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Association of Single-Nucleotide Polymorphisms of the Tau Gene With Late-Onset Parkinson Disease

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Abstract

Context—The human tau gene, which promotes assembly of neuronal microtubules, has been associated with several rare neurologic diseases that clinically include parkinsonian features. We recently observed linkage in idiopathic Parkinson disease (PD) to a region on chromosome 17q21 that contains the tau gene. These factors make tau a good candidate for investigation as a susceptibility gene for idiopathic PD, the most common form of the disease.

Objective—To investigate whether the tau gene is involved in idiopathic PD.

Design, Setting, and Participants—Among a sample of 1056 individuals from 235 families selected from 13 clinical centers in the United States and Australia and from a family ascertainment core center, we tested 5 single-nucleotide polymorphisms (SNPs) within the tau gene for association with PD, using family-based tests of association. Both affected ($n = 426$) and unaffected ($n = 579$) family members were included; 51 individuals had unclear PD status. Analyses were conducted to test individual SNPs and SNP haplotypes within the tau gene.

Main Outcome Measure—Family-based tests of association, calculated using asymptotic distributions.

Results—Analysis of association between the SNPs and PD yielded significant evidence of association for 3 of the 5 SNPs tested: SNP 3, $P = .03$; SNP 9i, $P = .04$; and SNP 11, $P = .04$. The 2 other SNPs did not show evidence of significant association (SNP 9ii, $P = .11$, and SNP 9iii, $P = .87$). Strong evidence of association was found with haplotype analysis, with a positive association with one haplotype ($P = .009$) and a negative association with another haplotype ($P = .007$). Substantial linkage disequilibrium ($P < .001$) was detected between 4 of the 5 SNPs (SNPs 3, 9i, 9ii, and 11).

Conclusions—This integrated approach of genetic linkage and positional association analyses implicates tau as a susceptibility gene for idiopathic PD.

Parkinson Disease (PD) IS A progressive neurologic disorder that affects more than 500000 people in the United States.¹ The etiology of PD is complex and likely involves several different environmental and genetic factors. Familial aggregation and sibling risk studies^{2,3} have supported a genetic component for late-onset, idiopathic PD. Two genes (α -synuclein and parkin) have been identified that lead to very rare mendelian forms of the phenotype.^{4,5} However, our recent genome screen for linkage in PD produced the first strong evidence suggesting multiple genetic susceptibility loci for the common form of PD.⁵

One of the regions giving the highest logarithm of odds (lod) scores in our genomic screen for PD was on chromosome 17q21 in the late-onset PD subset. This region contains the gene for the microtubule-associated protein tau. Mutations in tau are known to cause frontotemporal dementia with parkinsonism (FTDP), a rare autosomal dominant disorder.^{7,8} A conserved haplotype spanning the tau gene and a series of sequence variants also have been found to be strongly associated with a second form of parkinsonism, progressive supranuclear palsy (PSP).^{9–11} Recently, significant associations were found with PD and an allele of a dinucleotide repeat polymorphism in a tau intron using unrelated cases and controls.^{12,13} Therefore, we believe the tau gene is an excellent candidate for positional association studies as the 17q21 susceptibility gene for idiopathic PD, extending its potential involvement from rare forms of parkinsonism to the more common form of late-onset PD.

METHODS

Sample Data

Families were selected from the Duke Center for Human Genetics (DCHG)/ GlaxoSmithKline Parkinson Disease Genetics Collaboration (composed of 13 centers) and the DCHG Morris K. Udall Parkinson Disease Center of Excellence family ascertainment core center. Patients were selected through participating movement disorders and neurology clinics, referrals, and advertisements. All study participants provided written informed consent, according to protocols approved by the institutional review board at each center.

All participants were examined by a board-certified neurologist or a physician assistant trained in neurologic disease and supervised by neurologists. Diagnostic and exclusion criteria, based on previously published diagnostic criteria for PD,^{14,16} were adopted by all participating clinicians before beginning ascertainment of families. Participants were classified as affected, unclear, or unaffected based on neurologic examination and clinical history. Affected individuals had at least 2 cardinal signs of PD (eg, rest tremor, bradykinesia, and rigidity) and no atypical clinical features or other causes of parkinsonism. Unclear individuals had only one sign, a history of atypical clinical features, or both. Unaffected individuals had no signs of PD. To ensure diagnostic consistency across sites, clinical data for all participants were reviewed by a clinical adjudication board, consisting of a board-certified neurologist with fellowship training in movement disorders (B.L.S.), a dually board-certified neurologist and medical geneticist (J.M.V.), and a certified physician assistant (J.M.S.). Forms with missing data or data inconsistent with the diagnosis assigned to the individual were referred back to the site for clarification.

