Association of polymorphisms in the *Tau* and *Saitohin* genes with Parkinson's disease

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Background: The *Saitohin* gene has recently been identified in intron 9 of the *Tau* gene. Because an association between Parkinson's disease and *Tau* has been described, *Saitohin* represents a candidate gene for Parkinson's disease.
Objective: To test these two genes for their association with Parkinson's disease in a large community based case–control study.
Results: Cases (n = 208) were more often homozygotes for the *Tau* H1 haplotype than controls (n = 483; odds ratio (OR) = 1.71 (95% confidence interval, 1.20 to 2.43); p = 0.003), and the saitohin Q allele was in complete linkage disequilibrium with the H1 haplotype. This association was stronger among cases with Parkinson's disease onset below 65 years (⩽65 years: OR = 2.52 (1.49 to 4.25); p<0.001) than among those with older onset (>65 years: OR = 1.20 (0.73 to 1.98); p<0.47).
Conclusions: The data suggest that there is a functional polymorphism at this locus involved in Parkinson's disease.

S everal polymorphisms scattered through the *Tau* gene have been associated with tauopathies sharing motor symptoms with Parkinson's disease.[1] [2] Moreover, mutations have been identified indicating that tau dysfunction is sufficient to cause these disorders.[1] [3] [4] Interestingly, several studies have also found an association between the *Tau* gene and Parkinson's disease.[5–10]

A gene called *Saitohin* was recently described in intron 9 of the *Tau* gene.[11] Despite the association observed between *Tau* and Parkinson's disease, tau aggregates are rarely observed in brains from affected patients. Thus a gene near the *Tau* gene may explain the association. This hypothesis has not been tested yet, so we examined the association between the *Tau* gene and Parkinson's disease in a community based study, and evaluated the implication of the *Saitohin* gene.

METHODS

Participants were recruited through a French health insurance organisation, the Mutualité Sociale Agricole (MSA), which is responsible for the reimbursement of health related expenses to workers connected with agriculture. The study has been described in detail previously.[12] [13] Cases of Parkinson's disease were recruited from among subjects submitting their first application for benefit from the free health care coverage for Parkinson's disease. The diagnosis of Parkinson's disease was established using standard criteria after examination by a neurologist or, when such an examination was not possible, using information provided by the patient's neurologist.[12–14] Controls were recruited among MSA affiliates. A maximum of three controls were matched with each case for age (±2 years), sex, and region of residency at the time of the study.

Participants provided information on a family history of Parkinson's disease among first degree relatives and on the country of birth of their parents. Information on smoking was obtained through a face to face questionnaire. Information on pesticide exposure was obtained using a two stage, case by case exposure assessment procedure.[12] [13] Subjects were classified as ever or never smokers and as ever or never pesticide users (for gardening or professional use) before the onset of Parkinson's disease (in cases) or before the index date in controls (the index date is the date of onset in the matched case).

The ethics committee of the Hôpital du Kremlin-Bicêtre approved the research protocol, and all subjects signed their informed consent.

Three exonic polymorphisms were genotyped in exons 3, 9 (variation 9iii), and 13 of the *Tau* gene.[2] The *Tau* haplotypes were constructed as reported previously.[2] The A/G polymorphism in *Saitohin* was genotyped as described.[11] Sequencing of the coding region of the *Saitohin* gene was done using the primers 5'-CCCTGTAAACTCTGACCACAC-3' and 5'-GCCACTTTTCCACACTTCGT-3'.

Statistics

Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression. Analyses were based on subjects with both parents of European origin to reduce the risk of population stratification bias; we also carried out analyses restricted to subjects with both parents born in France. Analyses of the interaction between *Tau* polymorphisms and other variables were undertaken by estimating individual and joint effects of the variables and by introducing multiplicative terms in the models.

RESULTS

Cases (n = 208) and controls (n = 483) had a mean (SD) age of 67 (7) years. The mean age at Parkinson's disease onset was 63 (7) years. Most of the cases (91%) were sporadic. The parents of 92% of cases and 93% of controls were born in France. Twenty five per cent of cases and 33% of controls were ever-smokers; 71% of cases and 68% of controls had been pesticide users.[13]

Tau haplotypes were constructed and their frequencies calculated (table 1). Polymorphisms in exon 3 and 13 of *Tau* are in complete linkage disequilibrium, and the polymorphism in exon 9 only occurs on the H1 haplotype. There were no differences in this polymorphism's frequency between cases and controls (p = 0.59); we therefore pooled carriers of the exon 9 polymorphism with the H1 group.

