Published in final edited form as:
Neurology. 2008 July 1; 71(1): 28–34. doi:10.1212/01.wnl.0000304051.01650.23.

Haplotypes and gene expression implicate the *MAPT* region for Parkinson disease:

The GenePD Study

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Abstract

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Disclosure: The authors report no disclosures.

Background—Microtubule-associated protein tau (*MAPT*) has been associated with several neurodegenerative disorders including forms of parkinsonism and Parkinson disease (PD). We evaluated the association of the *MAPT* region with PD in a large cohort of familial PD cases recruited by the *Gene*PD Study. In addition, postmortem brain samples from patients with PD and neurologically normal controls were used to evaluate whether the expression of the 3-repeat and 4-repeat isoforms of *MAPT*, and neighboring genes Saitohin (*STH*) and *KIAA1267*, are altered in PD cerebellum.

Methods—Twenty-one single-nucleotide polymorphisms (SNPs) in the region of *MAPT* on chromosome 17q21 were genotyped in the *Gene*PD Study. Single SNPs and haplotypes, including the H1 haplotype, were evaluated for association to PD. Relative quantification of gene expression was performed using real-time RT-PCR.

Results—After adjusting for multiple comparisons, SNP rs1800547 was significantly associated with PD affection. While the H1 haplotype was associated with a significantly increased risk for PD, a novel H1 subhaplotype was identified that predicted a greater increased risk for PD. The expression of 4-repeat *MAPT*, *STH*, and *KIAA1267* was significantly increased in PD brains relative to controls. No difference in expression was observed for 3-repeat *MAPT*.

Conclusions—This study supports a role for *MAPT* in the pathogenesis of familial and idiopathic Parkinson disease (PD). Interestingly, the results of the gene expression studies suggest that other genes in the vicinity of *MAPT*, specifically *STH* and *KIAA1267*, may also have a role in PD and suggest complex effects for the genes in this region on PD risk.

Parkinson disease (PD) (MIM 168600) is a chronic and progressive adult-onset neurodegenerative disorder that results from the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. The characteristic symptoms of PD are a tremor at rest, bradykinesia, rigidity, and postural instability. The *MAPT* gene on chromosome 17q21 was initially implicated in PD by linkage analysis[1] and an independent candidate gene study.[2] *MAPT* encodes microtubule-associated protein tau, which regulates microtubule dynamics and assembles microtubules into parallel arrays within axons. In the class of neurodegenerative diseases called tauopathies, which includes Alzheimer disease and progressive supranuclear palsy (PSP), tau abnormally aggregates to form intracellular inclusions.[3] The association of *MAPT* to PD remains controversial since the results of genetic association studies are mixed and there is no widespread tau pathology found in idiopathic PD.[4]

The H1 and H2 *MAPT* haplotypes were originally defined by eight single nucleotide polymorphisms (SNPs) and a 238-basepair deletion in complete linkage disequilibrium (LD).[5] The H1 haplotype is significantly associated with increased risk of PSP but also occurs in a majority of control populations.[5] The complete LD characteristic of the H1/H2 haplotypes and the association of the H1 variants to PSP has been shown to extend for 1.8 Mb (megabases).[6] Indeed, the longest region of LD identified in the human genome is reported to be the chromosomal region surrounding *MAPT*.[7] The divergence of the H1/H2 haplotypes may result from a 900-kilobase (kb) inversion that spans the entire coding region of *MAPT* and several nearby genes.[8] There is no evidence of recombination between the H1 and H2 haplotypes, suggesting that these extended haplotypes are chromosomal backgrounds on which other variants may occur.[6,8,9]

Previous studies have evaluated the association of the H1 haplotype to PD susceptibility but the results have been varied.[9-14] Given the strong LD across this region, genes near *MAPT*, including Saitohin (*STH*) or the hypothetical protein *KIAA1267*, may influence the development of PD. *STH*, a single-exon gene located within intron 9 of *MAPT*, has shown similar tissue distribution to *MAPT*, suggesting that the two genes may be co-regulated in certain tissue types.[15]

