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ORIGINAL ARTICLE

α-T-Catenin Is Expressed in Human Brain and Interacts With the Wnt Signaling Pathway But Is Not Responsible for Linkage to Chromosome 10 in Alzheimer's Disease

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Abstract

The gene encoding α-T-catenin, *CTNNA3*, is positioned within a region on chromosome 10, showing strong evidence of linkage to Alzheimer's disease (AD), and is therefore a good positional candidate gene for this disorder. We have demonstrated that α-T-catenin is expressed in human brain, and like other α-catenins, it inhibits Wnt signaling and is therefore also a functional candidate. We initially genotyped two single-nucleotide polymorphisms (SNPs) in the gene, in four independent samples comprising over 1200 cases and controls but failed to detect an association with either SNP. Similarly, we found no evidence for association between *CTNNA3* and AD in a sample of subjects showing linkage to chromosome 10, nor were these SNPs associated with Aβ deposition in brain. To comprehensively screen the gene, we genotyped 30 additional SNPs in a subset of the cases and controls ($n > 700$). None of these SNPs was associated with disease. Although an excellent candidate, we conclude that *CTNNA3* is unlikely to account for the AD susceptibility locus on chromosome 10.

Index Entries: CTNNA3; α-T-catenin; Alzheimer's disease; chromosome 10; amyloid; Aβ; age of onset; APOE; Wn.

Introduction

Previously, we observed strong evidence for linkage to Alzheimer's disease (AD) susceptibility on chromosome 10 at the marker D10S1211, with a LOD score of 3.9 (Myers et al., 2000, 2002). Concurrently, others found that the quantitative trait of plasma Aβ42 levels linked to the same marker (Ertekin-Taner et al., 2000). Other genome scans have also implicated chromosome 10 in AD, albeit at markers distal to D10S1211 (Bertram et al., 2000). Given the biological complexity of AD, few genes in the region can be confidently excluded as possible candidates and we have therefore adopted a strategy of screening genes closest to the region of maximal linkage. D10S1211 is positioned in a region that is comparatively sparse in relation to known annotated genes and unknown transcripts. The transcript closest to D10S1211 D10S1211 is *VR22*, now known as *CTNNA3*, coding for α-T-catenin (Janssens et al., 2001, 2003). α-T-Catenin is expressed predominantly in heart and testis but also in other tissues. Association between single-nucleotide polymorphisms (SNPs) in the *CTNNA3* gene and plasma amyloid β (Aβ) levels have previously been reported (Ertekin-Taner et al., 2003). We have extended these studies, determining the expression pattern of α-T-catenin in brain and genotyping four large AD case-control populations for two polymorphic variants in *CTNNA3*.

Materials and Methods

Plasmids

Plasmid pEF6MH-haTctn(1–2860) containing cDNA for human α-T-catenin was previously described (Janssens et al., 2001). We used the GATEWAY™ Cloning Technology (Invitrogen Life Technologies) to clone the mouse α-T-catenin cDNA into vector pdCS3, which was obtained by inserting the GATEWAY conversion cassette A (Invitrogen) into the *Stu*I site of plasmid pCS3 (Rupp et al., 1994). We polymerase chain reaction (PCR)-amplified the 5′ end of the murine α-T-catenin open reading frame (Janssens et al., 2003), from mouse heart cDNA, using primers MCBU#3464 (5′-CGGATCC CCAAAGCTCATTTACTTGTA-3′) and MCBU#2918 (5′ACAGCCAGAAAGTCGTCAATG-3′), yielding a product of 1573 bp. This PCR fragment was cloned into the pGEMTeasy® vector (Promega) and then transferred into the *Bam*HI–*Not*I-digested pENTR2B vector (Invitrogen), yielding plasmid pENTR2B-PCR3464+2918. The 3′ end of the mouse α-T-catenin–cDNA was obtained by *Nco*I–*Not*I digestion of plasmid pGEMTe-maTctn (1–2979)

(Janssens et al., 2003) and ligated into the *Nco*I-*Not*I-digested pENTR2B-PCR3464+2918, yielding plasmid pENTR2B-maTctn (120–2979). With the LR-CLONASE enzyme (GATEWAY; Invitrogen Life Technologies), the mouse α-T-catenin–cDNA was transferred to the pdCS3 vector, containing the attR1 and attR2 recombination sites, yielding plasmid pdCS3–maTctn (120–2979).

