Tau gene and Parkinson's disease: a case-control study and meta-analysis

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Objective: To investigate whether the tau H1 haplotype is a genetic risk factor in Parkinson's disease and to report a meta-analysis on all previously published data

Methods and results: In a sample of 580 patients with Parkinson's disease and 513 controls there was an increased risk of Parkinson's disease for both the tau H1 haplotype ($p \leq 0.0064$; odds ratio (OR) 1.34 (95% confidence interval (CI), 1.04 to 1.72)) and the H1H1 genotype ($p \leq 0.0047$; OR 1.42 (1.1 to 1.83)). Under a fixed effect model, the summary OR for this showed that individuals homozygous for the H1 allele were 1.57 times more likely to develop Parkinson's disease than individuals carrying the H2 allele (95% CI 1.33 to 1.85; $p < 0.00001$). The population attributable risk for the tau variant, for the main comparison of H1H1 against H2 carriers, was 24.8% for all studies combined.

Conclusions: Homozygosity for the tau H1 is associated with an increased risk of Parkinson's disease. This adds to the growing body of evidence that common genetic variation contributes to the pathogenesis of this disorder.

There is good evidence implicating the tau gene (MAPT) in the pathogenesis of Parkinson's disease. A large, family based genome-wide search of late onset cases reported linkage to the tau locus on chromosome 17q,¹ and, while no tau gene mutations have been found in typical Parkinson's disease, mutations have been reported in cases of fronto-temporal dementia with parkinsonism on chromosome 17 (FTDP-17).²

Recent evidence suggests that tau can co-aggregate with α synuclein in the Lewy body,³ and Giasson *et al*⁴ have shown that tau and α synuclein interact to promote and propagate the polymerisation of each component into fibrils. In addition, tau aggregation—sometimes in the absence of Lewy body pathology—has been demonstrated in autosomal recessive Parkinson's disease caused by parkin gene mutations.⁵

Nine genetic case-control association studies have evaluated the association between the tau gene and Parkinson's disease.⁶⁻¹⁴ However, the results from these individually underpowered studies have failed to resolve this question. We have therefore carried out a new and larger case-control association study assessing tau and Parkinson's disease in the British population. We then combined our results with all previous studies in a meta-analysis to provide a more precise estimate of the tau-Parkinson's disease association.

METHODS

The project was approved by the joint research ethics committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery (NHNN), London, UK.

Study population

In all, 580 patients with Parkinson's disease and 513 controls participated in the study. The Parkinson's disease cohort consisted of 193 sporadic cases, 263 unrelated patients with onset of disease before the age of 50 years (YOPD), and 124 index familial cases with one or more relative also affected by Parkinson's disease (FPD). The following were excluded from the study:

- familial cases where the family history was clearly autosomal dominant or recessive;
- individuals harbouring parkin (PARK2) gene mutations ($n = 12$);
- individual carriers for pathogenic DJ-1 mutations ($n = 2$).

However, not all cases were screened for mutations in parkin and only 400 cases were screened for mutations in DJ-1. Sixty patients had pathologically proven Lewy body Parkinson's disease.

All living patients were diagnosed clinically by at least one consultant neurologist with an interest in movement disorders and in accordance with the modified Queen Square Brain Bank criteria for Parkinson's disease.¹⁵

The control cohort consisted of unrelated patient spouses ($n = 168$), healthy volunteers ($n = 66$), and unrelated patients seen at the NHNN for other neurological disorders including peripheral neuropathy ($n = 104$), Huntington's disease ($n = 69$), ataxia ($n = 41$), and epilepsy ($n = 65$). All control subjects had been assessed by a consultant neurologist, and none had parkinsonism.

Patients and controls were all white British and lived in south east England. Both groups were matched for age (Parkinson's disease, 53.8 years (age of onset); control, 56.1 years) and sex (male 52% v 49%). A similar proportion of patients and controls were in the age categories <45 years, 45 to 65 years, and >65 years. Neither group had previously participated in studies relating to the tau gene.

Genotyping

The tau haplotype was inferred as previously described by assessing for the presence or absence of a 238 base pair deletion in intron 9 of the H2 haplotype,¹⁶ with presence defining H1 and absence defining H2. Genotyping was conducted blinded to the clinical status.

Some reports included in the meta-analysis predate Baker *et al*,¹⁶ showing the extended H1 and H2 tau haplotypes.