Excluded from participation were individuals with a history of encephalitis, neuroleptic therapy within the year before diagnosis, evidence of normal pressure hydrocephalus, or a clinical course with unusual features suggestive of atypical or secondary parkinsonism. To exclude PSP, FTDP, and other parkinsonian conditions from the PD affected group, all subjects in the PD affected group had to meet strict clinical criteria, including asymmetrical motor symptoms at onset, no postural instability with falls early in the disease course, and no supranuclear downward- or lateral-gaze palsy. The presence of any of these exclusion criteria was sufficient to prevent inclusion in the PD affected group and excluded subjects with clinical features of PSP and other atypical parkinsonian syndromes. Subjects with FTDP were excluded from the PD affected group by clinical criteria that required the absence of dementia at onset and the presence of asymmetrical onset of motor symptoms. Other parkinsonian syndromes were screened by additional clinical criteria such as absence of severe autonomic neuropathy or signs of significant cerebellar dysfunction (multiple system atrophy); absence of abrupt symptom onset or a stepwise course (vascular parkinsonism); and absence of unilateral dystonia with apraxia or cortical sensory loss (cortical-basal ganglionic degeneration).

Molecular Analyses

Five single-nucleotide polymorphisms (SNPs) in tau, previously tested by Baker et al¹⁰ for association with PSP, were chosen for analysis of association in our PD family sample. Two SNPs were intronic: one in intron 3 (SNP 3) and one in intron 11 (SNP 11). The other 3 SNPs chosen were all in exon 9 (SNPs 9i, 9ii, 9iii). The dinucleotide repeat polymorphism between exons 9 and 10 also was tested.⁹

The SNPs were genotyped using a modification of the gel-based oligo-nucleotide ligation assay (OLA).¹⁷ Table 1 shows polymerase chain reaction (PCR) primers and OLA probes for SNPs used in this study. The PCR amplification was performed in 10- μ L reactions (30 ng of DNA, 1 \times PCR buffer [Gibco, Carlsbad, Calif], 0.6 mM of dinucleotide triphosphates,

3.0 mM of magnesium, 0.5 units of platinum Taq [Gibco], and 0.04 µg of forward and reverse primers) using thermocyclers (MJ PTC200 or Primus96Plus; MWG-Biotech, Ebersberg, Germany) for 40 cycles (at 94°C for 4 minutes; 5 times at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; 20 times at 94°C for 5 seconds, 55°C for 30 seconds, and 72°C for 45 seconds; 15 times at 94°C for 5 seconds, 55°C for 45 seconds, and 72°C for 80 seconds; and 72°C for 7 minutes) followed by a 30-minute incubation at 94°C to heat kill the enzyme.

Two microliters of the PCR reaction mix was transferred and dried before being resuspended in 10 µL of ligation mix (1× Taq DNA ligase buffer, 4 units of Taq DNA thermostable ligase; New England BioLabs, Beverly, Mass). Allele-specific probes were fluorescently labeled using Fam or Cy3, and common probes were phosphorylated on the 5' end. Ligations were performed in a thermocycler (40 times at 94°C for 20 seconds; 50°C for 1 minute). Reactions were stopped with the addition of 20 µL of loading or stop dye (98% deionized formamide, 10 mM of EDTA, 0.025% xylene cyanol, 0.025% bromphenol blue).

Approximately 4 µL of each sample was loaded onto a 6% polyacrylamide gel, run for approximately 40 minutes, and scanned (Hitachi FMBio II fluorescent static scanner, MiraiBio Inc, Alameda, Calif). Images were analyzed using BioImage software (Genomic Solutions Inc, Ann Arbor, Mich). To ensure correct OLA genotyping, representative OLA genotypes were checked for accuracy using sequencing (CEQ2000XL). Genotyping of the microsatellite marker was performed by fluorescence imaging using the FASST method previously described.¹⁸

The genotyping was subjected to extensive quality control using a set of blinded internal quality control samples, and each pair of markers within each gene was tested for recombination among siblings.^{6,19,20} All samples from individuals in families who showed evidence of recombination between markers were checked for genotype misreads. Because 4 of these SNPs have been reported elsewhere¹⁰ to be in strong linkage disequilibrium, we also evaluated genotypes of individuals showing evidence of haplotypes that were not expected. In each case, rereads or direct sequencing resolved the recombination or haplotype discrepancy.