The finding of modest evidence for linkage to PD affection in the *Gene*PD Study (nonparametric lod score = 1.7 on chromosome 17 at 63.8-63.9 cM) justified an examination of the *MAPT* region. We analyzed the association of 21 SNPs spanning *MAPT*, *STH*, and *KIAA1267* to PD in the *Gene*PD Study. We performed haplotype analyses, including analysis of a two-SNP haplotype that is representative of the extended H1 and H2 haplotypes. We evaluated the mRNA expression of *MAPT* isoforms, as well as *STH* and *KIAA1267*, in a set of 32 PD and 28 control postmortem human cerebellar samples. *MAPT* isoforms that include exon 10 have 4 copies, or repeats, of a microtubule-binding domain while isoforms without exon 10 only have 3 repeats of this domain.[16] The ratio of 4-repeat (4R) to 3-repeat (3R) *MAPT* mRNA isoforms is increased in several parkinsonian disorders including PSP, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), and corticobasal degeneration (CBD).[17,18] Therefore, we also evaluated this ratio in idiopathic PD.

METHODS

The *Gene*PD Study is a multicenter study of affected relative pairs with idiopathic PD.[19,20] Individuals with PD and at least one living first-degree relative with PD were recruited for this study. The diagnosis of idiopathic PD was confirmed by participating neurologists according to the United Kingdom PD Society Brain Bank Criteria, omitting the criterion requiring sporadic occurrence.[21] DNA was isolated from blood samples collected from study participants and was screened for known mutations in parkin,[22] LRRK2,[23,24] PINK1,[25] DJ-1, SNCA, and NR4A2.[26] Mutations in PINK1, DJ-1, SNCA, and NR4A2 were not identified in this sample[26] and individuals with parkin and LRRK2 mutations were removed from analyses. A total of 543 familial PD cases from 296 families and 245 unrelated, unaffected controls as previously described were used in this study.[19,27] The PD cases were 55% male with an average enrollment age of 70.3 ± 10.4 and the controls were 52% male with an average enrollment age of 62.6 ± 11.8. This research was approved by the Institutional Review Boards of all participating institutions.

RNA isolated from the fresh-frozen cerebellum specimens of 32 neuropathologically confirmed PD cases and 28 neurologically and neuropathologically confirmed controls, as previously described,[28] was reverse transcribed into cDNA in order to study gene expression.[28] There was no difference in the postmortem interval (Student *t* test, $p = 0.3$) or age at death between the PD cases and controls ($p = 0.6$). No family history data were provided for the majority of autopsied cases.

Twenty-one SNPs (table 1) were genotyped in all individuals using TaqMan technology on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA). Three of the genotyped SNPs are nonsynonymous coding polymorphisms: rs2258689 (Tyr > His at *MAPT* amino acid 441), rs7220988 (Leu > Pro at *KIAA1267* amino acid 1010), and rs17662853 (Ile > Thr at *KIAA1267* amino acid 221).

Real-time RT-PCR was performed in technical triplicates of each brain cDNA sample using TaqMan Gene Expression Assays on the ABI PRISM 7900HT Sequence Detection System. Target assays were specific to the 3R (ABI Assay id: Hs00902192_m1) and 4R (Hs00902312_m1) isoforms of *MAPT*, *STH* (Hs02340552_s1), and *KIAA1267* (Hs01077436_m1). An assay for the endogenous control gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; Hs99999905_m1), was run simultaneously with each target assay.

SNPs were screened for Mendelian errors using the software program INFER, part of the PEDSYS package. When Mendelian inconsistencies were found, SNP genotypes for the entire family were removed. SNPs were examined for departures from Hardy-Weinberg equilibrium (HWE) in PD cases and for double recombination events using the software program MERLIN.

