Glycogen Synthase Kinase-3β and Tau Genes Interact in Alzheimer's Disease

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Objective: We examined the epistatic effect between haplotypes of *glycogen synthase kinase-3β (GSK3B)* gene and *microtubule-associated protein Tau (MAPT)* gene in Alzheimer's disease (AD).

Methods: A genetic association study of three AD cohorts was made. Linear regression analyses were used to examine effects of *MAPT* polymorphisms on gene expression and alternative splicing. β-Catenin levels and signaling were determined using Western blot and luciferase reporter assays in cells transfected with a combination of *GSK3B* and *MAPT* complementary DNA.

Results: Consistent interaction between *GSK3B* and *MAPT* genes in three late-onset AD cohorts was observed, with the *GSK3B* haplotype (T-T) significantly increasing the risk for AD in individuals with at least one H2 haplotype (odds ratio, 1.68–2.33; $p = 0.005–0.036$). The *GSK3B* haplotype was significantly protective in the Chinese cohort (odds ratio, 0.33; $p = 0.016$), after adjusting for the effect of age and sex. There are significant differences in in vivo transcriptional efficiency between the two *MAPT* haplotypes (H1 and H2) as determined by measurement of cerebellar transcripts ($p < 0.001$). Overexpression of either *MAPT* or *GSK3B* resulted in decreased β-catenin levels compared with a control vector ($p < 0.001$). Conversely, cotransfection of both of these molecules increased β-catenin signaling.

Interpretation: Our genetic and biochemical analyses have identified a novel interaction between Tau and GSK-3β in late-onset AD causative factors. Our data are consistent with an epistatic model of interaction where discordant levels of *GSK3B* and *MAPT* gene expression can lead to altered β-catenin levels and pathogenicity.

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Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder (OMIM 104300), affecting approximately 13% of people at 80 years of age.[1] Neurofibrillary tangles, composed of hyperphosphorylated microtubule-associated protein Tau (MAPT), are one of two key neuropathological and diagnostic features of AD.[1] Several genetic studies have shown an inconsistent association between the *MAPT* gene[2] and AD.[3–7] These reports have utilized a set of polymorphisms in complete linkage disequilibrium that differentiate the two major *MAPT* haplotypes, H1 and H2, as Baker and colleagues[8] first defined. Recent reports have shown that a subhaplotype of H1 (H1c), as defined by a series of single nucleotide polymorphisms (SNPs) including rs242557 and rs2471738,[9,10] was the major susceptibility variant associated with sporadic tauopathies including AD.[8–11] Functional analyses of the H1 haplotype and subhaplotypes suggest that the pathogenic mechanism is due to increased *MAPT* expression using in vitro promoter assays[12,13] or in vivo measurements of transcripts in brain samples with tauopathies.[11,13] Fine mapping of the *MAPT* locus demonstrated that the SNP rs242557 within the H1c subhaplotype is the probable functional variant associated with tau levels in cerebrospinal fluids.[13] An additional effect of the H1 and H1c subhaplotype is on alternative splicing of exon 10 of *MAPT* gene, in which two studies demonstrated increased inclusion of exon 10 using allele-specific expression assays on H1(c)/H2 heterozygotes from diseased brains.[11,14]

Glycogen synthase kinase-3β (GSK-3β) is serine/

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threonine-specific kinase that phosphorylates Tau.[15] GSK-3β is also an essential modulator of multiple signaling pathways, including growth factor and Wnt signaling.[16] The substrate for GSK-3β in the Wnt pathway is β-catenin, whose intracellular level is tightly regulated by phosphorylation.[16] Several studies have highlighted the role of the Wnt pathway and β-catenin signaling in AD. GSK-3β and β-catenin form multiprotein complex with early-onset AD linked proteins such as the presenilins.[17] Moreover, β-catenin levels are also decreased in brains from patients bearing presenilin mutations.[18] We have shown that the polymorphisms in *GSK3B* interact with the *MAPT* haplotypes to increase the risk for idiopathic Parkinson's disease (PD).[19] Here, we show that the same *GSK3B* and *MAPT* haplotypes are associated with an increased risk for AD. We also confirm the postulated effect of the *MAPT* haplotypes on gene transcription in vivo. Finally, based on further experimental data, we proposed a model for the epistatic interaction between *GSK3B* and *MAPT* haplotypes for discordance of gene expression and subsequent effect on β-catenin levels and signaling.