Abbreviations: FPD, familial Parkinson's disease; LD, linkage disequilibrium; PSP, progressive supranuclear palsy; YOPD, young onset Parkinson's disease

These reports used an intronic dinucleotide repeat (IVS 9), comprising five alleles (A0, A1, A2, A3, and A4 alleles). The A0, A1, and A2 alleles constitute H1 haplotype, while A3 and A4 constitute the H2 haplotype.

Statistical analysis

For the case-control study, tau haplotype and genotype frequencies were calculated using the standard Pearson χ^2 test. To test for association between tau H1 and Parkinson's disease, univariate and multivariate odds ratios (OR), the corresponding 95% confidence intervals (CI), and probability (p) values were obtained by logistic regression methods.

We then carried out a meta-analysis which included our data. Two electronic databases (Medline and Embase) were searched up to August 2003 for all studies evaluating the tau H1 and Parkinson's disease. For inclusion, the following criteria had to be fulfilled:

- the study was a case-control design (retrospective or nested);
- the subjects were unrelated and white;
- the diagnosis of Parkinson's disease was made clinically or pathologically confirmed;
- the study involved examining the associations between Parkinson's disease and tau H1.

Studies were excluded if they were reported only in abstract form, or if genotype or haplotype frequency was not reported.

Terms used for the search were both MeSH terms and text words: "tau" and "Parkinson's disease" in combination with "genetic," "haplotype," "H1," and "association study." The search results were limited to "human" and "English language." We searched for any additional studies in the references of all identified publications. Two investigators independently reviewed study eligibility, and inconsistencies were resolved by consensus.

As in the case-control study, the a priori hypothesis in the meta-analysis was that H1 homozygosity compared with carriers of H2 (H1H2+H2H2) would be associated with an increased risk of Parkinson's disease. In a separate analysis, odds ratios for other genetic models were also calculated. We calculated a fixed effect and a random effect summary odds ratio and 95% confidence interval for the association. Fixed effect summary odds ratios were calculated using the Mantel-Haenszel method,^{17,18} and the DerSimonian and Laird method was used to calculate random effect summary odds ratio.¹⁹ The DerSimonian and Laird *Q* test was also used to evaluate the degree of heterogeneity between studies, and *I*² was used as a measure to describe the percentage of variability in point estimates resulting from heterogeneity rather than sampling error.²⁰ A funnel plot was used to assess for publication bias.²¹ In addition, the influence of individual studies on the summary odds ratio was evaluated by re-estimating and plotting the summary odds ratio in the absence of each study.

The population attributable risk—reflecting the proportion of Parkinson's disease in the population that can be attributed to a particular risk factor—was calculated for the tau variant using the following formula:

$$100 \times [\text{prevalence (OR-1)} / \text{prevalence (OR-1)+1}].$$

For this calculation we used the OR derived from the fixed method from the meta-analysis, and the proportion of the total population exposed to the putative risk (that is, H1) was calculated from genotype frequencies in the control group. Data were analysed using the Review Manager software (version 4.2) from the Cochrane Collaboration 2003.

We also undertook a secondary analysis in which we attempted to control for a possible misdiagnosis of

progressive supranuclear palsy (PSP) in our Parkinson's disease population. We made the assumption, as reported by another study from this institution,²² that 6% of our patients had PSP but had been clinically misdiagnosed as Parkinson's disease. We also assumed that 87.5% of those PSP patients were H1H1 and 12.5% H1H2, based on other studies.¹⁶ We therefore randomly removed 35 patients with Parkinson's disease (6%), 31 with the H1H1 genotype (87.5%), and four with the H1H2 genotype (12.5%).

RESULTS

Case-control study

Analysis of our control population showed no deviation from Hardy-Weinberg equilibrium ($p = 0.54$), but our Parkinson cohort was not in Hardy-Weinberg equilibrium ($p = 0.03$).

We observed the tau H1 haplotype in 74.8% of our controls and 79.7% of our overall Parkinson's disease population. This result was highly significant (OR 1.32 (95% CI, 1.10 to 1.62); $p = 0.006$).