[29] Departure from HWE was evaluated in the control population using SAS (Statistical Analysis Software, Cary, NC). Linkage analysis to PD using the genotyped SNPs was performed with the program MERLIN in all families and adjusted for LD by clustering markers with $r^2 > 0.05$. SNPs were analyzed using an additive genetic model for minor allele association to PD affection in a logistic regression model. Generalized estimating equations (GEE) were used to account for the correlated genotypes within families.[30] *p* Values were adjusted using the Bonferroni correction for 16 tests using the haplotype block strategy.[31] Six SNPs in strong LD as defined by an $r^2 > 0.8$, which are representative of the H1/H2 haplotypes, were counted as a single test. There were 15 singleton SNPs that were not in strong LD with one another ($r^2 < 0.8$) that were considered as independent tests for a total of 16.

No evidence of recombination between the H1 and H2 haplotypes has been shown[6,8] and therefore we were able to study a two-SNP haplotype that is representative of the LD observed across the extended H1/H2 haplotypes. In this study, the major alleles at both rs1800547 and rs1052553 defined the H1 haplotype, while the minor alleles at these loci defined H2. Haplotype analyses comparing an unrelated case sample to controls were performed using the haplo.stats software.[32,33] Global *p* values for each haplotype were calculated using the haplo.score algorithm, while haplotype-specific odds ratios (ORs) (relative to the most common haplotype) and haplotype-specific *p* values were calculated using the haplo.glm algorithm. LD was evaluated in the PD cases and graphically presented using Haploview software.[34]

Relative levels of target gene expression were evaluated using the standard curve method because two of the transcripts studied (*STH* and *KIAA1267*) failed the amplification efficiency test necessary to perform the comparative cycles to threshold (Ct) calculations.[35] The average Ct for each sample was converted into quantity units using a standard curve of pooled cDNA samples for each transcript. The quantity of each target transcript was normalized to the quantity of *GAPDH*. Samples with a normalized quantity outside of 2 standard deviations from the mean normalized quantity for cases or controls were considered outliers and were subsequently dropped from analysis. The normalized quantity of each sample was calibrated to the mean normalized quantity of the control brain samples to give the relative quantity of each transcript. The ratio of 4R to 3R isoforms of *MAPT* (4R:3R) was calculated using the normalized quantity of each isoform.[18] A Student *t* test was used to evaluate differences in relative gene expression and 4R:3R between PD cases and controls. Since the relative quantity and 4R:3R did not follow a normal distribution based on visual inspection of the data and the Shapiro-Wilk test, a log transformation was used to normalize these values for statistical analysis. In order to evaluate the relationship between *STH* and *MAPT* transcript expression, a Pearson correlation was performed using the log normalized quantity of these transcripts.

RESULTS

One SNP, rs17662853, exhibited departure from HWE in the PD cases ($p = 0.019$) but not in the controls ($p > 0.05$), and was therefore not removed from analyses. No other SNP showed departure from HWE in PD cases or controls. Linkage analysis of the genotyped SNPs revealed a nonparametric lod score of 1.7 at *MAPT*, suggesting modest linkage to PD affection in our cohort. After adjusting for multiple comparisons, the minor allele at rs1800547 was significantly associated with a decreased risk for PD using an additive genetic model (table 1). A number of the SNPs genotyped that represented the H1/H2 haplotypes were in strong pairwise LD in the PD cases assessed by $r^2 > 0.8$ (figure 1).

The association of the H1/H2 haplotypes to PD was evaluated in this study. The SNPs used to define this haplotype, rs1800547 and rs1052553, were in almost perfect LD in our PD cases (figure 1, $r^2 = 0.98$). The H1 haplotype, present in 87% of the PD cases and 77% of the controls,

was associated with a significantly increased risk for PD compared to the H2 haplotype (table 2). Additional haplotype analysis identified a novel six-SNP subhaplotype of H1 (hCV3202946, rs1800547, rs3785883, rs2435207, rs11568305, rs1078997) that was significantly associated with an increased risk for PD (table 3). This high-risk subhaplotype was present in 6.7% of cases and 1.7% of controls (OR = 4.48, $p = 0.003$).

