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Report

GAB2 Alleles Modify Alzheimer's Risk in *APOE* ε4 Carriers

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SUMMARY

The apolipoprotein E (*APOE*) ε4 allele is the best established genetic risk factor for late-onset Alzheimer's disease (LOAD). We conducted genome-wide surveys of 502,627 single-nucleotide polymorphisms (SNPs) to characterize and confirm other LOAD susceptibility genes. In ε4 carriers from neuropathologically verified discovery, neuropathologically verified replication, and clinically characterized replication cohorts of 1411 cases and controls, LOAD was associated with six SNPs from the *GRB*-associated binding protein 2 (*GAB2*) gene and a common haplotype encompassing the entire *GAB2* gene. SNP rs2373115 ($p = 9 \times 10^{-11}$) was associated with an odds ratio of 4.06 (confidence interval 2.81–14.69), which interacts with *APOE* ε4 to further modify risk. *GAB2* was overexpressed in pathologically vulnerable neurons; the Gab2 protein was detected in neurons, tangle-bearing neurons, and dystrophic neuritis; and interference with *GAB2* gene expression increased tau phosphorylation. Our findings suggest that *GAB2* modifies LOAD risk in *APOE* ε4 carriers and influences Alzheimer's neuropathology.

INTRODUCTION

Alzheimer's disease (AD) afflicts about 10% of persons over 65 and almost half of those over 85 (Evans et al., 1989) and the number of afflicted persons continues to grow. To date, researchers have firmly established associations between four genes and AD risk. Whereas more than 150 mutations of the presenilin 1 (*PS1*), presenilin 2 (*PS2*), and amyloid precursor protein (*APP*) genes account for many early-onset AD cases with autosomal dominant inheritance, the apolipoprotein E (*APOE*) ε4 allele accounts for many cases of late-onset AD (LOAD), with dementia onset after age 60 (Papassotiropoulos et al., 2006; Corder et al., 1993, 1994; Farrer et al., 1997). The *APOE* gene has three common variants, ε2, ε3, and ε4. The ε2 allele is associated with the lowest LOAD risk, while each copy of the ε4 allele in a person's *APOE* genotype is

Table 1. *GAB2* SNPs Implicated in the Neuropathological Discovery, Neuropathological Replication, and Clinical Replication Cohorts

			Neuropathology Discovery Cohort			
dbsnp RS ID	Position	Risk Allele	Freq. Cases	Freq. Controls	p Value[a]	OR (95% CI)
rs901104	77608147	C	0.89	0.72	4.56E-07	3.24 (2.01–5.20)
rs1385600	77613814	T	0.89	0.71	5.02E-07	3.18 (1.99–5.08)
rs1007837	77618724	C	0.89	0.73	4.78E-06	3.18 (1.99–5.08)
rs2510038	77643682	C	0.90	0.74	8.92E-05	2.44 (1.37–4.36)
rs4945261	77667908	G	0.89	0.72	5.66E-07	3.21 (1.99–5.15)
rs7101429	77670615	A	0.89	0.73	4.62E-05	2.13 (1.21–3.75)
rs10793294	77674051	C	0.82	0.66	2.98E-05	2.45 (1.60–3.77)
rs4291702	77678896	C	0.88	0.70	3.73E-06	3.19 (2.00–5.07)
rs7115850	77722719	C	0.86	0.67	1.60E-07	3.21 (2.04–5.05)
rs2373115	77768798	G	0.88	0.70	4.60E-07	3.21 (2.04–5.05)

[a] p values were computed using χ^2 tests. For the overall comparison, the Cochran-Mantel-Haenszel χ^2 test was used. All p values are uncorrected.

associated with a higher LOAD risk and a younger median age at dementia onset (Corder et al., 1994; Farrer et al., 1997). Although twin studies suggest that there are several susceptibility genes which, along with the *APOE* ε4 allele, contribute to up to 80% of LOAD cases (Gatz et al., 2006), discovery of other susceptibility genes has been elusive (Bertram et al., 2007).