Statistical Analyses

Single-locus tests for association were conducted using a likelihood ratio test implemented in the program Transmit and the pedigree disequilibrium test (PDT).^{21,22} We used a version of the PDT based on the PDT-sum statistic.²³ The robust variance estimator was used in the likelihood ratio test to ensure validity as a test of association in sib-ships of arbitrary size. The data set used for our association analyses consists of few extended pedigrees; thus, we report the analysis based on all nuclear families. Haplotype analyses were conducted using Transmit. *P* values for a global test of significance were computed using the χ^2 distribution with $h - 1$ *df*, where *h* is the number of distinct haplotypes observed ($h=2$ for single-locus tests).

A single affected and unaffected individual were selected at random from each family for tests of Hardy-Weinberg disequilibrium and linkage disequilibrium between markers. Analyses were conducted in the affected sample and unaffected sample separately using a permutation test with 3200 permutations to estimate each *P* value.²⁴ *P* .05 was set as the level of significance.

RESULTS

Our sample consisted of 1056 individuals from 235 families. The sample was composed of 48% females and 52% males. Among 426 affected individuals, 56% were females and 44% were males. Among 579 unaffected individuals, 43% were females and 57% were males. Because we used family-based tests of association in sibships, the association analyses include both males and females and could not be stratified by sex. Most families in this study were discordant sibships (at least 1 affected sibling and 1 unaffected sibling) without both parental samples (family type A, $n = 158$). A smaller number were nuclear families with at least 1 affected individual and both parents (family type B, $n = 40$) or only 1 parent (family type C, $n = 2$) sampled. The remaining families were more complex, containing more than a single nuclear family or sibship (family type D, $n = 35$).

This data set contains many of the families used in our PD genomic screen⁶ and more recently ascertained families. Table 2 details the numbers of individuals in each of the 4 family types used in the genomic screen and new individuals for this association study. Age at onset was self-reported, defined as the age at which the affected individual could first recall noticing one of the cardinal signs of PD. The mean age at onset in affected individuals in the sample was 57.5 years, and the mean age of unaffected individuals was 66.8 years. When possible, we sampled unaffected siblings who were older at the age of examination than the age at onset of their affected siblings. Approximately 50% of the unaffected individuals sampled met his criterion. Because of the nature of the study design, most affected individuals had their conditions diagnosed before study enrollment and thus represent prevalent cases rather than incident cases.

Three SNPs (3, 9i, and 11) showed significant association with PD ($P < .05$) using the likelihood ratio test (Table 3). For each marker, the more common allele 2 (nucleotide A, A, and G) was positively associated with PD in our sample. Similar results were obtained for the PDT analysis (results not shown). For comparison, P values for SNPs 3, 9i, and 11 were .06, .08, and .05, respectively.

The high frequency of allele 2 (G) at SNP 9iii (Table 3) offers an explanation for why no association was detected with this allele. If the positively associated allele is at high frequency in the population, it will be difficult to detect the association because there cannot be a large difference between the allele frequency in the population and in the affected individuals, even if the allele has a frequency of 1.0 in the affected individuals.

As has been reported elsewhere,¹⁰ there was considerable linkage disequilibrium between the SNPs. In all individuals, the 2 haplotypes H1 and H2 observed by Baker et al¹⁰ were the only haplotypes directly observed for the 4 SNPs 3, 9i, 9ii, and 11. There was no evidence of the existence of other haplotypes for these 4 markers in our sample. $P < .001$ was estimated for linkage disequilibrium for all combinations of these markers. As a consequence, each marker carries the same information, which is why the results for the single-locus association tests at these 4 SNPs were similar (Table 3). Differences in P values were due only to variable amounts of missing genotype data at the different SNPs. For SNP 9iii, the rare 1 (A) allele occurs almost exclusively on the H1 haplotype, suggesting other haplotypes are old and this allele at 9iii arose more recently on the common H1 haplotype. Significant linkage disequilibrium was not detected between SNP 9iii and the other 4 markers. No evidence for deviation from Hardy-Weinberg equilibrium was found in affected or unaffected individuals for any of the markers.