All relative gene expression data are reported as the mean ± SEM fold difference in expression in PD cases relative to controls and are visually represented in figure 2A. The expression of 4R *MAPT* was higher in PD relative to controls (1.43 ± 0.09, $p = 0.002$), while no change in expression was detected for 3R (1.12 ± 0.05, $p = 0.13$). The relative expression of both *STH* (1.97 ± 0.34, $p = 0.001$) and *KIAA1267* (1.85 ± 0.14, $p < 0.0001$) was also higher in PD cases (figure 2A). Additionally, the 4R:3R ratio of *MAPT* isoforms was significantly higher in PD cases than controls (figure 2B). Pearson correlation analysis across all samples demonstrated that as the expression of *STH* increases, the expression also increases for both 3R (r = 0.55, $p < 0.0001$) and 4R *MAPT* (r = 0.59, $p < 0.0001$).

DISCUSSION

This study provides strong evidence that the *MAPT* region is associated with PD in the *Gene*PD Study. We identified a novel haplotype that defines a greater increased risk for PD than that observed for the H1 haplotype. As expected, the risk variants of this haplotype occurred on the H1 background, as defined by the major allele at rs1800547. Furthermore, expression analysis revealed increased expression of 4R *MAPT*, *STH*, and *KIAA1267* in PD brains relative to control brains.

It is important to note that the SNPs in our H1 subhaplotype are neither in strong LD ($r^2 < 0.8$) with one another nor with the H1 SNPs in our PD cases. It has been suggested that the association of *MAPT* to PD is localized in the 5′ end of the gene containing exons 1-4.[9] While rs1800547 is located in intron 4 of *MAPT*, our high-risk haplotype extends from intron 1 (hCV3202946) to over 4 kb 3′ of *MAPT* (rs1078997), into *KIAA1267*. Our results suggest that SNP variants associated with increased PD risk are not confined to the 5′ portion of the gene but rather, they span the entire *MAPT* region and may even extend 3′ beyond *MAPT* to implicate other nearby genes.

The high LD across this chromosomal region has made it difficult to distinguish the polymorphisms that are contributing to disease from those that are merely in LD with the "functional" polymorphism. Thus, it is possible that genes near *MAPT* may also be involved in PD. For example, *KIAA1267* has yet to be fully characterized. It is located approximately 1,500 base pairs downstream of *MAPT* (NCBI Build 36.1) and the two genes are transcribed in opposite directions. We demonstrate that *KIAA1267* mRNA is expressed in both PD and normal human cerebellum. While no SNP in *KIAA1267* showed significant association to PD after Bonferroni correction, one of the SNPs in the high-risk PD haplotype (rs1078997) is located in intron 12. These results support the hypothesis that the *KIAA1267* protein may be implicated in PD and other parkinsonian syndromes thus deserving further study.

STH is an intronless gene nested in intron 9 of *MAPT* that encodes a 128 amino acid protein with no clear homology.[15] There is immunohistochemical evidence that *STH* produces a protein product,[15] but studies have demonstrated that *STH* may be involved in the regulation of 3R and 4R *MAPT* splicing.[36,37] In the current study, it was found that higher expression of *STH* was strongly correlated with higher expression of both 3R and 4R *MAPT* isoforms across all samples. While the role of *STH* in the splicing and expression of *MAPT* transcripts has yet to be determined, our study provides evidence that the expression of these genes may not be independent of one another.

The 4R:3R *MAPT* ratio is increased in several parkinsonian disorders, including FTDP-17, PSP, and CBD, and thus it is noteworthy that we observed an increase of this ratio in PD cases compared to controls.[17,18] Based upon the expression of the individual *MAPT* isoforms, the higher 4R:3R ratio in PD cases may be a consequence of increased 4R expression in PD relative to controls. This suggests that some forms of PD may share pathogenic mechanisms with other related parkinsonian disorders.