To identify susceptibility genes for common and genetically complex disorders like LOAD, it has been proposed that it would help to conduct genome-wide surveys of at least 300,000 single-nucleotide polymorphisms (SNPs) in unrelated cases and controls, compare the most homogeneous samples, and consider interactions between major and minor genes (Papassotiropoulos et al., 2006; Kruglyak, 1999; Coon et al., 2007). We individually genotyped 502,627 SNPs to characterize and confirm LOAD susceptibility genes in three separate cohorts of LOAD cases and controls, including a discovery cohort of clinically and neuropathologically characterized brain donors, a replication cohort of similarly characterized brain donors, and a replication cohort of clinically characterized living subjects. The brain donor cohorts were selected to exclude clinically misdiagnosed cases and cognitively normal but neuropathologically affected elderly controls; the clinical cohort was selected to confirm genetic associations independent of any brain donor selection bias. Within each cohort, LOAD cases and controls were stratified into subgroups of APOE ε4 carriers and noncarriers, permitting us to investigate genes that modify LOAD risk in the ε4 carriers and genes that might otherwise be masked by disproportionately large ε4 effects.

RESULTS AND DISCUSSION

We recently demonstrated the feasibility of high-density genome-wide association studies in our neuropathologically characterized cases and controls, providing empirical support for the suggestion that the *APOE* locus is unparalleled in its contribution to LOAD risk (Coon et al., 2007). With the exception of an SNP only 14 kb pairs distal to and in linkage disequilibrium (LD) with the *APOE* ε4 variant on chromosome 19, no other SNP distinguished LOAD cases from controls after Bonferroni correction for multiple comparisons (Figure S1A at http://www.tgen.org/neurogenomics/data). For the previously noted reasons, we divided each cohort into two subgroups: allelic APOE ε4 carriers (Figure S1B) and APOE ε4 noncarriers (Figure S1C). We now report associations between a common gene and LOAD in *APOE* ε4 carriers in our three cohorts; we show that the implicated gene is associated with AD neuropathology in neuronal microarray and immunohistochemical studies; and we consider a possible mechanism by which *GAB2* modifies AD risk in a small-interfering RNA (siRNA) study. Finally, we deposit all of the data into the public domain for use by the community (http://www.tgen.org/neurogenomics/data).

High-Density Genome-Wide Association Studies

Genome-wide genotyping was performed on each individual sample from a "neuropathological discovery cohort" of 736 brain donors, a "neuropathological replication cohort" of 311 brain donors, and an additional "clinical replication cohort" of 364 living subjects who were at least 65 years old at the time of their death or last clinical assessment and who were independently assessed for their APOE genotype. For the two neuropathological cohorts, brain tissue for DNA extraction, neuropathological diagnoses, and data were supplied by investigators from 20 of the National Institute on Aging (NIA)-sponsored Alzheimer's Disease Centers (ADCs) (in accordance with agreements with the NIA, the ADCs, and the National Alzheimer's Coordinating Center) and from the Netherlands