Including SNP 9iii, 3 common haplotypes were observed for all 5 SNPs. The 2 most common haplotypes were significantly associated with PD (Table 3). The haplotype

carrying alleles 11121 (at SNPs 3,9i, 9ii, 9iii, and 11, respectively) was significantly undertransmitted to affected individuals, whereas the haplotype carrying alleles 22222 was significantly overtransmitted to affected individuals. The 22222 haplotype corresponded to the H1 haplotype previously associated with PSP.¹⁰ There was no evidence for association with the H1 subhaplotype carrying the allele 1 (A) at 9iii (haplotype 22212), suggesting that the putative susceptibility allele may occur with increased frequency on the H1 haplotype carrying allele 2 (G) at 9iii. A fourth haplotype carrying alleles 21121 was inferred with a frequency of 0.001 in the likelihood analysis. This haplotype was pooled with haplotype 22212 for calculation of the test statistic because of the low frequency of the 21121 haplotype.

We also examined a dinucleotide repeat polymorphism, previously associated with PSP,¹⁰ located between exons 9 and 10 in the tau gene for association with PD. The repeat has 2 common alleles: *a0* occurring on the H1 haplotype and *a3* occurring on the H2 haplotype. We found a significant association with the likelihood ratio test (global test $P = .02$), with the common allele, *a0*, being significantly overtransmitted ($P = .004$) to affected individuals and allele *a3* being significantly undertransmitted ($P = .02$). These results are consistent with the findings of Baker et al¹⁰ for PSP and are supported by the recent reports^{12,13} of a difference in *a0* allelic frequency between PD patients and controls.

COMMENT

The integrated approach of linkage and subsequent positional association studies strengthens our conclusion that the finding of allelic and haplotype association with tau represents a biologically meaningful association. The previously identified linkage in a subset of these families is not expected to inflate the false-positive rate for the association tests because the linkage and association tests are largely independent under the null hypothesis of no linkage disequilibrium with the disease locus.^{25,26} As pointed out by Tan et al,²⁷ significant association of candidate genes for idiopathic PD chosen only because of their biological function has not proven to be routinely reproducible or successfully implied causality.

Tau is a microtubule-associated protein that normally functions to promote assembly and stability of the microtubule. Although both PSP and FTDP have demonstrated abnormal tau structure, no abnormal tau structure has been reported in PD. However, as is the case for *APOE*, a susceptibility gene does not have to cause gross pathologic conditions to be important. Its effect may well be toward triggering the pathway to the disease. Thus, the in vitro studies by Jensen et al²⁸ that demonstrate that α -synuclein binds tau at the microtubule-binding site are of interest. In addition, those authors found that α -synuclein can modulate the phosphorylation of tau.

Patients who presented with dementia initially in their clinical course were excluded from our study. However, given the known tau mutations in FTDP-17, it would be of interest to know if the genetically associated tau haplotype shown herein has any clinical association with dementia in our PD patients. Unfortunately, our initial clinical analysis of the patients included in this study did not allow us to explore this possibility. We are currently collecting those data as part of our subsequent patient data set and in follow-up of our initial patients. Our finding reported herein, along with such pathologic findings as Lewy body dementia, suggests future studies may find that PD and Alzheimer disease represent different outcomes of the same disease continuum.

Two tau SNP haplotypes show strong evidence of association, with one significantly overtransmitted and the other significantly undertransmitted. This may be similar to *APOE*, for which the *APOE*- ϵ 4 allele is a risk allele for developing Alzheimer disease, whereas the

APOE-ε2 allele provides a protective effect.²⁹ However, the third haplotype in our study is fairly uncommon, and thus this undertransmission of the 11121 haplotype may reflect only the expectation of a functionally 2-haplotype system. Therefore, further work will be needed to determine the significance of this finding.

Given that the common haplotype is positively associated with PD, it is likely that a yet unidentified polymorphism is generating this association. However, because tau is a large gene with 16 coding exons, additional research will be needed to identify this specific polymorphism. Furthermore, despite the strong evidence for association at markers within the tau gene, it is still possible that another nearby gene might be the true susceptibility gene and these markers are merely in linkage disequilibrium with causal changes in that gene. In summary, we have shown that the convergence of significant association results, the evidence of linkage in the region, and biological function point strongly to tau as a susceptibility gene for common idiopathic PD.