Subjects and Methods

Subjects

Clinical data and DNA samples from a UK case–control cohort were collected from 604 individuals (68% women) with AD and 589 control subjects (69% women).[20] Mean age of AD patients was 82 years (standard deviation [SD], 6.4). The samples were recruited from both community and hospital settings and an assessment battery was used to form a diagnosis of probable AD according to National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association criteria.[21] Mean age of control subjects was 77 years (SD, 6.5). Control subjects were recruited in two ways. Spouse control subjects were recruited from the participation of their AD partner. Control subjects were also recruited from general practices in the same areas as the AD patients. Differences in age and sex will be adjusted for in our analysis using logistic regression. The Sydney Older Person Study (SOPS) cohort is a population-based longitudinal cohort of 300 community-living elderly people (>75 years old) within a specific geographical region of Sydney, comprising 129 affected individuals and 171 cognitively normal individuals.[22] The cohort was examined in five waves over 11 years.[22] Mean age at last assessment is 88.0 (SD 4.3) years, and 48% were women. Probable AD and senile dementia status was determined using both Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R) and National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association criteria. A total of 179 Chinese AD patients (93% were women; mean age at study, 84.3 years [SD, 6.2]) with National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association diagnosis of probable or possible AD were recruited from the Psychogeriatric clinics and outreach services of the Department of Psychiatry at the New Territories East Cluster Hospitals in Hong Kong.[23] A group of 188 Chinese elderly control subjects (73% were women; mean age at study, 73.8 years [SD 7.1]) were recruited from local elderly social centers for comparison. All control subjects were evaluated by the Chinese versions of the Mini-Mental State Examination, Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog), and were evaluated by a specialist psychiatrist as clinically not demented before recruitment (Clinical Dementia Rating < 1). Both the AD and control subjects were ethnically from the Guangdong province. Informed consent was obtained from all participants, and the project was approved by the relevant institutional ethics committee.

Genotyping of Single Nucleotide Polymorphisms

The three functional *GSK3B* polymorphisms (rs375557, rs334558, rs6438552) were genotyped by commercially available Taqman Probe Genotyping Assays (ABI Biosystems, Foster City, CA) or by restriction-length fragment polymorphism, as described previously.[19] The primary *MAPT* haplotypes were determined from an exon 9 SNP (rs1052553) from Baker and colleagues' original description[8] using commercially available Taqman Probe Genotyping Assays (ABI Biosystems), or based on the promoter region -373 G/C SNP.[12] Two SNPs were used to define the H1c subhaplotype. rs242557 was amplified using primers F 5′-CTTCCTTACAAAGCAGTTGGCTTC-3′ and R 5′-ATGGCAGACCCTGTGAGATCATCC-3′ followed by restriction enzyme digest with *Rsa*I, and rs2471738 was amplified using primers F 5′-TGCTCAGGCATGTGGAGCTTGTAG-3′ and R 5′-AGGACTGGAAAGTCTGGAGACGAG-3′, followed by restriction enzyme digest with *Hinf*I.

Quantification of MAPT *Gene Expression and Alternative Splicing*

Total RNA was extracted and reverse-transcribed using Superscript III first-strand complementary DNA (cDNA) synthesis kit (Invitrogen, La Jolla, CA) from 92 frozen cerebellum samples of brains with no evidence of AD or Tauopathies. Tau transcript was amplified from brain cDNA using primers flanking the constitutively spliced exon 7 sequence (F: 5′-AGGGGGCTGATGGTAAAACG-3′; R: 5′-GGGGTTTTTGCTGGAATCC-3′), and expression was measured by real-time polymerase chain reaction (PCR; SYBR-Green chemistry; Invitrogen) under manufacturer's conditions. Total Tau messenger RNA level for each sample was measured twice and normalized by comparison with transcripts from the housekeeping gene GAPDH. To account for variability between real-time PCR runs, we then adjusted these normalized ratios against the mean value of the H1/H1 subset in each run.

Exon 10 splicing was analyzed using flanking PCR primers (F: 5′-GAAGATTGTCAAGTCCAAGATC-3′; R: 5′-GGAGGAGACATTGCTGAGAT-3′), resulting in two bands corresponding to four-repeat and three-repeat *MAPT* gene splice isoforms. The forward primer was ^{33}P-labelled, and intensities of these bands were quantified using PhosphoImager 445 S1 (Molecular Dynamics, Sunnyvale, CA).[24]

The ratio of the four-repeat band to the three-repeat band was used to assess splicing.