Neuropathology Replication Cohort				Clinical Replication Cohort				Overall	
Freq. Cases	Freq. Controls	p Value[a]	OR (95% CI)	Freq. Cases	Freq. Controls	p Value[a]	OR (95% CI)	p Value[a]	OR (95% CI)
0.87	0.73	4.52E-02	2.54 (0.90–7.17)	0.89	0.80	1.47E-01	2.08 (0.76–5.88)	1.99E-06	2.87 (1.84–4.50)
0.88	0.70	1.32E-02	3.00 (1.09–8.24)	0.95	0.78	4.63E-02	5.56 (0.89–33.33)	2.81E-09	3.65 (2.34–5.71)
0.89	0.73	3.20E-02	2.85 (1.00–8.11)	0.88	0.80	2.04E-01	1.89 (0.70–5.00)	3.97E-07	3.01 (1.94–4.68)
0.88	0.75	4.35E-02	2.53 (0.88–7.32)	0.89	0.80	1.47E-01	2.08 (0.76–5.88)	1.19E-05	2.72 (1.72–4.31)
0.88	0.73	4.20E-02	2.54 (0.90–7.17)	0.95	0.78	4.63E-02	5.56 (0.89–33.33)	3.06E-08	3.44 (2.18–5.43)
0.89	0.73	1.87E-02	2.84 (1.00–8.11)	0.88	0.75	4.99E-02	2.50 (0.98–6.25)	1.06E-06	2.96 (1.89–4.63)
0.80	0.59	1.34E-02	2.66 (1.06–6.57)	0.88	0.61	2.17E-02	4.45 (1.18–16.95)	1.59E-07	2.83 (1.90–4.21)
0.87	0.72	2.44E-02	2.70 (0.97–7.52)	0.88	0.80	2.04E-01	1.89 (0.70–5.00)	5.88E-07	2.96 (1.91–4.59)
0.86	0.72	3.91E-02	2.48 (0.90–6.81)	0.98	0.72	3.45E-03	14.93 (1.61–140.85)	2.80E-10	3.92 (2.51–6.11)
0.86	0.72	3.91E-02	2.48 (0.90–6.81)	0.98	0.72	3.45E-03	14.93 (1.61–140.85)	9.66E-11	4.06 (2.81–14.69)

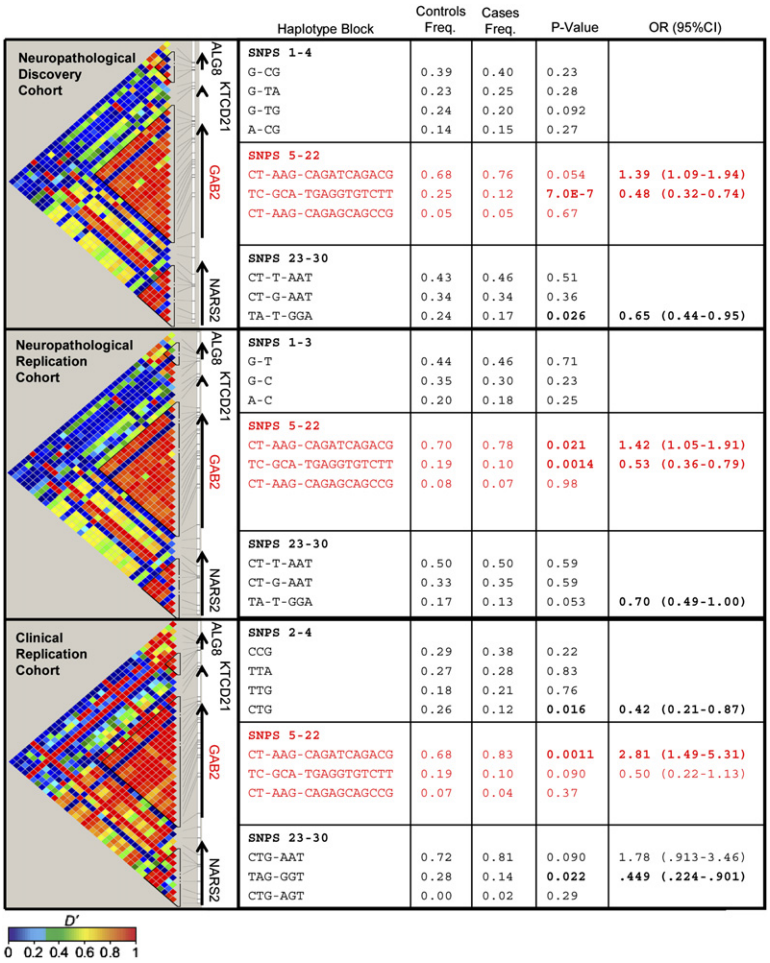
Brain Bank. For the hypothesis-testing clinical replication cohort, DNA extracted from blood, clinical diagnoses, and data from subjects assessed in Rochester, MN were supplied by investigators from the Mayo Clinic.