Increased 4R *MAPT* expression has been shown to have several adverse effects on neurons that could contribute to the development of PD. In neuronal cell culture, increased tau inhibited intracellular transport along microtubules, disrupting cell function and enhancing vulnerability to oxidative stress.[38] Transgenic mice that overexpress human 4R tau have pathologic dilations along axons throughout the brain, which are sites of accumulation of neurofilaments, microtubules, and organelles.[39] This mouse model demonstrated that increased 4R tau was sufficient to cause damage to CNS neurons. Furthermore, it has been shown that tau promotes the assembly of α-synuclein into fibrils, which can further aggregate into Lewy bodies, the pathologic hall-mark of PD.[40]

Dopaminergic neurotransmission occurs in the cerebellum[41] which has connections with areas of the brain more directly affected by PD pathology including substantia nigra, locus coeruleus, and various regions of the cortex. However, one of the limitations of this gene expression study is that we did not identify the specific cell populations from which the mRNA is derived. Additionally, there was very limited clinical information available about the brain specimens, with unknown family history of PD and medication exposure.

While our haplotype and gene expression results suggest that *MAPT* plays a role in PD, it is possible that all three genes, *MAPT, STH,* and *KIAA1267,* are together implicated in PD. Further studies are warranted in order to unravel the complex contributions of the genes in this region to the pathogenesis of idiopathic and familial PD.

Acknowledgements

Supported by the Bumpus Foundation, PHS grant R01 NS36711-05 (Genetic Linkage Study in PD), and NIA grant 5-T32-AG00277-05 (Neurobiology and Neuropsychology of Aging). DNA samples contributed by the Parkinson Institute-Istituti Clinici di Perfezionamento, Milan, Italy, were from the Human Genetic Bank of Patients Affected by PD and Parkinsonisms, supported by Italian Telethon grant GTF04007. The Harvard Brain Tissue Resource Center, which is supported in part by PHS grant R24 MH 068855, provided tissue used in this study. Boston University Alzheimer's Disease Center Brain Bank, is supported by the NIH, National Heart, Lung, and Blood Institute's Framingham Heart Study (NIH/NHLBI Contract N01-HC 25195), NIA 5R01-AG08122, NIA 5R01-AG 16495, and National Institute of Neurological Disorders and Stroke 2R01-NS17950, the Boston University Alzheimer's Disease Center NIAAA, P30 AG13846, and the Department of Veteran's Affairs. Dr. Stephen Kish at the Centre for Addiction and Mental Health at the University of Toronto provided additional brain tissue.

GLOSSARY

CBD, corticobasal degeneration; FTDP-17, frontotemporal dementia with parkinsonism linked to chromosome 17; GEE, generalized estimating equations; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; OR, odds ratio; PD, Parkinson disease; PSP, progressive supranuclear palsy; SNP, single-nucleotide polymorphism.