Statistics

Linear regression was used to determine correlation between trends in genotypes or haplotypes and quantitative traits.[25] Differences in means of quantitative traits were also compared using the two-sample *t* test assuming unequal variances. Mean and standard error of the mean are given for all variables. χ^2 contingency tables were used to test for significance of association between the *GSK3B/MAPT* haplotype interaction and risk for AD. χ^2 analyses were performed with a specific a priori hypothesis[19] on the interaction between specific functional *GSK3B* and *MAPT* haplotypes to avoid multiple testing. Given our previous finding of an association between PD and the *GSK3B/MAPT* haplotype interaction, a logistic regression model was performed to test the same hypothesis and take into account age and sex variations (Intercooled Stata 8.0 for Windows; StataCorp, College Station, TX). The dependent variable was disease status, and the five independent variables include age, sex, carriage of the *MAPT* H1 haplotype on both alleles, carriage of the *GSK3B* T-T haplotype (SNP rs334558 and SNP rs6438552) on both alleles, and an interaction variable for the *MAPT* and *GSK3B* variables. *GSK3B* haplotype pairs for individuals were estimated from population genotype data (rs334558-rs6438552) using the PHASEv2 program.[26] Genotypes from an additional *GSK3B* marker (rs3755557) were used to infer haplotype pairs in the Chinese population.

Overexpression of MAPT *and Glycogen Synthase Kinase-3β Complementary DNA in Transfected Cells*

A full-length *MAPT* cDNA with exon 10 included (four-repeat Tau) was isolated from a human adult hippocampal cDNA library (Stratagene, La Jolla, CA) and subcloned into the mammalian expression vector pCDNA3.1 (Invitrogen). A pCDNA3.1 construct expressing *MAPT* cDNA without exon 10 (three-repeat Tau) was generated by insertion of a Sfi1/Nhe1 restriction enzyme digest fragment of a *MAPT* cDNA library clone that lacked exon 10. The GSK3BΔexon9+11 splice isoform cDNA was subcloned into the mammalian expression vector pCDNA3.1, as described previously.[19] Each recombinant vector was transfected into the human neuroblastoma cell line, SK-N-MC (ATCC HTB 10) and human embryonic kidney 293 (HEK293) cells (ATCC CRL 1573) using Lipofectamine 2000 (Invitrogen). Cells were left for 48 hours before being lysed in 1X Lysis buffer (50mM Tris HCl [pH 7.4], 150mM NaCl, 1mM phenylmethylsulfonyl fluoride, 1X complete cocktail protease inhibitor [Roche, Mannheim, Germany] and 0.05% Triton X-100 [Sigma, St. Louis, MO]), snap frozen at −80°C, and microfuged at 13,000rpm for 20 minutes.

Western Blot Analysis

Approximately 25μg protein lysate underwent electrophoresis on a 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and was transferred to a nitrocellulose membrane (Trans-blot transfer medium; Biorad, Hercules, CA). A 1:2,000 dilution of a mouse monoclonal antibody (Clone 7; BD Bioscience, Paolo Alto, CA) was used to detect GSK-3β protein. A 1:2,000 dilution of mouse monoclonal antibody (Clone 15B8; Abcam, Cambridge, Cambridgeshire, United Kingdom) was used to detect total β-catenin levels. A 1:2,000 dilution of a mouse monoclonal antibody (Clone 5E2; Neomarkers, Fremont, CA) was used to detect total tau protein. Relative intensities of chemiluminescent bands on the blots were quantified directly using the Molecular Imager ChemiDoc XRS System (BioRad).

Luciferase Reporter Assay

The TOPFLASH luciferase vector (Upstate Cell Signaling Solutions, Lake Placid, NY) was used to measure the level of active β-catenin that is capable of initiating transcription of a luciferase reporter gene. HEK293 cells plated out in white-walled 96-well plates (Corning Glass Works, Corning, NY) and transfected with a series of construct combinations to determine the effects of overexpression of either Tau or GSK-3β, alone or in combination. A combination of 100ng of the TOPFLASH construct was transfected together with sufficient amount of the LacZ vector, *MAPT* or *GSK3B* (GSK3BΔexon9+11) cDNA, for a final total amount of 200ng DNA/well. Cells were lysed in situ and assayed for luciferase activity using the Bright-Glo Luciferase assay system (Promega, Madison, WI).

Results

Association of a GSK3B *Haplotype in Three Independent Alzheimer's Disease Cohorts*

We have previously shown that the *GSK3B* T-T haplotype, comprising the T allele of promoter SNP rs334558 and the T allele of intronic SNP rs6438552, is significantly associated with PD in individuals with at least one *MAPT* H2 haplotype.[19] We estimated haplotype pairs of each individual using the PHASE software[26] that allows us to examine the effect of genotypes on disease risk.