Expression Analysis by RT-PCR

For reverse transcription (RT)-PCR analysis of human α-T-catenin and its alternative transcript, cDNA was prepared from human brain using a commercial kit (Invitrogen). For human α-T-catenin (GenBank accession no. AF091606), an exon-1-specific primer MCBU#3573 (5′-CTGC CTCTCAATTTGGTACT-3′) and an exon-4-specific primer MCBU#3597 (5′-AGGGGTCATCTG TAAATCTC-3′) were designed, resulting in the amplification of a 478-bp product (corresponding to sequence 35–512 of GenBank accession no. AF091606). For the alternative transcript, an exon-1b-specific primer MCBU#3596 (5′-GTGGCACT GAATTTCTAAATAC-3′) was designed on the basis of three EST-sequences (GenBank accession nos. BI552791, BI549643, BG719928). PCR with primers MCBU#3596 and MCBU#3597 resulted in the amplification of a 503-bp product (GenBank accession no. AY198183). Other RT-PCR primer pairs used were as follows: MCBU#3575 (5′-AGCTGGAAAATTTGGATTATT-3′) plus MCBU#3576 (5′-AAAGCAAGTGCAGCAT TAATA-3′), resulting in an amplification product of 859 bp (corresponding to sequence 714–1572 of GenBank accession no. AF91606 and encoded by exons 5–11); MCBU#3577 (5′-GTTCCATGTCAACAAATGAA-3′) plus MCBU#3578 (5′-GCATCCAGCTTACTCTTTACT-3′), resulting in an amplification product of 769 bp (corresponding to sequence 1473–2241 of GenBank accession no. AF91606 and encoded by exons 10–15); MCBU#3579 (5′-ACCGAAGGGAAAACTGATAG-3′) plus MCBU#3580 (5′-AGCCTTCATTCTCCA CATC-3′), resulting in an amplification product of 600 bp (corresponding to sequence 2132–2731 of GenBank accession no. AF91606 and encoded by exons 14–18).

α-Catenin-Dependent LEF/TCF Transactivation Assay

SW480 human colon carcinoma cells were transiently transfected using LipofectAMINE PLUS (Invitrogen Life Technologies), whereas MCF7/AZ human breast cancer cells (Bracke et al., 1994) were transiently transfected with FuGENE 6 Reagent (Roche). After seeding 200,000 cells per 10-cm^2 well, cells were incubated for 24 h and then transfected with 500 ng of each of the following plasmids: an α-T-catenin expression plasmid, the luciferase reporter plasmid pTKTOP or its negative control pTKFOP (Roose et al., 1998), and the control β-galactosidase expressing construct pUT651 (Eurogentec). MCF7/AZ cells were also transfected with the mutated murine β-catenin expression plasmid $pCS2^+$S33A (Aberle et al., 1997). Two days after transfection, luciferase activity was measured using a Galacto-star kit (Tropix). Transfection normalization was done by measuring β-galactosidase (Galacto-Star kit; Tropix).

Subjects for Genetic Studies

The affected sibling pairs used for this study have been described before and come from the NIMH series, the NIA AD cell repository at Indiana University and the United Kingdom (Myers et al., 2002). In addition, we genotyped four independently collected case-control series comprised of white individuals of European descent. Ages of subjects and APOE status are shown in Table 1. Subjects in the Cardiff and London series are part of the MRC Genetic Resource collected by three of the authors (JW, MO, and SL). Subjects in the St. Louis series were participants in the Washington University Alzheimer's Disease Research Center (ADRC) patient registry. All cases had probable NINCDS-ADRDA AD with onset after the age of 60 yr (St. Louis samples have onset after 65 yr). Population dwelling controls were free from dementia (CDR 0), and most had MMSE scores greater than 27. DNA was extracted from blood or mouth swab samples. Informed consent was obtained for all patient samples used in the study and local research ethical committee approval was obtained.

Table 1
Sample Characteristics

	Status	Female (%)	Mean age (SD)	APOE ε4 +ve (%)
London	Case control	76.0	77 (4.48)	63.1
		76.0	76 (6.86)	31.1
Cardiff	Case control	81.7	74 (6.17)	64.5
		61.8	76 (6.52)	29.4
Mayo	Case control	67	74 (10.09)	56.2
		67	83 (7.47)	23.4
St. Louis	Case control	49.5	77 (6.87)	51.6
		48.9	83 (8.36)	24.3

Single-Nucleotide Polymorphism Detection

Primers were designed for each exon of *CTNNA3* (GenBank accession no. AF091606) to generate amplicon sizes of 200–500 bp (oligonucleotide sequences and PCR conditions are available on request from the authors). The PCR products were analyzed using denaturing high-performance liquid chromatography (dHPLC) using WAVE (Transgenomic). Samples showing altered dHPLC profiles were then sequenced using Perkin-Elmer Big Dye Sequence Terminator Chemistry and analyzed using an ABI 3100.

SNP Genotyping

Two SNPs were genotyped in all sample sets. SNP1 (forward primer: 5′-ttgagaaaaatctgctgctatcc-3′; reverse primer: 5′-gttcatctcctccaccaagg-3′) is a C>T change 15 bp beyond the 3′ of exon 4 (724+15). Genotyping in the 600 cases and 752 controls was performed using restriction fragment length polymorphism (RFLP) analysis with the restriction endonuclease *Sac*I (New England Biolabs). Digested products were separated on a 1.5% agarose gel at 120 V for 1 h, and then stained with ethidium bromide. For one sample (St. Louis), genotyping was performed using Pyrosequencing technology (www.pyrosequencing.com). A 10-µL PCR was genotyped on a PSQ 96 or a PSQ HS 96A using a Sepharose protocol.

SNP2 (4360 in Ertekin-Taner et al., 2003), a C>T change 1597-bp 5′ of exon 13 (2060–1597), was genotyped in 940 cases and 1031 controls (forward primer: 5′-ctgcaaagggatgggtattg-3′; reverse primer: 5′-gctctggtttgccttaatcg-3′). The enzyme used for RFLP analysis was HpyCH4IV (New England Biolabs), and fragments were visualized as earlier. Genotyping of the linkage sample was performed using Pyrosequencing technology.