H1|H1 homozygotes had a greater risk of Parkinson's disease than H1|H2 and H2|H2 carriers (table 1). Similar findings were observed in analyses restricted to subjects with French parents (OR = 1.68 (95% CI, 1.14 to 2.47); p = 0.008), or without a family history of Parkinson's disease (OR = 1.68 (1.15 to 2.45); p = 0.007). An interaction between the H1 haplotype and age at Parkinson's disease onset was observed.

Table 1 Association between Parkinson's disease and the H1 haplotype in the *Tau* gene

	n	H1|H1% (n)	H1|H2% (n)	H2|H2% (n)	OR (95% CI) H1|H1 v H1|H2 + H2|H2	p Value
Overall†						
Controls	483	52.4 (253)	41.0 (198)	6.6 (32)§		
Cases	208	64.4 (134)	31.3 (65)	4.3 (9)	1.71 (1.20 to 2.43)	0.003
Men						
Controls	284	51.8 (147)	42.9 (122)	5.3 (15)		
Cases	118	65.3 (77)	30.5 (36)	4.2 (5)	1.86 (1.15 to 2.99)¶	0.01
Women						
Controls	199	53.3 (106)	38.2 (76)	8.5 (17)		
Cases	90	63.3 (57)	32.2 (29)	4.5 (4)	1.54 (0.91 to 2.63)¶	0.11
*Onset ⩽ 65 years**						
Controls	264	53.4 (141)	39.4 (104)	7.2 (19)		
Cases	116	72.4 (84)	27.6 (32)	–	2.52 (1.49 to 4.25)‡	<0.001
*Onset >65 years**						
Controls	219	51.2 (112)	42.9 (94)	5.9 (13)		
Cases	92	54.3 (50)	35.9 (33)	9.8 (9)	1.20 (0.73 to 1.98)‡	0.47

*Median age at onset.
†24 H1|H1 controls and 19 H1|H1 cases carried the exon 9 polymorphism on one of the H1 haplotypes; 13 H1|H2 controls and 1 H1|H2 case carried the exon 9 polymorphism on the H1 haplotype. No H2|H2 cases or controls carried the exon 9 polymorphism.
‡Interaction between H1|H1 and age at onset, p=0.05.
§Tau haplotypes were in Hardy-Weinberg equilibrium among controls (p=0.66).
¶Interaction between H1|H1 and sex was not significant.

The association between the *Tau* gene and Parkinson's disease was stronger among cases with onset under 65 years (median age at onset) than among patients with later onset. Moreover, in analyses restricted to cases, the frequency of H1 homozygotes was higher among younger than among older patients (72.4% *v* 54.3%; p = 0.007), while there was no difference among controls (table 1).

Saitohin polymorphism showed the same frequencies as the H1 haplotype (controls: AA = 52.4%, AG = 41.0%, GG = 6.6%; cases: AA = 64.4%, AG = 31.3%, GG = 4.3%); thus the A allele was in complete linkage disequilibrium with the H1 haplotype (fig 1). Sequencing of the *Saitohin* gene in 15 patients (10 H1|H1, 5 H2|H2) failed to detect any other nucleotide variations.

We found no interaction between the H1 haplotype and pesticide exposure or cigarette smoking. In multivariate analyses, adjusting for these two variables did not modify our findings (OR = 1.72 (1.20 to 2.50); p = 0.004).

DISCUSSION

In this large community based study, we observed a higher proportion of H1 homozygotes for the *Tau* gene in Parkinson's disease patients than in controls, and showed that the A/G polymorphism in the *Saitohin* gene was in complete linkage disequilibrium with the H1 haplotype.
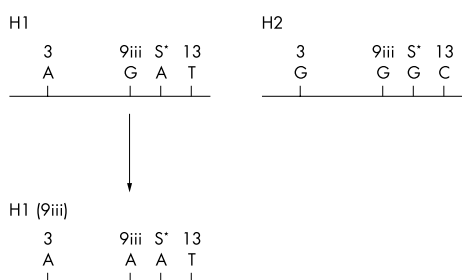

Figure 1 Schematic representation of *Tau* and *Saitohin* haplotypes. Polymorphism 9iii is only present on the more common H1 haplotype, and leads to the H1 (9iii) haplotype. S*, saitohin; the A to G substitution changes the amino acid from a glutamine (Q) to an arginine (R).