The first white AD cohort we examined was a late-onset AD cohort from the United Kingdom.[20] As shown in the Table, overall, individuals who were homozygous for the *GSK3B* T-T haplotype do not show an association with disease risk (odds ratio, 1.22; $p = 0.098$). However, in individuals with at least one *MAPT* H2 haplotype, the homozygous T-T haplotype was significantly associated with AD (odds ratio, 1.68; $p = 0.005$). Logistic regression, after taking into account age and sex, confirmed the genotypes and their interaction to be significant predictors of disease. The *MAPT* H1/H1 haplotype, *GSK3B* T-T/T-T haplotype, and their interaction term were found to have odds ratios of 1.49 ($p = 0.012$), 1.72 ($p = 0.007$), and 0.47 ($p = 0.004$), respectively. When apolipoprotein E4 carriage is included as an additional variable, these odds ratios become 1.45 ($p = 0.028$), 1.44 ($p = 0.090$) and 0.52 ($p = 0.022$), respectively.

The second white cohort we examined was a community-based cohort collected from a single geo-

Table. **Association between a *GSK3B* Haplotype (T-T) and Disease Risk in Three Independent Cohorts from Two Ethnic Groups**

AD Cohort	T-T Genotypic Frequency			OR (p value): T-T Haplotype (+/+) vs Others	OR	p
	+/+	+/−	−/−			
United Kingdom (white population)						
All subjects (N = 1,193)				1.22 (0.098)		
AD patients (n = 604)	235 (39%)	265 (44%)	104 (17%)			
Control subjects (n = 589)	202 (34%)	300 (51%)	87 (15%)			
H1/H1				0.956 (0.816)		
AD patients (n =353)	130 (37%)	157 (44%)	66 (19%)			
Control subjects (n = 329)	124 (38%)	164 (50%)	41 (12)%			
H1/H2+H2/H2				1.68 (0.005)[a]		
AD patients (n = 251)	105 (42%)	108 (43%)	38 (15%)			
Control subjects (n = 260)	78 (30%)	136 (52%)	46 (18%)			
ORs after adjusting for age and sex[b]						
MAPT (H1/H1)					1.49	0.012[a]
GSK3B (T-T/T-T)					1.72	0.007[a]
Interaction term					0.47	0.004[a]
Australia (white population)						
All subjects (n = 300)				1.66 (0.035)[a]		
AD patients (n = 129)	56 (43%)	50 (39%)	23 (18%)			
Control subjects (n = 171)	54 (32%)	80 (47%)	37 (22%)			
H1/H1				1.38 (0.292)		
AD patients (n = 81)	33 (41%)	30 (37%)	18 (22%)			
Control subjects (n = 111)	37 (33%)	53 (48%)	21 (19%)			
H1/H2+H2/H2				2.33 (0.036)[a]		
AD patients (n = 48)	23 (48%)	20 (42%)	5 (10%)			
Control subjects (n = 60)	17 (28%)	27 (45%)	16 (27%)			
OR after adjusting for age and sex[b]						
MAPT (H1/H1)					1.22	0.561
GSK3B (T-T/T-T)					2.54	0.034[a]
Interaction term					0.57	0.302
Hong Kong (Chinese)						
ORs after adjusting for age and sex[b]						
GSK3B (T-T/T-T)					0.33	0.016[a]

[a]Significance at $p < 0.05$.
[b]Odds ratios (ORs) derived from a logistic regression model using disease status as the dependent variable and five independent variables: age, sex, *MAPT* haplotype, *GSK3B* haplotype, and interaction term for the two haplotypes.
GSK3B = glycogen synthase kinase-3β; AD = Alzheimer's disease; MAPT = *microtubule-associated protein Tau.*

graphical region within Sydney, Australia (Sydney Older Person Study cohort) comprising 129 affected individuals and 171 individuals without dementia.[22] Individuals who were homozygous for the *GSK3B* T-T haplotype showed a significant association with disease risk (odds ratio, 1.66; $p = 0.035$). As with the UK AD cohort, the odds ratio for the T-T haplotype increased to 2.32 ($p = 0.036$) in individuals with at least one *MAPT* H2 haplotype. With logistic regression taking into account age and sex, the *MAPT* H1/H1 haplotype, *GSK3B* T-T/T-T haplotype, and their interaction term were found to have odds ratios of 1.22 ($p = 0.561$), 2.54 ($p = 0.034$), and 0.57 ($p = 0.302$), respectively. When apolipoprotein E4 carriage is included as an additional variable, these odds ratios become 1.12 ($p = 0.749$), 2.34 ($p = 0.057$), and 0.66 ($p = 0.452$), respectively.

Our final cohort comprised 179 Chinese patients with AD and 188 control subjects.[23] As found in previous studies, all individuals in this Chinese cohort were homozygous for the H1 haplotype. Because of imbalances in distribution of age and sex in the AD

and control groups, the effect of the homozygous *GSK3B* T-T haplotype was assessed using logistic regression. Consistent with the H1/H1 subsets in the white cohorts and a previously published Chinese PD cohort,[19] individuals who were homozygous for the *GSK3B* T-T haplotype were found to have a lower disease risk after adjusting for age and sex (odds ratio, 0.33; $p = 0.016$). Hardy–Weinberg equilibrium was found to be intact for control populations in all three cohorts.