The neuropathological discovery cohort included 446 LOAD cases (299 ε4 carriers and 147 ε4 noncarriers) and 290 controls (61 ε4 carriers and 229 ε4 noncarriers); the neuropathological replication cohort included 197 LOAD cases (113 ε4 carriers and 84 ε4 noncarriers) and 114 controls (27 ε4 carriers and 87 ε4 noncarriers); and the clinical replication cohort included 218 LOAD cases (115 ε4 carriers and 103 ε4 noncarriers) and 146 controls (29 ε4 carriers and 117 ε4 noncarriers). Brain donor cases satisfied clinical and neuropathological criteria for LOAD, and were age 73.5 ± 6.2 at death. Brain donor controls did not have significant cognitive impairment or significant neuropathological features of AD, and were age 75.8 ± 7.5 at death. Clinical cases satisfied criteria for probable AD, and were age 78.9 ± 7.8 at last clinical visit. Clinical controls did not have clinically significant cognitive impairment and were age 81.7 ± 6.6 at last clinical assessment.

We initially surveyed SNPs in the neuropathological discovery cohort to explore LOAD associations in the ε4 carrier and noncarrier subgroups. Within the discovery subgroup of *APOE* ε4 carriers, 10 of the 25 SNPs with the most significant LOAD-association significance levels (contingency test $p = 9 \times 10^{-5}$ to 1×10^{-7}; uncorrected for multiple comparisons) were located in the *GRB*-associated binding protein 2 (*GAB2*) gene on chromosome 11q14.1 (Table 1). LOAD associations in six of these SNPs were then confirmed in both the neuropathological replication and clinical replication cohorts (Table 1). These ten SNPs were not significantly associated with LOAD in the *APOE* ε4 noncarrier group (contingency test p = 0.08 to 0.97) (Table S1A). Combining data from all 644 *APOE* ε4-carrying cases and controls, we found highly significant associations between LOAD and all ten *GAB2* SNPs (contingency test $p = 1.19 \times 10^{-5}$ to 9.66×10^{-11}), with five of the six consistently implicated alleles surviving the highly conservative Bonferroni correction for 312,316 independent comparisons ($p = 1.55 \times 10^{-7}$) (Table 1). When data from the ε4 carriers and noncarriers were analyzed together, as in our previous report (Coon et al., 2007), the ten *GAB2* SNPs were still associated with LOAD (contingency test $p = 0.013$ to 2.7×10^{-6}, Table S1B), but these associations did not survive Bonferroni correction.

The PLINK analysis toolset (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) was used for whole-genome analysis. Haploview v3.32 was used to determine the LD structure of the chromosome 11q14.1 region surrounding *GAB2* in each of the three APOE ε4-stratified cohorts (Figure 1). Three haplotype blocks are present in this region: one block upstream of *GAB2*, roughly corresponding to the *ALG8* locus; one 189 kb-pair block encompassing most of the *GAB2* locus; and one downstream block corresponding to the *NARS2* locus. These blocks were consistent with the LD structure of the HapMap CEPH populations. The *GAB2* gene is completely encompassed by a single haplotype block extending from rs901104 to rs2373115 (SNPs 5–22 in Figure 1), which has three major haplotypes: an extremely common "*GAB2* risk haplotype," a common "*GAB2* protective haplotype," and a relatively uncommon *GAB2* haplotype unrelated to LOAD risk in *APOE* ε4 carriers (Figure 1). In all three cohorts, the *GAB2* CT-AAG-CAGATCAGACG haplotype was associated with *higher* LOAD risk, the *GAB2* TC-GCA-TGAGGTGTCTT haplotype was associated with a *lower* LOAD risk, and the CT-AAG-CAGAGCA GCCG was unrelated to LOAD risk in the *APOE* ε4 carriers (Figure 1).