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Table 1

PCR Primers and OLA Probes for SNPs Used in Association Analyses*

SNP	PCR Primer	Allele	OLA Probe [†]
3 , IVS3+9A→G	Forward: GGGCTGCTTTCTGGCATATG	1 (G)	5'-Cy3-AGGAACCACAGGTGAGGGTG
	Reverse: CCTCACTTCTGTACAGGTC	2 (A)	5'-Cy3-AGAAGGAACCACAGGTGAGGGTA
		Common	5'-Pho-AGCCCCAGAGACCCCCAGGCAGTC
9i , C1632A→G Ala544Ala	Forward: CCACCCGGGAGCCCAAGAAGGTGCC	1 (G)	5'-Fam-GGGAGCCCAAGAAGGTGGCG
	Reverse: CTGGTGCTTCAGGTTCTCAGTG	2 (A)	5'-Fam-CCCGGGAGCCCAAGAAGGTGGCA
		Common	5'-Pho-GTGGTCCGTACTCCACCAAGTCGCCGTCTCCGC
9ii , C1716T→C Asn572Asn	Forward: CGAGTCCTGGCTTCACTCC	1 (C)	5'-Cy3-CCATGCCAGACCTGAAGAAC
	Reverse: CTTCCAGGCACAGCCATAACC	2 (T)	5'-Cy3-TGCCCATGCCAGACCTGAAGAAT
		Common	5'-Pho-GTCAAGTCCAAGATCGGCTCCACTGAGA
9iii , C1761G→A Pro587Pro	Forward: CGAGTCCTGGCTTCACTCC	1 (A)	5'-Fam-AGAACCTGAAGCACCAGCCA
	Reverse: CTTCCAGGCACAGCCATAACC	2 (G)	5'-Fam-CTGAGAACCTGAAGCACCAGCCG
		Common	5'-Pho-GGAGGCGGGAAGGTGAGAGTGGCTGG
11 , IVS11+34G→A	Forward: GTCATTCTCTCTCCTCCTC	1 (A)	5'-Cy3-GGTGAGGGTTGGGACGGGAA
	Reverse: CCAGGACTCCTCCACCCCATGCAGC	2 (G)	5'-Cy3-GAAGGTGAGGGTTGGGACGGGAG
		Common	5'-Pho-GGTGCAGGGGTGGAGGAGTCTGGTGGAGGCTGGAAC

* PCR indicates polymerase chain reaction; OLA, oligonucleotide ligation assay; and SNP, single-nucleotide polymorphism.

[†] Probes were labeled using Fam or Cy3, and common probes were phosphorylated (Pho) on the 5' end. Letters in bold correspond with the SNP.

Table 2
 Individuals Included in Genomic Screen Linkage Study and Additional Individuals Included in the Association Analyses*

Family Type [†]	Enrolled in Linkage Study, No.			Enrolled in Association Analysis, No.			Total No.
	PD	Unaffected	Unclear	PD	Unaffected	Unclear	
A	190	224	14	83	108	9	628
B	14	28	0	48	67	8	165
C	0	0	0	2	1	1	4
D	50	89	5	39	62	14	259
Total	254	341	19	172	238	32	1056
							235

* Individuals are classified as affected with Parkinson disease (PD), unaffected, or with unclear disease diagnosis.

[†] A indicates discordant sibships; B, nuclear families with at least 1 affected and both parents; C, nuclear families with at least 1 affected and 1 parent; and D, complex pedigrees with more than 1 nuclear family.

Table 3

Results of Single-Locus and Haplotype Tests*

Single SNPs	Population Frequencies [†]	Case Frequencies	No. of Cases Used	P Value [‡]
3	0.794	0.826	768	.03
9i	0.793	0.824	744	.04
9ii	0.790	0.817	756	.11
9iii	0.955	0.958	756	.87
11	0.793	0.821	772	.04
Haplotypes for 3/9i/9ii/9iii/11				
11121	0.19	0.158	759	.007
22212	0.05	0.044		.86
22222	0.76	0.797		.009

* SNPs indicates single-nucleotide polymorphisms. The number of cases used in the global haplotype test was 759 ($P = .02$ overall).

[†] Maximum likelihood estimates for population frequencies for the common allele for single SNPs, and for population frequencies for 5 SNP haplotypes.

[‡] P values for single SNPs and individual haplotypes are from a χ^2_1 distribution. P values for global haplotype test are from a χ^2_5 distribution.