Functional Consequences of MAPT H1 *Haplotypes in Cerebellum Samples*

We have previously demonstrated that the H1/H2 primary *MAPT* haplotypes results in altered *MAPT* gene expression as assessed by in vitro reporter gene transcription studies.[12] We now test the hypothesis that the H1 *MAPT* haplotype is more efficient at driving *MAPT* gene expression in vivo. As shown in Figure 1A, samples homozygous for the H2 primary haplotype had significantly decreased *MAPT* gene expression compared with those without the risk variants (2.83-fold; $p = 0.0008$, two-sample t test, assuming unequal variances). Similarly, homozygosity for the G allele of rs242557 significantly decreased *MAPT* expression by 1.54-fold ($p = 0.044$, two-sample t test, assuming unequal variances; see Fig 1A). We used linear regression to determine trend effects of either SNPs or haplotypes on gene expression.[25] Both H1/H2 (rs1052553) and rs242557 were equally informative in explaining the variance in *MAPT* expression levels ($r^2 = 0.03$; $p > 0.05$; see Fig 1A). Linear regression combining information from both SNPs additively gave a positive significant correlation between genotypes and *MAPT* transcript levels ($r^2 = 0.04$; $p = 0.044$). The correla-

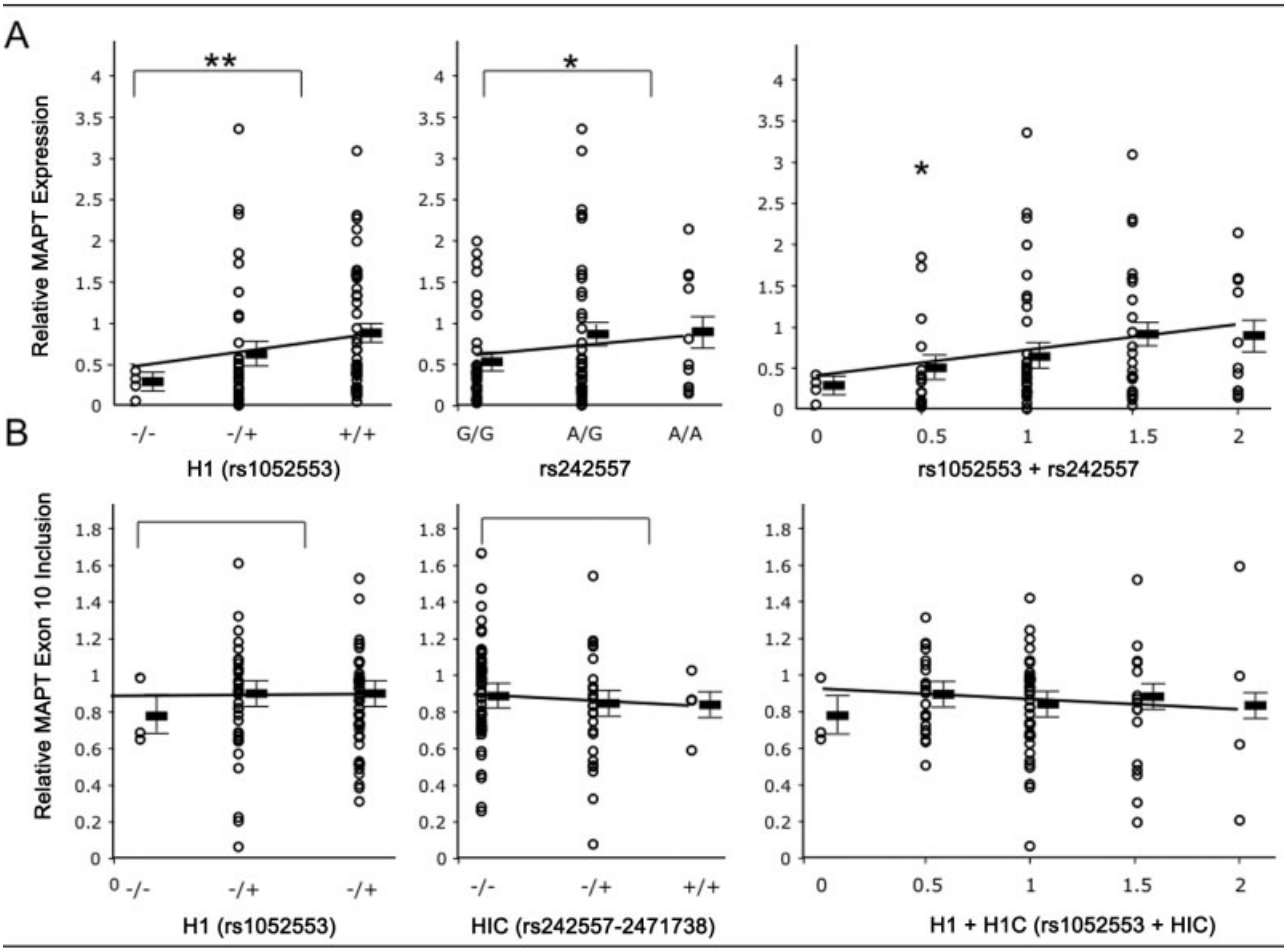

Fig 1. Linear regression analysis of two quantitative traits: (A) microtubule-associated protein Tau (MAPT) expression and (B) alternative splicing of MAPT exon 10. (A) Normalized MAPT transcript levels from 92 cerebellum samples were segregated according to genotypes for rs1052553, rs242557, or additive effects of both using linear regression. The correlation coefficient (r^2) is given for each linear regression analysis and significant p *value is indicated (*$p < 0.05$). Inheritance of at least one risk allele (H1 for rs1052553 and A for rs242557) is associated with increased MAPT transcript levels (**$p < 0.001$; *$p < 0.05$). (B) Relative levels of MAPT exon 10 inclusion from 88 samples were segregated using the same MAPT polymorphisms. No significant effect of the MAPT haplotypes on exon 10 splicing was detected.*

tion between H1c haplotype and expression levels was less than the single SNPs (data not shown).