Genotyping of the remaining SNPs was essentially as described elsewhere (Germer et al., 2000).

Statistical Analysis

Chi-square analysis was performed on the genotype and allele counts for each center individually and for the total dataset. Analysis of amyloid load within the frontal cortex by genotype was by analysis of variance (ANOVA) (Iwatsubo et al., 1994). We used the program 2LD for linkage disequilibrium analysis (Zapata et al., 2001) and Fast EH Plus for haplotype analysis (Zhao and Sham, 2002).

Neuropathology

Studies of α-T-catenin expression in the postmortem brain utilized nine autopsy-confirmed AD samples, four cognitively normal indiviuduals without any AD pathology, and three individuals with infarcts. All tissues were provided by the Brain Bank at the Institute of Psychiatry, London. In each case, brain samples were from both frontal and temporal lobes (including the hippocampus). A formalin-fixed brain was embedded in paraffin blocks and 10-µm-thick sections from each case were cut and stained with a polyclonal antibody against α-T-catenin (Ab952) (Janssens et al., 2001) using standard immunohistochemical procedures.

For studies of Aβ load, brains were obtained at autopsy from 46 patients with early- and late-onset sporadic AD (46% male, mean age at onset = 65.0 ± 10.5 yr, range = 35–85 yr; mean age at death = 73.3 ± 10.5 yr, range 44–92 yr). Genomic DNA was extracted from frozen brain tissue by standard methods. Amyloid load data (both as $A\beta_{40}$ and $A\beta_{42}$ loads) had been generated previously (Mann et al., 1997) by image analysis of sections immunostained using the end-specific monoclonal antibodies BA27 and BC05, respectively (Iwatsubo et al., 1994).

Results

α-T-Catenin Is Expressed in Human Brain

Previously, expression of α-T-catenin mRNA was reported predominantly in the heart and testis but also in the brain (Janssens et al., 2001). In order to determine the localization of α-T-catenin in the brain, polyclonal antibody #952 was used to examine nine AD brains, four normal control brains, and three with vascular infarcts. α-T-Catenin was expressed exclusively in the cytoplasm of neurons, predominantly those in layers IV and VI of cortex but also to some extent in those of the hippocampus. Some neuronal α-T-catenin staining was apparent in both control and AD brains, but the staining in neurons from the AD brain appeared somewhat stronger (Fig. 1). However, there was no association between α-T-catenin expression and the pathology of AD; nor did neurons affected by tangle formation appear to express α-T-catenin. Moreover, several reverse transcription (RT)-PCR reactions with primer pairs designed on the standard sequence of the α-T-catenin cDNA (Janssens et al., 2001) yielded the expected products on RNA templates from either normal or AD brains (Fig. 1B). All PCR products had the correct size and were sequence validated (not shown).

Identification of an Alternative Transcript, h-α-T-ctn Exon 1b and Exon 1c

Because the expression of α-T-catenin appeared to show some interindividual variation, we investigated whether the gene shows alternative splicing. On the basis of three EST sequences (GenBank accession nos. BI552791, BI549643, and BG719928), we identified an alternative transcript that lacks the noncoding first exon of the human α-T-catenin. Instead of this exon 1, two other exons are transcribed, designated exon 1b and exon 1c (Fig. 2). The location of these alternative exons in the *CTNNA3* gene was determined by a BLAST search (www.genome.ucsc.edu). The alternative transcript is apparently transcribed from a putative alternative promoter, which contains a possible binding site for the nervous-system-specific transcription factor N-Oct 3 (Brain-2) (Schreiber et al., 1993) (Fig. 2). An extended N-terminus could be predicted for the protein, encoded by this alternative transcript (GenBank accession no. AY198183). We did an expression analysis by RT-PCR, with specific primers for both transcripts, starting from human neural RNA and RNA from several AD patients. We could detect expression of both transcripts of human α-T-catenin in RNA from AD and control brains (data not shown). These data suggest that *CTNNA3* expression is regulated in a more complex way through transcriptional regulation and alternative splicing and possibly also through posttranscriptional regulation (Takahashi et al., 2000).

α-T-Catenin Inhibits Wnt Signaling

The role of α-catenin in the brain is not fully understood. It has been suggested that α-N-catenin contributes to the maintenance of positional information during forebrain development (Park et al., 2002). Along with β-catenin and their associated cadherines, α-N-catenin is localized to adherens junctions bordering active zones in developing and mature synapses throughout the brain. The cadherin–catenin complex has been proposed to be crucial in mediating adhesion between presynaptic and postsynaptic membranes, and expression of cadherin subtypes delineates specific neuronal circuits and could be involved in sensory gating (Hirano et al., 2003;Togashi et al., 2002). In addition, α-catenins participate in signaling, not least in Wnt signaling events (Giannini et al., 2000; Sehgal et al., 1997). As Wnt signaling has previously been implicated in AD, we examined the effects of overexpression of human α-T-catenin on Wnt signaling using the TCF/Lef-1 TOPflash luciferase reporter assay. In the colorectal cancer cell line SW480 cells, expression of human α-T-catenin reduced TCF/Lef-1 reporter activity by up to 50% (Fig. 3A). Mouse α-T-catenin also reduced TCF-Lef signaling, but by a lesser extent. In the human breast carcinoma cell