Using a recessive genetic model comparing the H1H1 genotype with H1H2 plus H2H2, subjects homozygous for H1 were 1.43 times more likely to develop Parkinson's disease than carriers of the H2 allele (95% CI, 1.12 to 1.82; $p = 0.004$). This increased risk remained significant after adjustment, as outlined above, for potential PSP misdiagnosis (OR 1.34 (1.04 to 1.70); $p = 0.02$). In fact we estimate that a PSP misdiagnosis rate of 26% would be required for the odds ratio to cross 1.0.

When we analysed YOPD separately, the H1 effect remained significant (OR 1.38 (1.06 to 1.80); $p = 0.01$). We observed a particularly strong association between the H1H1 genotype and YOPD (OR 1.6 (1.2 to 2.2); $p < 0.004$), although the 95% CI for all cases of Parkinson's disease and the YOPD group overlap. There was no association in the FPD group ($p = 0.4$). This may reflect the relatively small number of patients in this subgroup ($n = 124$), or may be accounted for by unidentified single gene mutations such as PARK2. These data are displayed in table 1.

Meta-analysis results

The primary search identified 10 potentially relevant articles, of which seven—including the present case-control study—met the selection criteria. Of the three articles excluded, one was a family based study,¹⁴ another did not provide enough data,¹¹ and the third was not reported as a full text paper.¹² No prospective, cohort, or nested case-control studies were identified.

Overall, the genotype frequency for individuals homozygous for the H1 allele in the control population was 57.9% (95% CI, 52.5% to 63.5%); for heterozygous individuals (H1H2) it was 36.9% (32.1% to 41.9%), and homozygous individuals for the H2 allele it was 5.0% (3.7% to 6.4%).

Figure 1 shows the results of all studies included in the present meta-analysis (1305 cases and 1194 controls). The summary odds ratio, under a fixed effect model, shows that individuals homozygous for H1 were 1.57 times more likely (95% CI, 1.33 to 1.85; $p < 0.00001$) to develop Parkinson's disease than H2 carriers (H1H2+H2H2).

We did identify significant interstudy heterogeneity (p value for heterogeneity (P_{Het}) = 0.01). Likewise, a modest degree of interstudy variability was also observed ($I^2 = 62.1\%$). However, a sensitivity analysis showed that the study of Farrer *et al*,⁷ conducted in an isolated Norwegian population, largely accounted for this heterogeneity. This was also the study with the largest odds ratio. Nevertheless, a random effect summary odds ratio that takes into account the intrastudy and interstudy variability resulted in a similar and significant overall estimate (OR 1.71 (1.25 to 2.36); $p = 0.009$). When this study⁷ was excluded from the summary

Table 1 Haplotype and genotype frequency for the present case-control study

	Controls (n = 513)	Total PD (n = 580)	YOPD (n = 263)	Sporadic PD (n = 193)	FPD (n = 124)
<i>Genotype</i>					
H1H1	290 (56.5)	377 (65)	177 (67.3)	125 (64.8)	75 (60.5)
H1H2	188 (36.6)	171 (29.5)	69 (26.2)	57 (29.5)	45 (36.3)
H2H2	35 (6.9)	32 (5.5)	17 (6.5)	11 (5.7)	4 (3.2)
<i>Allele frequency</i>					
H1 (%)	74.9	79.7	80.4	79.5	78.6
H2 (%)	25.2	20.3	19.6	20.5	21.4

Values are n (%) unless specified.

FPD, familial Parkinson's disease (index familial cases with one or more relative also affected by Parkinson's disease); PD, Parkinson's disease; YOPD, young onset Parkinson's disease (onset before the age of 45 years)

odds ratio, the individual odds ratios were no longer heterogeneous ($P_{\text{Het}} = 0.57$), no interstudy variability was observed ($I^2 = 0\%$), and the summary odds ratio remained statistically significant (summary OR 1.46 (1.23 to 1.73); $p < 0.0001$).

The distribution of the odds ratios from individual studies compared with their respective standard errors (funnel plot) was slightly asymmetrical, suggesting moderate positive publication bias (fig 2).

When H1 carriers (H1H1+H1H2) were compared with H2H2 (dominant genetic model), no significant association was observed (summary OR 1.27 (0.98 to 1.82); $p = 0.19$). Furthermore, when a co-dominant model was evaluated, only the H1H1 v H2H2 comparison was significant (summary OR 1.46 (1.02 to 2.10); $p = 0.04$), while heterozygosity for the H1 allele did not increase the risk of Parkinson's disease (summary OR 0.96 (0.66 to 1.40); $p = 0.83$). These data support the hypothesis that homozygosity for H1 (recessive model) increases the risk of Parkinson's disease.