As genetic susceptibility is hypothesised to play a greater role in patients with a younger onset of Parkinson's disease,[15][16] we analysed the implication of the *Tau* haplotype according to age at onset. We observed a significantly stronger association among Parkinson's disease patients with onset below median age than among patients with an older onset. In addition, in case-only analysis, H1|H1 carriers were significantly more common among patients with a younger disease onset. This finding is in agreement with a previous study in which the frequency of H1 homozygotes among cases with an age at examination (which is correlated with the age at onset) before 71 years was higher than among older patients, although the difference did not reach significance.[8]

An association between the *Tau* gene and Parkinson's disease has been found in several studies, but not all. It has been hypothesised that in some studies the association may have occurred because they included patients with progressive supranuclear palsy (PSP) misdiagnosed as Parkinson's disease. It is important to note that our study was community based. Thus the frequency of other causes of parkinsonism misdiagnosed as Parkinson's disease is likely to be lower than in previous studies that were hospital based (where atypical cases are found more often). A correction for potential diagnostic misclassification has been carried out in several studies. We did a similar correction by assuming that 6% (n = 12) of the cases had PSP, with 88.7% of them being H1|H1.[8] After excluding these cases and their matched controls at random, the odds ratio remained significantly greater than 1 (OR = 1.60 (1.11 to 2.20), p = 0.01). Finally, some studies were underpowered to detect an odds ratio of 1.70 with 80% power.

As *Tau* and *Saitohin* polymorphisms are in linkage disequilibrium and both are associated with Parkinson's disease, our data confirm that there may be genetic susceptibility factors at this locus. This hypothesis is in agreement with the results of a complete genome screen among 174 families with idiopathic Parkinson's disease (mean age at onset, 60 (13) years) which detected significant evidence for linkage on chromosome 17q, about 8 cM from the *Tau* gene.[17]

Several observations support the potential involvement of *Saitohin* or *Tau* in the development of Parkinson's disease. On the one hand, associations between the H1 haplotype in the *Tau* gene and PSP, FTDP-17, or Parkinson's disease have been shown.[1 2 5 7–10] In FTDP-17, most of the mutations located in or around exon 10 affect the splicing regulatory element structure and lead to an increased ratio of four repeat to three repeat tau isoforms.[18 19] Exon 10 and/or downstream intronic sequences also contain a complex array of enhancer or inhibitor *cis*-acting elements that modulate the use of a weak 5' splice site.[20] FTDP-17, PSP, and Parkinson's disease are all characterised by parkinsonian symptoms. The association of FTDP-17 and PSP with the H1 haplotype may be explained by the role of tau in these disorders, but tau is not a major component of Lewy bodies, and no neurofibrillary tangles are usually observed in Parkinson's disease. However, it has been shown that α-synuclein can bind to tau and stimulate tau phosphorylation.[21] Furthermore, the overexpression of transgenic *Tau* in *Drosophila* results in neuronal death without formation of inclusions.[22] Thus *Tau* overexpression may be sufficient to cause neuronal death, without the need for the formation of fibrillar aggregates.

On the other hand, *Saitohin* appears as an interesting candidate gene for Parkinson's disease because of its gene localisation and brain expression. Both genes could share some regulatory elements. Indeed there is an overlap in their tissue expression distribution, including that in the brain. Regulatory elements (CpG islands) are associated with exon 1 of *Tau* gene, and also exon 9 near the *Saitohin* gene, suggesting the importance of this region in regulating transcription of tau or saitohin.[23] In order to identify a potential mutation explaining the observed association, we sequenced the coding region of the *Saitohin* gene in 15 patients but we did not detect other variations. However, we cannot exclude the presence of polymorphisms in the regulatory regions of the *Saitohin* gene, or a change in methylation in the CpG islands around exons 1 and 9.

Our data do not allow us to discriminate between *Saitohin* and *Tau* genes. Additional work is therefore needed before we can fully understand the associations observed at this locus with the development of Parkinson's disease, and implicate one of these two genes or another nearby. It would be of interest to sequence the entire gene of this locus and to search for the presence of saitohin in Lewy bodies.

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