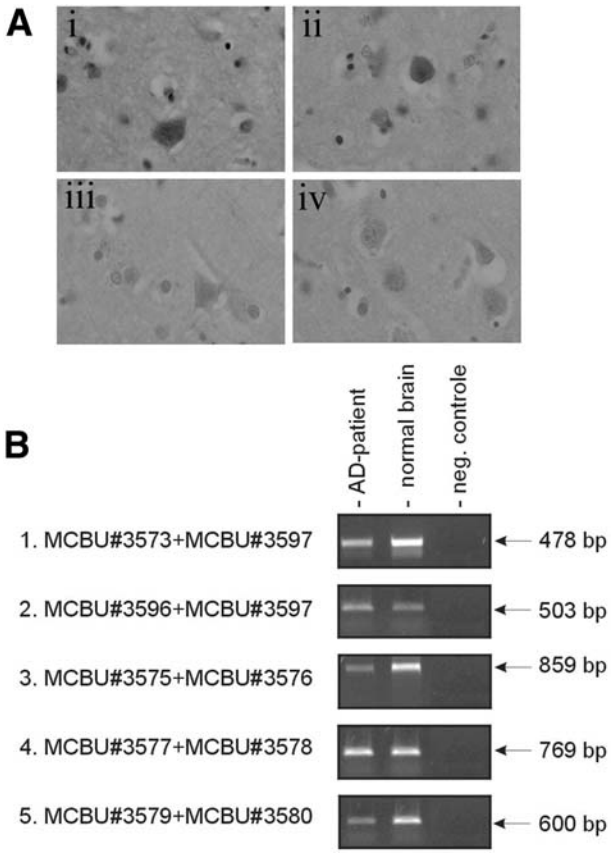

Fig. 1. Expression of α-T-catenin in the brain. **(A)** Expression of α-T-catenin protein. A polyclonal antibody recognizing human α-T-catenin stained neurons in layers IV and VI of frontal lobe cortex. Although both AD (i, ii) and control (iii, iv) brains showed some neuronal staining this appeared intense in some but not all AD cases but was weak in all control cases. **(B)** Expression analysis by RT-PCR. Several RT-PCR experiments, using different primer pairs together covering the whole α-T-catenin transcript, were executed on RNA templates from either AD or normal brains. Representative data are shown. The primers were designed on the standard sequence of the human α-T-catenin cDNA (MCBU#3573, MCBU#3597, MCBU#3575, MCBU#3576, MCBU#3577, MCBU#3578, MCBU#3579, MCBU#3580) or on the basis of three human EST sequences (MCBU#3596; *see also* Fig. 2). All PCR products obtained had the correct size and were sequence validated.

line MCF7/AZ, TCF/Lef-1 reporter activity induced by mutationally stabilized β-catenin was substantially reduced (~50%) by both human and mouse α-T-catenin (Fig. 3B).

Polymorphic Variation in CTNNA3 Is Not Responsible for the Chromosome 10 Linkage With AD

As we have demonstrated, α-T-catenin is expressed in the human brain and interacts with the Wnt signaling pathway, which has been postulated to be involved in AD pathogenesis. Furthermore, the *CTNNA3* gene is a strong positional candidate because it is located under the linkage peak on chromosome 10. Therefore, we tested for an association between variation in the gene and susceptibility to AD in our linkage sample and four independent case-control series. Initially, we genotyped two variants in the entire dataset. SNP1 is located 3′ to exon 4 toward the 5′ end of the gene (SNP1; 724+15). whereas SNP2 is 5′ to exon 13, toward the 3′ end of the gene (SNP2; 2060–1597). First, we performed an independent case-control association study for each