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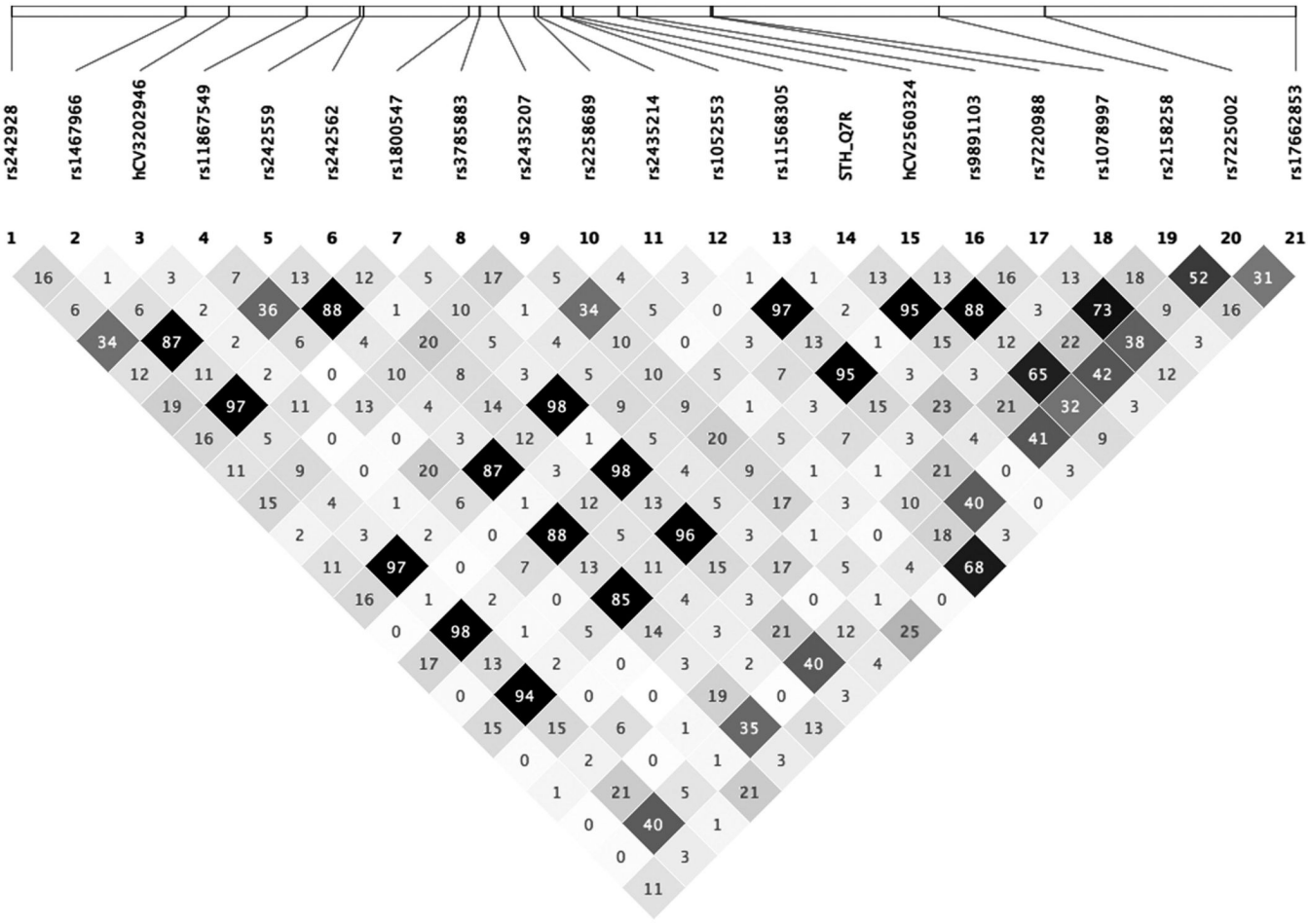

Figure 1. Linkage disequilibrium plot

Linkage disequilibrium of single-nucleotide polymorphisms in Parkinson disease cases was visualized as r^2 values with darker squares representing higher r^2 values. The pairwise r^2 value is printed in each box of the grid. This plot was created using Haploview.[34]