The population attributable risk for the tau variant, for the main comparison of H1H1 against H2 carriers, was 24.8% for all studies combined.

DISCUSSION

The principal observation of this study, and of the meta-analysis, was that homozygosity for the tau H1 genotype (H1H1) increased Parkinson's disease risk by 57% (95% CI, 33% to 85%; $p < 0.00001$).

Furthermore, as the population frequency of the "at risk" tau-H1H1 genotype in this meta-analysis was 58% in the

Figure 2 Funnel plot data assessing publication bias in studies evaluating the association between the tau gene and Parkinson's disease. OR, odds ratio.

control samples, the population attributable risk was considerable (24.8%). Thus given that the reported incidence of Parkinson's disease ranges between 16 and 19 per 100 000 inhabitants per year,²³ and assuming that our estimate is correct, the tau gene contributes between 2400 and 2900 cases of Parkinson's disease in the United Kingdom each year.

We acknowledge that these findings are difficult to reconcile with the lack of significant tau pathology in idiopathic Parkinson's disease. However, in the normal brain, tau and α synuclein are concentrated in the axon where in principle they could interact. It is possible to hypothesise that such an interaction could influence the propensity of α synuclein to self assemble. In addition, tau aggregation

Figure 1 Published association studies between tau and Parkinson's disease. Odds ratio for the outcome compared H1H1 genotype subjects against H2 carriers (H1H2 and H2H2). CI, confidence interval.

can occur in patients with parkin gene mutations, sometimes in the absence of Lewy bodies,⁵ which raises the possibility that tau dysfunction may also act independently of α synuclein to accelerate dopaminergic neuronal degeneration, and may be a necessary but insufficient factor in the pathogenesis of many cases of Parkinson's disease, especially YOPD.

The genetic architecture of tau is also worth considering. The two extended tau haplotypes, H1 and H2, are defined by a small number of polymorphisms that are inherited in complete linkage disequilibrium (LD). If, however, the H1 haplotype is a misnomer concealing greater genetic variability on the H1 backbone, or if the "block" of LD—reportedly encompassing the entire tau gene—has different boundaries in different populations, then this may influence the power of the 238 base pair deletion used to define H1 and H2 to capture the causal variant in genetically diverse populations.

It has been proposed that the mechanism whereby PSP causes neuronal loss is through the H1 influencing the excess deposition of the four microtubule binding repeat isoforms of the tau protein. However, given that this histopathological finding is not typically seen in Parkinson's disease, it is possible that another molecular pathway is responsible for the pathogenic effect of the H1 allele in this condition. If there is more than one subhaplotype within H1, then a plausible hypothesis would be that one subhaplotype influences PSP pathology while another influences Lewy body pathology. Further work is needed to determine the haplotypic structure of the region around the tau gene, especially given the evolutionary implausibility of just two ancient haplotypes, H1 and H2, covering a region of at least 670 kb in size²⁴ and encompassing genes other than the tau, including the recently described saitohe gene.²⁵

One perceived problem with case-control design has been the possibility of false positive results from subtle genetic diversity in a given population—that is, population stratification. However, the studies in our meta-analysis used populations of European origin, and recent evidence suggests that, with carefully designed studies and meta-analysis, such populations are unlikely to contain levels of stratification sufficient to cause an inflated number of false positive associations.^{26–27}

Positive publication bias, shown by asymmetry in the funnel plot, may have led us to overestimate the tau H1 risk in Parkinson's disease. However, while an empirical assessment of publication bias has shown that in most cases the bias does not affect meta-analyses,²⁸ more studies would help refine our estimate of the true effect of the tau H1.

The observed heterogeneity in this meta-analysis was largely accounted for by a small study in a Norwegian population⁷; the random effect estimation of the summary odds ratio was nevertheless significant, and when this study was excluded from the analysis, the association was still preserved.

Conclusions

Our new data, as well as the combined data from a meta-analysis, show that subjects homozygous for tau H1 have an increased risk of Parkinson's disease compared with H2 carriers (H1H2 and H2H2). This adds to the growing body of evidence that common genetic variation contributes to the pathogenesis of Parkinson's disease. We propose that the tau gene is implicated in the pathogenesis of this disorder. Whether and how it interacts with other Parkinson's disease genes has yet to be elucidated.

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