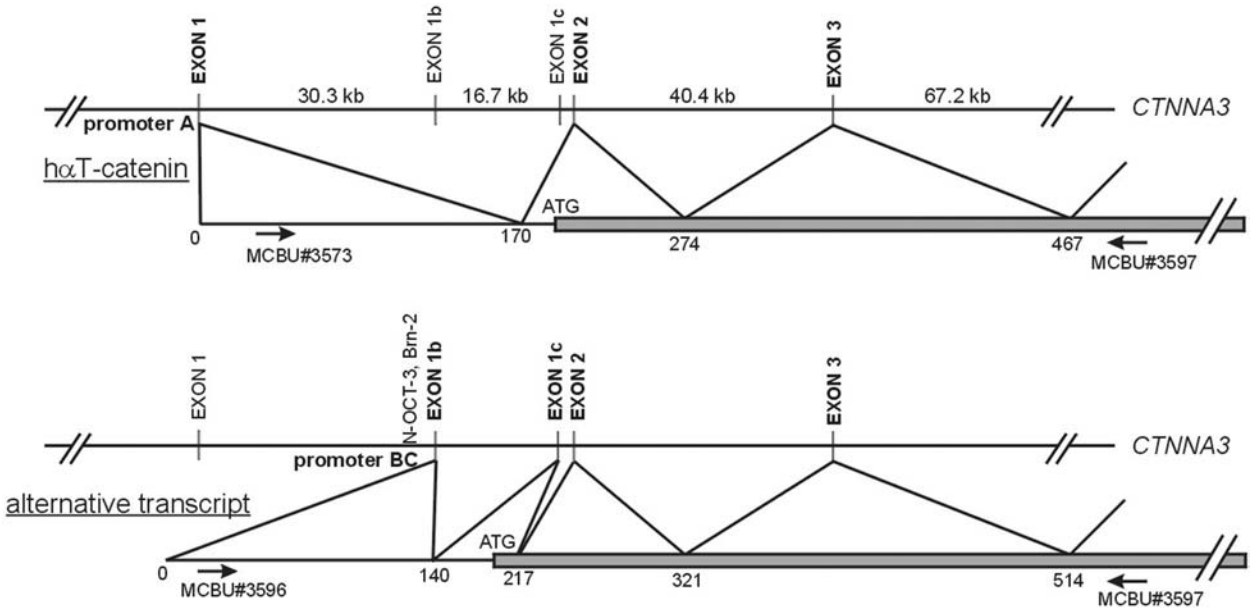

Fig. 2. Identification and localization of two alternative exons in the *CTNNA3* gene. The localization of novel exons 1b and 1c is indicated in the scheme representing the 5′ part of the *CTNNA3* gene. Transcription of human α-T-catenin mRNA comprising standard exon 1 is regulated by promoter A, whereas the synthesis of the alternative transcript (comprising exon 1b and 1c) is regulated by a putative alternative promoter BC. The open reading frame of the alternative transcript is extended as it encodes 12 more amino acid residues at the N-terminus (GenBank accession no. AY198183). The locations of primers MCBU#3573, MCBU#3596, and MCBU#3597 are as indicated.

SNP; then, we tested for an interaction with age of onset and variation in APOE.

The results are shown in Tables 2 and 3, both SNPs were in Hardy–Weinberg equilibrium. There was no allelic or genotypic association of SNP2 with AD in any of the samples individually or in the combined sample. SNP1 showed no genotypic association with AD but the C allele was marginally increased in AD cases (13.5% vs 10.8%, $\chi^2 = 4.47$, df = 1, $p = 0.03$) in the full sample, a finding that was evident in the Cardiff and St. Louis subsamples.

Haplotype analysis showed that there was no significant association of any haplotype with the disease. The two loci are in almost complete linkage equilibrium with low D′ values (the proportion of the maximum possible disequilibrium).

We then stratified the samples independently by APOE genotype and by onset of AD before or after the age of 75 yr. Neither genotypic nor allelic analysis for SNP2 showed a difference in cases vs controls in APOE ε4 positive or APOE ε4 negative samples (data not shown). SNP1 showed no genotypic association with AD in APOE ε4 positive or APOE ε4 negative samples but did show a small increase in C allele frequency in the APOE ε4 positive cases relative to APOE ε4 positive controls (14.2% vs 9.9%, $\chi^2 = 1.03$, df = 1, $p < 0.05$). Age of onset (onset after age 60 but before 75 yr compared to age of onset older than 75) did not affect the lack of association with SNP2, but when both cases and controls were stratified by age of onset or current age (controls), both genotypic (CC/CT 25.8% vs 19.8%, $\chi^2 = 8.49$, df = 2, $p = 0.01$) and allelic analyses (C allele 10.5% vs 13.8%, $\chi^2 = 7.25$, df = 1, $p = 0.01$) showed small but significant differences.

To avoid the possibility of a false-negative association, we also genotyped SNP1 and SNP2 in the sibling pair sample in which the original chromosome 10 linkage was obtained. Both SNPs were genotyped in a single individual from each sibship where both sibs shared alleles (Identity by descent [IBD] ≥ 1.8) for all five markers located underneath the linkage peak (sharers; 102 alleles) and compared with a series of age, gender, and ethnically matched