Data from the 1411 subjects (including 644 *APOE* ε4 carriers and 767 noncarriers) in all three cohorts were combined to characterize odds ratios (ORs) and 95%



Haplotype Block	Controls Freq.	Cases Freq.	P-Value	OR (95%CI)
SNPS 1-4				
G-CG	0.39	0.40	0.23	
G-TA	0.23	0.25	0.28	
G-TG	0.24	0.20	0.092	
A-CG	0.14	0.15	0.27	
SNPS 5-22				
CT-AAG-CAGATCAGACG	0.68	0.76	0.054	1.39 (1.09-1.94)
TC-GCA-TGAGGTGTCTT	0.25	0.12	7.0E-7	0.48 (0.32-0.74)
CT-AAG-CAGAGCAGCCG	0.05	0.05	0.67	
SNPS 23-30				
CT-T-AAT	0.43	0.46	0.51	
CT-G-AAT	0.34	0.34	0.36	
TA-T-GGA	0.24	0.17	0.026	0.65 (0.44-0.95)
SNPS 1-3				
G-T	0.44	0.46	0.71	
G-C	0.35	0.30	0.23	
A-C	0.20	0.18	0.25	
SNPS 5-22				
CT-AAG-CAGATCAGACG	0.70	0.78	0.021	1.42 (1.05-1.91)
TC-GCA-TGAGGTGTCTT	0.19	0.10	0.0014	0.53 (0.36-0.79)
CT-AAG-CAGAGCAGCCG	0.08	0.07	0.98	
SNPS 23-30				
CT-T-AAT	0.50	0.50	0.59	
CT-G-AAT	0.33	0.35	0.59	
TA-T-GGA	0.17	0.13	0.053	0.70 (0.49-1.00)
SNPS 2-4				
CCG	0.29	0.38	0.22	
TTA	0.27	0.28	0.83	
TTG	0.18	0.21	0.76	
CTG	0.26	0.12	0.016	0.42 (0.21-0.87)
SNPS 5-22				
CT-AAG-CAGATCAGACG	0.68	0.83	0.0011	2.81 (1.49-5.31)
TC-GCA-TGAGGTGTCTT	0.19	0.10	0.090	0.50 (0.22-1.13)
CT-AAG-CAGAGCAGCCG	0.07	0.04	0.37	
SNPS 23-30				
CTG-AAT	0.72	0.81	0.090	1.78 (.913-3.46)
TAG-GGT	0.28	0.14	0.022	.449 (.224-.901)
CTG-AGT	0.00	0.02	0.29	

Figure 1. Linkage Disequilibrium Structure and Haplotype Significance Levels for the Region Encompassing *GAB2*

Plots follow the Haploview GOLD heatmap color scheme. SNP numbers correspond to the following dbSNP ID identification numbers: 1:rs579711, 2:rs977978, 3:rs637149, 4:rs977977, 5:rs901104, 6:rs1385600, 7:rs11237419, 8:rs1007837, 9:rs2450130, 10:rs2510054, 11:rs11237429, 12:rs2510038, 13:rs2511170, 14:rs4945261, 15:rs7101429, 16:rs10793294, 17:rs4291702, 18:rs11602622, 19:rs10899467, 20:rs2458640, 21:rs10793302, 22:rs2373115, 23:rs12280198, 24:rs12287010, 25:rs17136630, 26:rs4945276, 27:rs1996172, 28:rs7395344, 29:rs11237522, and 30:rs7950813. In all three cohorts, there was a *GAB2* risk haplotype, a protective haplotype, and a haplotype unrelated to LOAD risk in *APOE* ε4 carriers.

confidence intervals (CIs) for rs2373115, the most significant SNP in our screen (Table 2). In ε4 carriers, LOAD cases had a risk genotype frequency of 0.88 and controls had a frequency of 0.71. In comparison with the other ε4 carriers, those with rs2373115 genotype GG had a significantly higher risk of LOAD (OR 2.36, 95% CI 1.55–3.58) than those with genotype GT and TT. In contrast, *APOE* ε4 noncarriers with rs2373115 genotype GG did not have a higher LOAD risk than the other ε4 noncarriers (OR 1.01, 95% CI 0.74–1.38).

Whereas we confirmed a younger age at dementia onset in the *APOE* ε4 carriers than in noncarriers (age 75.5 ± 7.2 versus 77.8 ± 7.9, $p = 2.4 \times 10^{-4}$, two-tailed unpaired t test; unequal variance is statistical test used for all tests), there was no significant effect of rs2373115 genotype on age at dementia onset in either the ε4 carriers (t test, p = 0.32) or noncarriers (t test, p = 0.84).