We performed the splicing study in 88 of our brain samples using a semiquantitative real-time PCR technique using primers that flank exon 10.[24] This allows us to measure both splice isoforms from each sample as a single reaction and to normalize for differences in sample concentration. We did not find any significant association between *MAPT* gene splicing ratio and the *MAPT* haplotypes (see Fig 1B). Linear regression combining information from both rs105266 and the H1c haplotype additively did not show stronger correlations between haplotypes and alternative splicing of exon 10 (see Fig 1B). Samples carrying at least one copy of the H1c haplotype had a 1.09-fold lower *MAPT* gene splicing compared with individuals without the risk variant, again without reaching statistical significance.

Altered Expression of Tau and Glycogen Synthase Kinase-3β Lead to Decease Stability of β-Catenin

We examined the effect of overexpression of Tau and GSK-3β on β-catenin levels in two assay systems. First, we measured total β-catenin levels (normalized against endogenous GSK-3β levels) in HEK293 and SK-N-MC cells overexpressing either tau with three (three-repeat Tau) or four (four-repeat Tau) microtubule-binding domains using Western blotting. As shown in Figures 2A and B, only overexpression of the four-repeat Tau resulted in a significant 1.3-fold decrease of the total levels of endogenous β-catenin in both HEK293 ($p = 0.010$, Student's t test) and SK-N-MC cells ($p = 0.001$, Student's t test) compared with cells overexpressing the lacZ gene. Overexpression of the three-repeat Tau had a lesser (1.1-fold), nonsignificant effect. Second, we measured the level of active β-catenin in transfected cells using the luciferase reporter construct, TOPFLASH, which contains a promoter element that binds to nuclear β-catenin. As shown in Figure 2C, overexpression of the four-repeat Tau resulted in a significant 1.4-fold decrease of β-catenin signaling and subsequent luciferase activity ($p = 0.001$). As expected, overexpression of the disease-associated isoform of GSK-3β (GSK3BΔexon9+11) also resulted in a decrease in β-catenin signaling. Of interest, coexpression of either three-repeat ($p = 0.001$, Student's t test) or four-repeat Tau ($p = 0.003$, Student's t test) significantly reversed the negative impact of GSK-3β overexpression on β-catenin signaling.