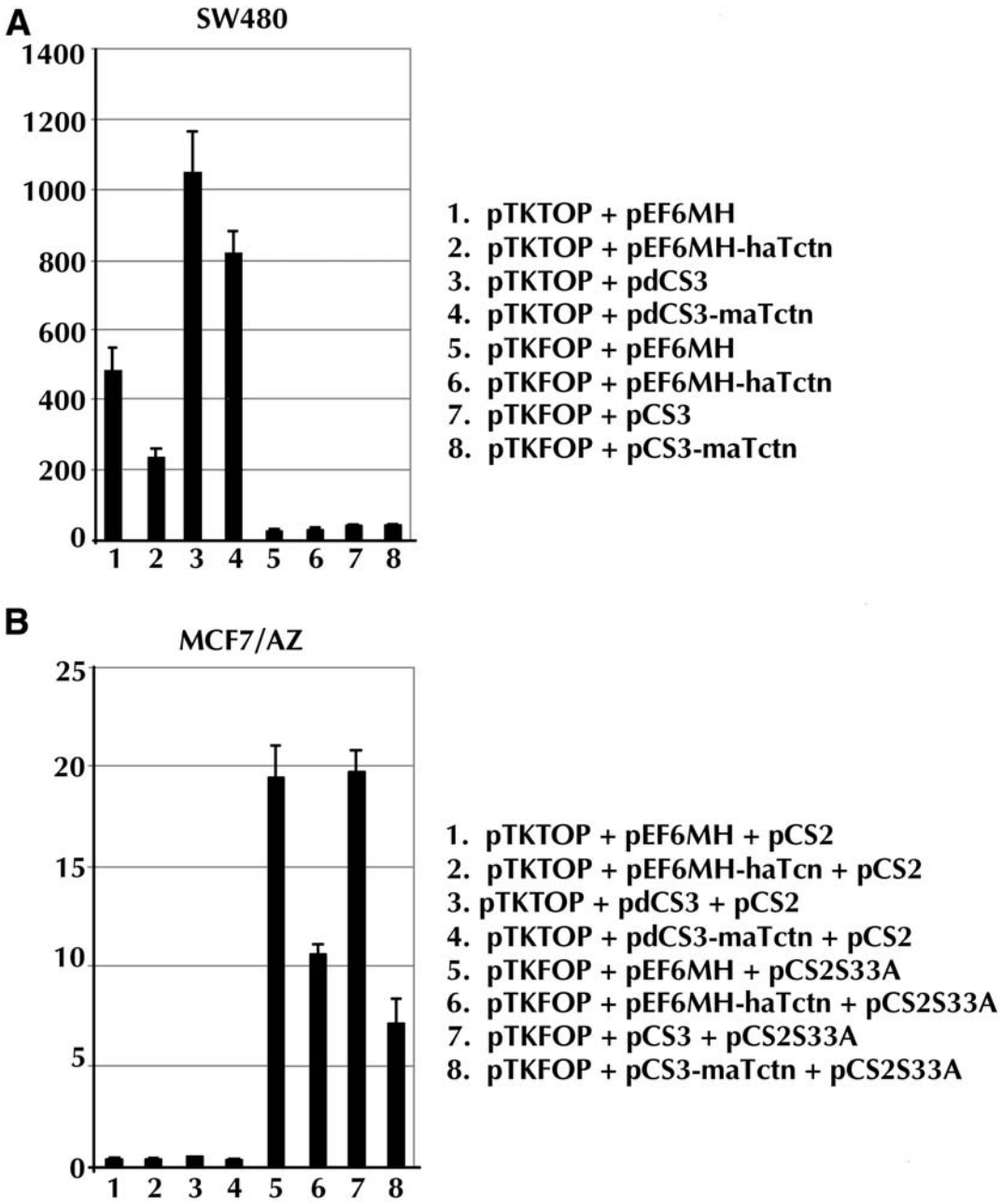

Fig. 3. α-T-Catenin reduces the LEF/TCF–β-Catenin-dependent transactivation in SW480 and MCF7/AZ cells. **(A)** Transfection of human (haTctn) and murine α-T-catenin (maTctn) expression plasmids into SW480 cells. The pTKTOP plasmid allows measurement of the endogenous LEF/TCF–β-catenin-dependent transactivation, whereas pTKFOP serves as a negative control. Luciferase values representing the nuclear β-catenin activity were normalized vs β-galactosidase activities. Values were determined in triplicate and bars show the mean values and the standard deviation. **(B)** Transfection of human (haTctn) and murine α-T-catenin (maTctn) expression plasmids into MCF7/AZ cells. The cells were also transfected with a plasmid encoding an activated mutant of β-catenin, pCS2^{+}S33A *(lanes 5–8)*. The empty vector, pCS2 *(lanes 1–4)*, serves as a negative control. Luciferase values representing the nuclear β-catenin activity were normalized vs β-galactosidase activities.

Table 2
Allele Frequencies

		SNP1			SNP2		
		T	C	Stats	T	C	Stats
London	Case	223 (83.8%)	43 (16.2%)	$p = 0.83$	207 (71.4%)	83 (28.6%)	$p = 0.49$
	control	186 (84.5%)	34 (15.5%)	$\chi^2 = 0.05$ df = 1	166 (68.6%)	76 (31.4%)	$\chi^2 = 0.488$ df = 1
Cardiff	Case	183 (84.7%)	33 (15.3%)	$p = 0.05$	153 (71.5%)	61 (28.5%)	$p = 0.63$
	control	189 (90.9%)	19 (9.1%)	$\chi^2 = 3.72$ df = 1	147 (69.3%)	65 (30.7%)	$\chi^2 = 0.24$ df = 1
Mayo	Case	473 (89.2%)	57 (10.8%)	$p = 0.67$	392 (73.7%)	140 (26.3%)	$p = 0.98$
	control	806 (90%)	90 (10%)	$\chi^2 = 0.18$ df = 1	624 (73.8%)	222 (26.2%)	$\chi^2 = 0$ df = 1
St. Louis	Case	159 (84.6%)	29 (15.4%)	$p = 0.22$	613 (72.6%)	231 (27.4%)	$p = 0.79$
	control	160 (88.9%)	20 (11.1%)	$\chi^2 = 1.48$ df = 1	549 (72.0%)	213 (28.0%)	$\chi^2 = 0.07$ df = 1
Total	Case	1038 (86.5%)	162 (13.5%)	$p = 0.03$	1365 (73.0%)	515 (27.0%)	$p = 0.74$
	control	1341 (89.2%)	163 (10.8%)	$\chi^2 = 4.47$ df = 1	1486 (72.1%)	576 (27.9%)	$\chi^2 = 0.11$ df = 1

