in the proband. A novel mutation of \( \tau \), G303V, was found in the proband and other family members. \( \tau \) isoforms with 4 microtubule-binding repeats were overexpressed in the proband brain.

Conclusions: The G303V mutation of \( \tau \) is associated with autosomal dominant PSP. Expression of 4 microtubule-binding repeat \( \tau \) isoforms is increased in the proband.

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The proband’s brain was divided into 2 halves for neuropathological and biochemical analyses. Paraffin-embedded sections were stained with hematoxylin-eosin, Nissl, and Gallyas, and immunostained with a polyclonal antibody against the C-terminal region of human H9270 (A024; Dako, Trappes, France).

### PROTEIN EXTRACTS AND WESTERN BLOTS

Protein samples from the frontal cortex, temporal cortex, striatum (St), and thalamus were obtained from the proband, a patient with Alzheimer disease, and a control subject without dementia. Soluble and particulate protein samples from Sarkosyl extracts, containing aggregated τ phosphoproteins, were separated in sodium dodecyl sulfate–polyacrylamide gels, electroblotted onto nitrocellulose sheets, incubated with specific antibodies, and developed with the ECL system (Amersham, Braunschweig, Germany).

Antibodies used include 7.51, raised against a phosphorylation-independent epitope located at the microtubule-binding region of τ, and PHF-1, recognizing phosphorylated τ at serine positions 396 and 404.

### REVERSE TRANSCRIPTION–PCR ANALYSIS OF τ ISOFORMS

The expression of messenger RNA containing or lacking exon 10 was studied by reverse transcription–PCR on complementary DNA samples obtained from striatal samples of the previously mentioned cases.

### RESULTS

#### DESCRIPTION OF THE FAMILY

This family was partially described earlier (Figure 1A). Three members were diagnosed as having PSP, according to neuropathological or clinical criteria. The clinical features were as follows.

The proband (individual III:5), at the age of 37 years, developed akinetic-rigid syndrome, gait disturbance, frequent falls and micrographia, dysarthria, difficulty in convergence, abolition of upgaze, apraxia of eyelid opening, and exaggerated stretch reflexes with extensor left plantar response. The result of a cognitive examination showed a Mini-Mental State Examination score of 22 of 30 and a Quick Frontal Efficiency Scale score of 11 of 18.

The mean fluorodopa F 18 Ki dopa influx rate constant (Kᵢ) values were 3.3 × 10⁻³ min⁻¹ (caudate) and 3.2 × 10⁻³ min⁻¹ (putamen) (Figure 2A), reduced by 70.8% and 70.9%, respectively, compared with controls. Fluorodopa F 18 uptake was asymmetrical in the patient (Kᵢ values were 4.4 × 10⁻³ min⁻¹ and 2.3 × 10⁻³ min⁻¹ for the right and left sides of the caudate nucleus, respectively, and 3.7 × 10⁻³ min⁻¹ and 2.8 × 10⁻³ min⁻¹ for the right and left sides of the putamen, respectively). Mean raclopride C 11 uptake was similar and normal in the striatum (Figure 2B), with values of 3.5 × 10⁻³ min⁻¹ and 3.8 × 10⁻³ min⁻¹ for the putamen and caudate nucleus, respectively.

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**Figure 1.** Genealogical tree of family members (A) and single-stranded conformational polymorphism (SSCP) analysis (B). In A, the schematic family tree shows the presence of progressive supranuclear palsy (PSP) in the proband (individual III:5), with a G303V mutation of τ, and in 2 members of the family (II:3 and III:1). In B, SSCP analysis of exon 10 in family members revealed an abnormal band pattern in the proband (III:5) and in individuals 4 through 6, who represent asymptomatic heterozygote carriers. The sex and genealogical order of the members of the family were masked for confidentiality. Shaded symbols with a diagonal line indicate deceased individuals who had PSP; unshaded symbols, healthy individuals.