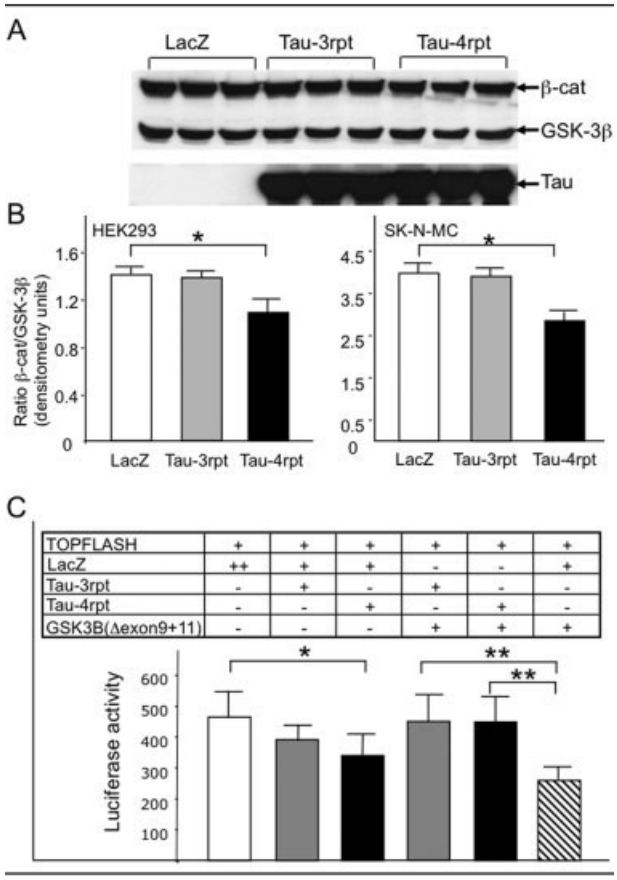

*Fig 2. Effect of overexpression of microtubule-associated protein Tau (MAPT) and glycogen synthase kinase-3β (GSK3B) complementary DNA (cDNA) on β-catenin levels and activity in cultured cells. (A) Western blot analysis of GSK-3β and β-catenin (products in cells transfected with expression vectors that constitutively express a control lacZ gene (LacZ), the three-repeat (Tau-3rpt) or four-repeat (Tau-4rpt) splice isoforms of Tau. (B) Quantification of total β-catenin levels (normalized to GSK-3β) in transfected human embryonic kidney 293 (HEK293) and SK-N-MC cells. Significance is indicated (*p < 0.05). (C) A luciferase reporter assay for β-catenin–induced gene transcription in HEK293 cells. Each treatment has equal amounts of DNA (*plus sign *indicates 50ng DNA) per transfection. Mean (± standard error of the mean) derived from five independent experiments. Significance is indicated (*p < 0.05; **p < 0.001).*

Discussion

A large body of evidence suggests that GSK-3β has a seminal role in neurodegeneration.[16,27] Our findings provide both genetic and functional evidence to support the role of GSK-3β in AD. We have previously shown that there was an interaction between *GSK3B* haplotypes and *MAPT* in PD.[19] The *GSK3B* functional T-T haplotype is associated with maximal transcription of the GSK-3β splice isoform that lacks exons 9 and 11 (GSK3BΔexon9+11).[19] The splice isoform has an increased ability to phosphorylate Tau, and thus is likely to be a pathogenic isoform.[19] Consistent with the effect of the *GSK3B* T-T haplotype in the white PD cohort, we observed that there is an increased risk for AD in individuals who are homozygous for the T-T haplotype and at least one copy of the H2 *MAPT* haplotype (see the Table). Also consistent with our previous findings in a Chinese PD cohort,[19] we observe a trend that the T-T haplotype is protective for disease risk in our Chinese AD cohort. We have previously

postulated that this is due to the fact that the H2 haplotype frequency is extremely low in East-Asian populations,[19,28] and a protective trend of the T-T haplotype is also seen in white individuals who are homozygous for the H1 haplotype (see the Table), although the effect does not reach significance within individual cohorts. Thus, our pooled findings indicate that *GSK3B* and *MAPT* interact in a consistent manner in at least five independent cohorts with two neurodegenerative diseases, AD and PD.[19]

An independent study reported the significant association between *GSK3B* rs334558 polymorphism (homozygous for the T allele) and late-onset AD (odds ratio, 1.99; $p = 0.003$) in a cohort of Spanish patients older than 72 years. Moreover, the authors discovered that the odds ratio was significantly increased (odds ratio, 4.58; $p = 0.028$)[29] in individuals who have the R allele of the Saitohin gene (which forms part of the *MAPT* H2 haplotype[6]). Given the high degree of linkage disequilibrium between the two markers (rs334558 and rs6438552) that comprise the T-T haplotype in white populations ($D' = 0.89–0.90$, $p < 0.001$ for our two white cohorts), this study serves as a further independent validation of the interaction between *GSK3B* and *MAPT* haplotypes.