Table 3
Genotype Frequencies

		SNP1				SNP2			
		TT	TC	CC	Stats	TT	TC	CC	Stats
London	Case	94 (70.7%)	35 (26.3%)	4 (3%)	$p = 0.62$	72 (49.7%)	63 (43.4%)	10 (6.9%)	$p = 0.72$
	control	81 (73.6%)	24 (21.8%)	5 (4.6%)	$\chi^2 = 0.96$ df = 2	54 (44.7%)	58 (47.9%)	9 (7.4%)	$\chi^2 = 0.67$ df = 2
Cardiff	Case	79 (73.1%)	25 (23.2%)	4 (3.7%)	$p = 0.08$	56 (52.3%)	41 (38.3%)	10 (9.4%)	$p = 0.88$
	control	85 (81.7%)	19 (18.3%)	0 (0%)	$\chi^2 = 4.96$ df = 2	53 (50%)	41 (38.7%)	12 (11.3%)	$\chi^2 = 0.26$ df = 2
Mayo	Case	214 (80.8%)	45 (16.9%)	6 (2.3%)	$p = 0.30$	141 (53%)	110 (41.4%)	15 (5.6%)	$p = 0.98$
	control	362 (80.8%0	82 (18.3%)	4 (0.9%)	$\chi^2 = 2.40$ df = 2	226 (53.4%)	172 (40.7%)	25 (5.9%)	$\chi^2 = 0.05$ df = 2
St. Louis	Case	68 (72.3%)	23 (24.5%)	3 (3.2%)	$p = 0.45$	222 (52.6%)	169 (40.1%)	31(7.4%)	$p = 0.69$
	control	71 (78.9%)	18 (20%)	1 (1.1%)	$\chi^2 = 1.59$ df = 2	195 (51.2%)	159 (41.7%)	27 (7.1%)	$\chi^2 = 0.16$ df = 2
Total	Case	455 (75.8%)	128 (21.3%)	17 (2.8%)	$p = 0.07$	491 (52.2%)	383 (40.7%)	66 (7.0%)	$p = 0.93$
	control	599 (79.7%)	143 (19%)	10 (1.3%)	$\chi^2 = 5.30$ df = 2	528 (51.2%)	430 (41.7%)	73 (7.1%)	$\chi^2 = 0.15$ df = 2

controls (184 alleles). Using this approach, we are able to address not only whether variation in *CTNNA3* is associated with AD but, more importantly, whether it is responsible for the linkage to a locus on chromosome 10 previously observed in this sample. There was no association between either SNP variant and sharers with AD compared to controls. Indeed, the minor allele of SNP1, that was more common in the association sample, was actually less common in the sharers (C allele in sharers 7.8% vs 12.0% in nonsharers, $\chi^2 = 1.183$, df = 1, $p > 0.05$). The allelic association analysis of all cases vs all controls in Table 2 gave estimates of 0.108 for the frequency of the C allele in controls and 1.28 (95% confidence interval [CI] 1.02–1.62) for the odds ratio. Assuming a multiplicative model for genotypic relative risks, these values predict a frequency of allele C of 0.167 in affected sibs sharing two alleles IBD with a case. The observed frequency of C in the sharers (8 out of 102 alleles) is significantly lower than the predicted frequency ($\chi^2 = 6.71$, df = 1, $p < 0.01$) and is thus inconsistent with the C allele having an odds ratio as large as 1.28.

Polymorphic Variation in CTNNA3 *Is Not Associated With Amyloid Deposition in AD*

The locus at chromosome 10 was independently associated with plasma Aβ (Ertekin-Taner et al., 2000), suggesting that the gene responsible for linkage is a modifier of either APP metabolism or Aβ clearance. Subsequently, SNP2 (4360) was also associated with plasma Aβ (Ertekin-Taner et al., 2003). We therefore correlated the amount of amyloid-related pathology in 46 AD brains with *CTNNA3* polymorphisms (Table 4). Previous analysis has demonstrated an association between APOE ε4 and Aβ40, demonstrating the utility of this type of study (Mann et al., 1997). Neither total Aβ40 nor Aβ42 showed any difference when compared by SNP2 or by SNP1 genotype.

Polymorphic Variation in Other Regions of the CTNNA3 Gene Is Not Associated With AD in a Case-Control Series

To eliminate the possibility of association between AD and SNPs in other parts of the *CTNNA3* gene, we analyzed a total of 31 SNPs (full details upon request) in one of the case-control samples (St. Louis; $n = 416$ cases, 376 controls). All but one SNP were intronic and spanned the entire gene, from 64.53 to 66.40 Mb (details available on request). One SNP showed a barely significant allelic difference between cases and controls ($p = 0.047$), but there was no genotype difference in this or any of the other SNPs, and no correction for multiple testing was made. No SNP showed a significant allelic or genotypic difference at the $p < 0.01$ level when stratified by APOE genotype.