**Figure 2.** Representative transaxial positron emission tomographic images of the proband: a fluorodopa F 18 scan shows a major reduction of fluorodopa F 18 uptake in the striatum, with clear asymmetry (A; caudate), and raclopride C 11 uptake in the striatum is normal (B; putamen).
This patient worsened slowly, developing axial dystonia, mutism, complete vertical gaze palsy, slow horizontal saccades, severe dysphagia, and weight loss requiring percutaneous gastrostomy. She died at the age of 45 years.

The proband’s mother (individual II:3) developed PSP at the age of 41 years and died at the age of 45 years.

The proband’s sister (individual III:1) developed PSP in her late 30s and died at the age of 41 years.
Seven family members were tested for the G303V mutation (Figure 1B). Three asymptomatic family members, younger than the average age at onset of disease in this family, are carriers of the mutation (Figure 1B). Two of them were examined and showed normal neurological and neuropsychological examination results, brain magnetic resonance imaging findings, and fluorodopa F 18 uptake.

NEUROPATHOLOGICAL EXAMINATION

There was atrophy of the mesencephalon, pons, striatum, and subthalamic nuclei and depigmentation of the substantia nigra. The lateral ventricles were enlarged, and the frontotemporal cortex showed mild atrophy.  
τ Protein accumulation took place in neurons and glia, predominantly in the mesencephalon. In neurons, τ aggregated into neurofibrillary tangles detectable by immunostaining and silver staining. τ Immunostaining was also intense in affected astrocytes. Neuronal loss, mild spongiosis and gliosis, and numerous neurofibrillary tangles were observed in the substantia nigra (Figure 3A). No Lewy bodies were seen. Similar lesions were present in the red nucleus, locus coeruleus (Figure 3B), nuclei of the third (Figure 3C and D) and fifth cranial nerves, and nucleus ambiguous. Globose and flame-shaped neurofibrillary tangles were present in these nuclei but not in the nuclei of the 4th and 12th cranial nerves. In addition to tangles, “grumous-degenerative” neurons were observed in the superior colliculus.

Atrophy and neuronal loss were also severe in the globus pallidus and subthalamic nucleus, mild in the hippocampus and parahippocampal gyrus, and moderate in the striatum. In all of these regions, τ accumulation was shown in neurons and glia (Figure 3E). Mild frontal and temporal atrophy was observed with neuronal loss, gliosis, and microvacuolation surrounded by reactive astrocytes and a few τ-positive cells. No significant atrophy was observed in the parietal and occipital cortices, but gliosis and a few τ protein–positive cells were present.

In the dentate nucleus, neurons appeared achromatic, accumulated swollen eosinophilic material, and showed grumous degeneration with accumulation of τ protein (Figure 3F). The lesions did not affect cerebellar white matter or the cerebellar cortex.

MOLECULAR ANALYSIS

Single-stranded conformational polymorphism analysis of τ exon 10 in the proband revealed a DNA band with an abnormal electrophoretic pattern. Direct sequencing analysis identified a mutation that consists of a G→T transversion at position 2095 of the complementary DNA (Figure 4A). This mutation changes the glycine residue at position 303 of the protein to valine (G303V). The proband and 3 asymptomatic young relatives were heterozygotes for the mutation (Figure 1B). The deceased and clinically affected members of this family were obligate carriers of the mutation. The mutation was not found in 194 unrelated control chromosomes. The absolute conservation of glycine at position 303 in all human τ repeats, in MAP2 and MAP4 (microtubule-associated proteins), and in the second repeat of τ from different species (Figure 4B) and the absence of this mutation in 194 unrelated control chromosomes suggest that G303V is a mutation and not a polymorphism.

