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

C Leveque, A Elbaz, J Clavel, J S Vidal, P Amouyel, A Alpérovitch, C Tzourio, M C Chartier-Harlin

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.

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.12–17 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 in several neurological centres in France. 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 9ii), 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′-CCCTGAAAACCTGACCACAC-3′ and 5′-GCCACTTTCACACCACCTTCGT-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.
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% vs 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.

As genetic susceptibility is hypothesised to play a greater role in patients with a younger onset of Parkinson’s disease, 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 analyses, H1|H1 carriers were significantly more common among patients with a younger disease onset. This finding is in agreement with previous studies which showed a significantly higher 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.

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 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% of cases had PSP, with 88.7% of them being H1|H1. 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.
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.\(^1\)\(^\text{2}^\text{5}\)\(^\text{7}^\text{10}\) 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.\(^\text{19}^\text{20}\) 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.\(^\text{21}\) 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.\(^\text{21}\) Furthermore, the overexpression of transgenic Tau in Drosophila results in neuronal death without formation of inclusions.\(^\text{22}\) 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.\(^\text{23}\) 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.

**ACKNOWLEDGEMENTS**

We thank all the MSA (Mutualité Sociale Agricole) physicians who interviewed the participants and the MSA employees who helped with the coordination of the study. Drs David Gervais and Lamia Benslima participated in the examination of Parkinson’s disease patients. Mrs Chantal Cordelier and Mrs Carline Amiel, Frédérique Brunner, and Jean-Philippe Galanaud helped with the assessment of exposure to pesticides. Dr Safia Zenagui participated in the coordination of the study. We thank Prakash Rucktooa, Aurélie Melchior, Xavier Hermant, and Vincent Mourgues for their technical assistance. The study was supported by the INSERM, the Institut Pasteur de Lille, the MSA, and the French Ministry of the Environment. CL is a recipient of the Ministère de la Recherche et de la Technologie.

**Authors’ affiliations**

C Leveque, P Amouyel, M C Chartier-Harlin, INSERM Unit 508, Institut Pasteur de Lille, Lille, France

A Elbaz, A Alperovitch, C Tzourio, INSERM Unit 360, Hôpital de la Salpêtrière, Paris, France

J Clavel, INSERM Unit 170, 16 Avenue Paul Vaillant-Couturier, Villejuif, France

J S Vidal, Service de Neurologie, Hôpital Saint-Antoine, Paris, France

Competing interests: none declared

**Correspondence to:** Dr M C Chartier-Harlin, INSERM Unit 508 Institut Pasteur de Lille, 1, rue du Pr Calmette, BP 85901 Lille Cedex, France; marie-christine.chartier@pasteur-lille.fr

 Received 31 March 2003

In revised form 11 July 2003

Accepted 11 July 2003

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