Discussion

Numerous candidate genes have been studied in an effort to determine susceptibility loci for AD. With the exception of APOE, none of these has been consistently and unequivocally replicated. In order to avoid adding to this confusing literature, we have followed a strategy of combining both linkage and biology to determine candidate genes and then to genotype multiple SNPs in replication samples. The linkage peak associated with AD on chromosome 10 is the most promising locus to start this search for three reasons: First, it demonstrates strong linkage, replicated in a coarse (20 cM) screen (Kehoe et al., 1999) and finer mapping (5 cM) (Myers et al., 2002); second, because the same linkage is reported for plasma Aβ (Ertekin-Taner et al., 2000); and third, because linkage to chromosome 10 has been replicated in an overlapping sample (Bertram et al., 2000), albeit distal to the D10S1211 marker.

CTNNA3, an α-catenin gene expressed most strongly in the heart and testis, but also in the brain, is a strong candidate by virtue of position—being the closest known gene to D10S1211. We have now demonstrated that *CTNNA3* is also a candidate by virtue of biology. We have determined that *CTNNA3* is expressed intraneuronally and that expression appears to be increased in AD brains relative to controls, although we found no spatial relationship between *CTNNA3* expression and either plaque or tangle pathology. Nonetheless, this strong expression suggests an important role for α-T-catenin in the brain.

Other α-catenins have multiple functions, both structural ones and in signaling. We have shown that α-T-catenin acts like other α-catenins (Giannini et al., 2000; Sehgal et al., 1997) to inhibit Wnt

Table 4
Brain Amyloid Load by Genotype

Genotype	*n*	Aβ40 (% mean area occupied [SD])	Aβ42 (% mean area occupied [SD])
SNP2 CC	10	4.7 [4.6]	10.8 [4.9]
SNP2 CT	16	2.7 [2.7]	9.4 [4.8]
SNP2 TT	20	3.0 [3.0]	9.2 [4.6]
SNP1 TT	34	3.8 [3.6]	10.5 [4.8]
SNP1 TC	8	2.7 [1.1]	10.1 [5.6]
SNP1 CC	1	1.4	4.7

Note: $p > 0.05$ in all cases.

signaling to the TCF/Lef responsive genes. Wnt signaling has been implicated in AD pathogenesis on a number of fronts. The presenilins, like α-catenin, inhibit Wnt signaling as measured by β-catenin nuclear transfer or by TCF/Lef-mediated gene transcription (Sehgal et al., 1997; Soriano et al., 2001; Van Gassen et al., 2000). In addition, Wnt signaling increases sAPPα production (through activating PKC and JNK) and decreases tau phosphorylation (through inhibiting GSK-3) (Mudher et al., 2001). However, it is not known how PS-1 and α-catenins inhibit Wnt signaling. It is possible that they do so by acting as β-catenin-binding proteins, thus inhibiting nuclear transfer and thereby inhibiting β-catenin-induced TCF/Lef-dependent gene transcription. Such a mechanism would bypass those elements of the canonical Wnt pathway that have been shown to alter APP processing and tau phosphorylation. Nonetheless, as α-catenin functions similarly to PS-1 to reduce TCF/Lef-dependent gene transcription, and given that α-T-catenin is strongly expressed in neurons in AD brain, α-T-catenin meets the criteria of both a positional and functional candidate gene for AD susceptibility.

Given the chromosome 10 linkage results, we would predict (1) a strong association with variation in *CTNNA3*, (2) that such association is not modified by APOE (given that the chromosome 10 locus association is not so modified), (3) that variation would be strongest in a sample set previously shown to be associated with chromosome 10 (sharers), and (4) that variation in *CTNNA3* would be associated with Aβ deposition in the brain. None of these criteria were met in this study. Out of four independent samples, we find an association with one SNP (SNP1), but not another, in one of the four sample sets. However, the fact that the difference is small, barely significant, not replicated in all samples, and not observed in the linkage sample suggests to us that this SNP is not responsible for the observed linkage to chromosome 10. Indeed, the SNP1 C allele is marginally more common in the association samples but less common in the linkage sample. Moreover, when the sample is stratified by APOE genotype, the increase in the frequency of the SNP1 C allele is present only in those with an APOE ε4 allele, whereas linkage to the region on chromosome 10 is APOE independent. Finally, if *CTNNA3* was responsible for the chromosome 10 linkage, we would expect to observe an association with cerebral Aβ load, as the linkage peak is also associated with increased peripheral Aβ. However there was no association with Aβ40 or Aβ42 in a large sample of brains.

Previously, Ertekin-Taner et al. (2003) reported two intronic SNPs (one of which was SNP2 in this study) that are in close linkage disequilibrium and that show strong association with plasma Aβ. Ertekin-Taner and colleagues note that "a specific variant with a strong influence on plasma Aβ42 may sometimes have little or no influence on the AD phenotype" and, indeed, that appears to be the case, as variation in *CTNNA3* in our study shows no influence on Aβ pathology in the brain and does not appear to be responsible for the linkage to chromosome 10 previously found for AD. One possible explanation of these findings is that plasma Aβ may

be more related to kidney than to brain function (Arvanitakis et al., 2002).

Therefore, we conclude that although α-T-catenin is expressed in neurons and although it inhibits the Wnt signaling pathway—a pathway implicated in AD through PS-1 function, APP processing, and tau phosphorylation—variation in the *CTNNA3* gene does not appear to be associated with AD and does not explain the linkage observed in late-onset AD families on chromosome 10, although we cannot completely exclude the gene as a genetic susceptibility factor for AD.

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