τ PROTEIN ISOFORMS IN THE PROBAND BRAIN

The patterns of bands of total τ protein, immunoreactive to antibody 7.51 (Figure 5A), and the fraction immunoreactive to PHF–1 (Figure 5B) indicate that the proteins with higher electrophoretic mobility present in the Alzheimer disease–affected brain are absent in the proband. PHF–1 immunoreactivity in Sarkosyl-insoluble pro-
We analyzed the expression of \(\tau\) isoforms containing or lacking exon 10 by reverse transcription–PCR analysis of messenger RNA samples from the striatum. A proband sample showed an increased proportion of \(\tau\) complementary DNA containing exon 10 compared with the other samples (Figure 7). Amplified control DNA samples containing, or lacking, exon 10 (4R and 3R) are indicated.

Herein, we describe a family with early-onset autosomal dominant PSP due to a novel mutation in the \(\tau\) protein, G303V. The pathogenesis of this disease seems related to the overexpression of the 4R \(\tau\) isoform and its hyperphosphorylation. These findings result in a pattern of proteins with relative mobility of 68 and 64 kDa and, in a small amount, of a 72-kDa protein.

In Alzheimer disease and other FTDP-17 cases, an additional band around 60 kDa is present. These 72-, 68-, 64-, and 60-kDa protein bands may correspond to the 6 different central nervous system \(\tau\)-phosphorylated isoforms,\(^{13,14}\) but only the 72-, 68-, and 64-kDa proteins contain 4R \(\tau\) (Figure 5D). Thus, the G303V mutation results in 4R \(\tau\), as in other PSP cases.

Mutations of the \(\tau\) gene are excluded in most patients with PSP,\(^{13,16}\) but PSP is on linkage disequilibrium for certain polymorphisms and haplotypes of the \(\tau\) gene. Moreover, patients with mutations in the \(\tau\) gene may develop atypical PSP features.\(^7,17\)

Around 100 families with mutations in the \(\tau\) gene have been described. Most of these patients are characterized by either frontal dementia with apathy, desinhibition, hyperorality, or hypersexuality or by atypical, atremoric parkinsonism, axial dystonia, and poor response to dopaminomimetic agents. Typical PSP and FTDP-17 patients are easy to distinguish because they first develop early gate disturbance and prominent brainstem and basal ganglia signs, while the latter usually develop early cognitive deficits or atypical parkinsonian syndromes, while gait palsy and gait disorders appear late. Atypical cases of both disorders are difficult to differentiate because the clinical phenotypes are variable.\(^{18}\)

The patients described herein had clinicopathological features of PSP with early age at onset. Supranuclear gaze palsy and gait disorders began early, and dementia appeared late or was questionable. The reduced fluorodopa F 18 uptake was typical of PSP.\(^9\) However, binding of raclopride C 11 was normal and did not suggest dropout of intrinsic striatal neurons. Normal raclopride binding has also been described in some patients with FTDP-17.

The novel G303V mutation of \(\tau\) seems responsible for this familial disease for the following reasons. (1) It cosegregates with the disease, because it was present in the proband and the other 2 affected members, who were obligate carriers. The 3 asymptomatic gene carriers of the
Mutation are younger than the mean age at onset for developing symptoms and may become involved in the future. (2) The mutation does not occur in the healthy population. (3) It takes place in a highly conserved codon of protein in several mammal species. (4) The mutation may produce a change in the function of protein.

Mutations interfere with protein function by modifying the splicing of exon 10, which alters the normal proportion between the 4R and 3R isoforms, and the degree of phosphorylation, which makes less efficient in its binding to tubulin and favors its self-assembly.

The mutation G272V, described in an FTDP-17–affected patient, changes the same amino acids in the same motif (PGGG) as the G303V mutation. However, the consequences of both mutations are different. For the G272V mutation, was hyperphosphorylated, but self-assembly in vitro was modest and the 4R/3R ratio was unchanged. For the G303V mutation, aggregates were present and the 4R/3R ratio was increased, typical features of PSP cases. Thus, other pathogenic mechanisms of action of the G303V mutation should be further investigated.

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