Our functional analyses suggest that a major effect of the H1 haplotype was on gene expression, with the H1 haplotype and the rs242557 polymorphisms both contributing to increased *MAPT* transcript levels, consistent with previous reports.[11,13] We did not find a significant effect of the H1 haplotypes on alternative splicing of the *MAPT* gene. This discrepancy may be because we were using a different methodology and brain region (cerebellum) compared with the other groups. Another possibility is that by using brain samples from patients with tauopathies, one cannot differentiate between *cis*-acting elements and *trans*-acting splicing factors that increase splicing in of *MAPT* exon 10.[30,31]

From the known effect on gene expressions by the *GSK3B*[19] and *MAPT* haplotypes (see Fig 1A), we propose the following model for the epistatic interaction between the two genes as shown in Figure 3. We propose that the downstream effect of discordant GSK-3β and Tau levels is altered interaction with other GSK-3β substrates, where excess Tau is able to sequester GSK-3β and shunt it toward cellular pools that will have functional effects on other substrates of GSK-3β. GSK-3β regulates the activity of multiple molecules that have a role in neurodegeneration including the Toll-like receptors and interleukin-6 involved in the inflammatory response, glycogen synthase in diabetes, and microtubule-associated proteins involved in cell migration.[32] We have focused on β-catenin because perturbations in its signaling have dramatic effects on brain development and on apoptotic pathways. Overexpression of β-catenin in transgenic mice resulted in increased neuronal production leading to enlarged forebrains and altered neuronal migration during embryonic development.[33] Conversely, conditional knockout of β-catenin resulted in increased neuronal apoptosis and absence of the midbrain and hindbrain in transgenic mice.[34] Consistent with our proposed model is our demonstration that overexpression of Tau or GSK-3β alone results in a decrease of total β-catenin levels and subsequent β-catenin–driven gene transcription (see Fig 2). When both risk genes are coexpressed, the effect on β-catenin levels is reversed. Li and coworkers'[35] study notes an identical effect on β-catenin levels when both Tau and GSK-3β were overexpressed. They postulated that this was due to competition between Tau and β-catenin as substrates for GSK-3β. However, we demonstrate that overexpression of Tau alone also resulted in a decrease in β-catenin activity (see Fig 2), an observation that contradicts Li and coworkers' postulate. Although the exact manner in which Tau and GSK-3β interact to modulate β-catenin levels remains to be elucidated, our observation that the four-repeat Tau has a stronger effect on β-catenin levels than three-repeat Tau (see Fig

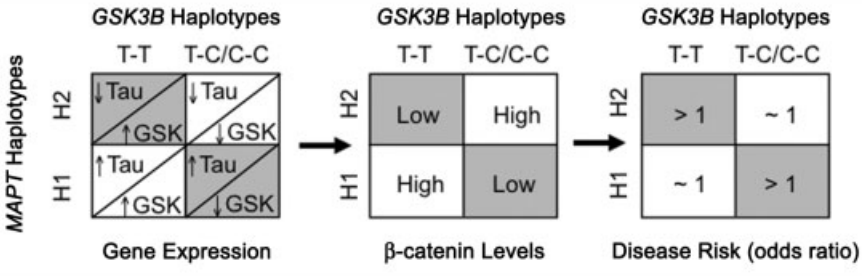

Fig 3. Model for the epistatic interaction between glycogen synthase kinase-3β (GSK3B) and microtubule-associated protein Tau (MAPT). Different levels of gene expression result from inheritance of the GSK3B[19] *and MAPT functional haplotypes. Discordance in the levels of the two proteins* (gray boxes), *that is, high levels of GSK-3β and low levels of Tau, or vice versa, has an effect on downstream GSK-3β signaling pathways (such as β-catenin levels and activity). This, in turn, may lead to altered disease risk* (gray boxes). *Conversely, concordant changes in levels of the two proteins* (open boxes) *do not lead to altered disease risk.*

2) suggests that differences in biochemical properties between the two splice isoforms, such as their differential ability to bind microtubules,[36] may be an important factor in the GSK-3β/Tau interaction pathway.

Our data indicate that *GSK3B* and *MAPT* genes interact to increase risk for both AD (see the Table) and PD,[19] two apparently distinct neurodegenerative diseases.[1] Our study is supported by clinical and epidemiological evidence that shows a high degree of comorbidity of the two diseases in elderly patients. Approximately a third of AD patients show clinical evidence of parkinsonism, whereas 33% of PD patients experience development of dementia during the course of the disease.[37] A recent retrospective study of patients with clinical diagnosis of parkinsonism shows that 31.2% of these patients have dementia, of which 18% were confirmed to have AD pathology.[38] Moreover, PD confers a 10-fold risk for dementia versus control populations.[39] Finally, there is increasing awareness that gene-gene interactions, or epistasis, plays an important role in susceptibility to common human diseases[40] and may be responsible for nonreplication of single-locus association studies. Our demonstration of a consistent pattern of epistatic interaction between *GSK3B* and *MAPT* genes, and verification of their effects on β-catenin by biochemical analyses has implications for other diseases including bipolar disorder and diabetes where the Wnt signaling pathway has been hypothesized to play an important role.[27,32]

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