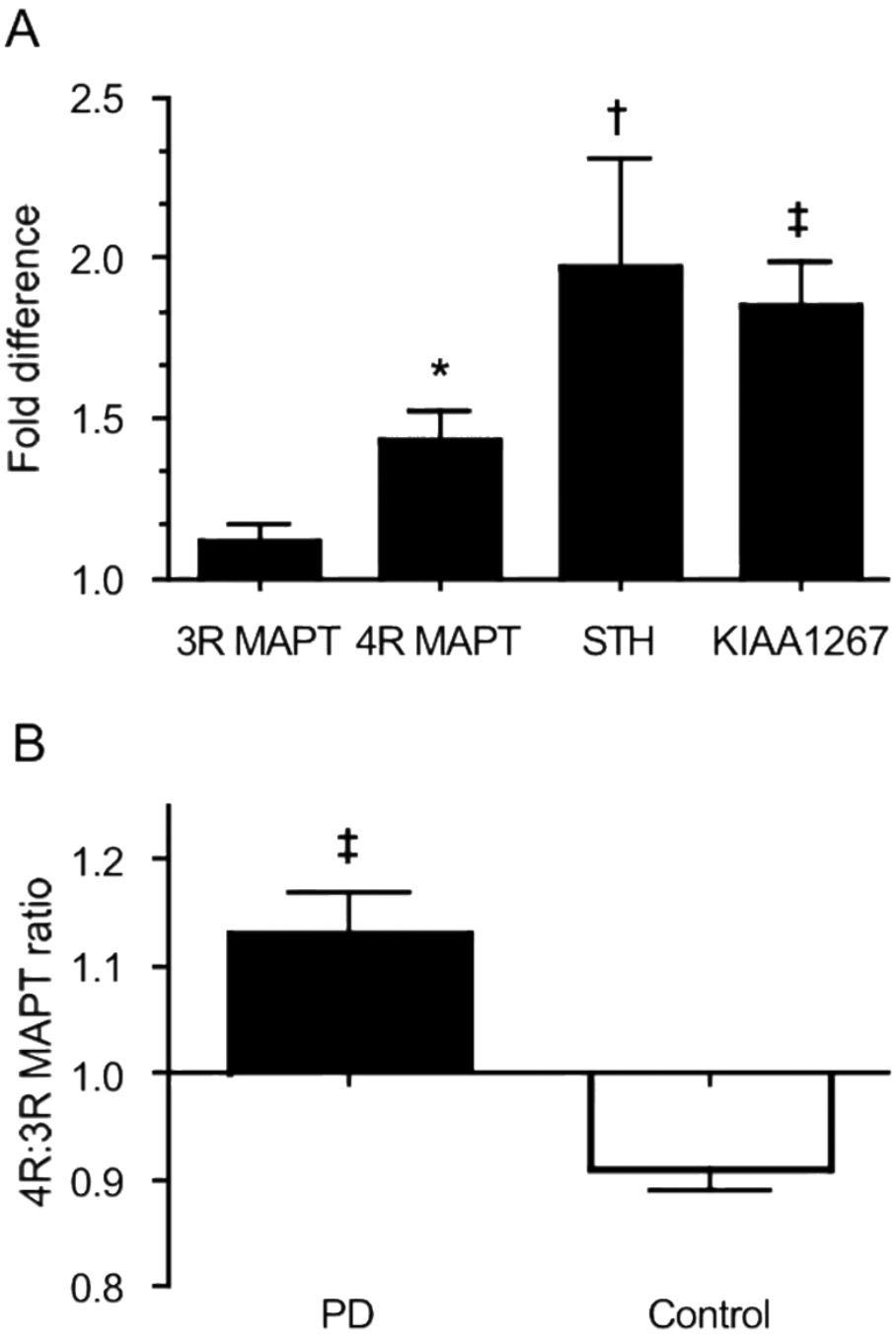

Figure 2. Relative gene expression

(A) The relative expression of MAPT, STH, and KIAA1267 represented as the mean ± SEM expression in Parkinson disease (PD) cases relative to controls. (B) The 4R:3R ratio of MAPT isoforms (mean ± SEM) in PD cases and controls. *$p = 0.002$, $^{\dagger}p = 0.001$, $^{\ddagger}p < 0.0001$.

Table 1

Single SNP association of the minor allele to Parkinson disease affection using an additive model and GEE to account for correlated observations

SNP	Position	SNP type	MAF PD	MAF control	OR	*p* Value	Adj *p* value
rs242928	41,298,840	3′ to *MAPT*	0.44	0.41	1.12	0.37	1
rs1467966	41,340,226	*MAPT* 3′ UTR	0.16	0.23	0.67	0.007*	0.11
hCV3202946	41,350,589	*MAPT* 3′ UTR	0.09	0.08	1.12	0.60	1
rs11867549	41,369,064	*MAPT* 3′ UTR	0.25	0.26	0.96	0.75	1
rs242559	41,381,748	*MAPT* 3′ UTR	0.17	0.25	0.63	0.013*	0.21
rs242562	41,382,599	*MAPT* 3′ UTR	0.36	0.35	1.06	0.64	1
rs1800547	41,407,682	*MAPT* intron 4	0.15	0.23	0.62	0.001*	0.02*
rs3785883	41,410,269	*MAPT* intron 4	0.21	0.15	1.52	0.008*	0.14
rs2435207	41,414,765	*MAPT* intron 5	0.32	0.28	1.24	0.09	1
rs2258689	41,423,219	*MAPT* exon 8 nonsyn	0.19	0.18	1.03	0.89	1
rs2435214	41,424,176	*MAPT* intron 8	0.14	0.16	0.83	0.38	1
rs1052553	41,429,726	*MAPT* exon 10 synon	0.16	0.23	0.67	0.007*	0.12
rs11568305	41,429,855	*MAPT* exon 10 synon	0.051	0.037	1.38	0.32	1
Q7R	41,432,503	*STH*	0.16	0.23	0.66	0.005*	0.09
hCV2560324	41,443,337	*MAPT* intron 10	0.43	0.41	1.12	0.48	1
rs9891103	41,447,723	*MAPT* intron 12	0.17	0.23	0.70	0.016*	0.25
rs7220988	41,465,320	*KIAA1267* exon 13 nonsyn	0.44	0.44	1.01	0.93	1
rs1078997	41,465,690	*KIAA1267* intron 12	0.14	0.11	1.40	0.082	1
rs2158258	41,519,626	*KIAA1267* intron 2	0.47	0.50	1.12	0.35	1
rs7225002	41,544,849	*KIAA1267* intron 1	0.33	0.40	0.75	0.018*	0.29
rs17662853	41,604,624	*KIAA1267* exon 1 nonsyn	0.15	0.15	1.03	0.88	1

Chromosomal positions are based on dbSNP Build 126. Raw GEE *p* values are presented along with *p* values adjusted using the Bonferroni correction for 16 tests.

OR = odds ratio; SNP = single-nucleotide polymorphisms; GEE = generalized estimating equations; MAF = minor allele frequency.

*Significant.

Table 2

Analysis of the H1/H2 haplotypes in the *Gene*PD Study

rs1800547	rs1052553	H1/H2	Freq. PD	Freq. C	OR	*p* Value
2	2	H2	0.13	0.23	1	0.0008*
1	1	H1	0.87	0.77	1.72	

The major allele is denoted as 1 and the minor allele as 2. Freq. PD = haplotype frequency in Parkinson disease cases; Freq. C = haplotype frequency in controls; OR = odds ratio.

*Significant.

Table 3

Haplotype analysis for risk of Parkinson disease in the *Gene*PD Study

hCV3202946	rs1800547	rs3785883	rs2435207	rs11568305	rs1078997	Freq. PD	Freq. C	OR	*p* Value
1	2	1	1	1	1	0.13	0.23	0.63	0.01*
1	1	1	1	1	1	0.41	0.41	1.00	
1	1	1	2	1	1	0.17	0.16	1.04	0.84
1	1	2	2	1	1	0.067	0.017	4.48	0.003*

Haplotypes present in less than 5% of the population are not shown in this table. The major allele is denoted as 1 and the minor allele as 2. Freq. PD = haplotype frequency in Parkinson disease cases; Freq. C = haplotype frequency in controls; OR = odds ratio. Global *p* value 0.003.

*Significant.