Neuronal Microarray Studies

To provide converging evidence that *GAB2* is biologically relevant to AD neuropathology, expression profiling using the Affymetrix Human Genome U133 Plus 2.0 array was used to characterize and compare *GAB2* expression in laser-capture microdissected non-tangle-bearing neurons of cases and controls in six brain regions differentially affected by AD. LOAD cases had significantly greater neuronal *GAB2* expression in the posterior cingulate cortex (9 cases, 13 controls, 4.50-fold change, t test, p = 0.00039) and hippocampus (9 cases, 13 controls, 2.94-fold change, t test, p = 0.00085), and no significant expression differences in the entorhinal cortex (10 cases, 13 controls, 1.20-fold change, t test, p = 0.46), middle temporal gyrus (13 cases, 12 controls, 1.44-fold change, t test, p = 0.14), superior frontal gyrus (22 cases, 11 controls, 1.25-fold change, t test, p = 0.47), or primary visual cortex (17 cases, 12 controls, 1.53-fold change, t test, p = 0.14).

The hippocampus is known to be especially vulnerable to AD-related neurofibrillary tangles (Braak and Braak, 1991), neuronal loss, and brain atrophy (Bobinski et al., 2000). It is preferentially involved in AD-related memory impairment (Jack et al., 1999) and is associated with the highest cerebral Gab2 expression in the rodent brain (Lein et al., 2007). The posterior cingulate cortex is known to be preferentially vulnerable to AD-related hypometabolic abnormalities and fibrillar amyloid deposition, and is

Table 2. *GAB2* LOAD Odds Ratios in *APOE* ε4 Carriers and Noncarriers

APOE ε4 group	*APOE* ε4 OR (95% CI)	rs2373115 genotype	Controls (n)	Cases (n)	% LOAD	rs2373115 OR (95% CI)[a]
APOE ε4−		GG	314	232	42.4%	1.12 (0.82–1.53)
		GT/TT	121	100	45.2%	
APOE ε4+		GG	63	406	86.6%	2.88 (1.90–4.36)
		GT/TT	54	121	69.1%	
All samples	6.07 (4.63–7.95)	GG	377	638	62.9%	1.34 (1.06–1.70)
		GT/TT	175	221	55.8%	

[a] All rs2373115 ORs are calculated using GG versus GT/TT.

also involved in AD-related memory impairment (Reiman et al., 1996, 2005; Johnson et al., 2006; Buckner et al., 2005; Mintun et al., 2006). While the entorhinal cortex and temporal and prefrontal regions are also affected by AD neuropathology, the visual cortex is relatively spared. Using a repeated measures analysis of variance to analyze neuronal *GAB2* gene expression data from the same eight LOAD cases and ten controls, there was a significant group-by-region interaction ($p = 0.011$), with LOAD-related increases in neuronal *GAB2* gene expression that were greater in the posterior cingulate cortex and hippocampus than in the visual cortex.

Tau Phosphorylation siRNA Study

In addition to its other properties, *GAB2* is the principal activator of the phosphatidylinositol 3-kinase (PI3K) signaling pathway (Pratt et al., 2001). PI3K activates Akt, which in turn promotes glycogen synthase kinase-3 (Gsk3) phosphorylation/inactivation. This mechanism suppresses Gsk3-dependent phosphorylation of tau at AD-related hyperphorylated tau residues, the principal component of neurofibrillary tangles, and prevents apoptosis of confluent cells (Baki et al., 2004; Kang et al., 2005). Based on these findings, we hypothesized that Gab2 might function to protect cells from neuronal tangle formation and cell death and that a loss-of-function *GAB2* haplotype would diminish such protection. We thus postulated that interference with *GAB2* expression using siRNA treatment would increase tau phosphorylation at the serine-262 residue known to be hyperphosphorylated in AD. As shown in Figure 2, *GAB2* siRNA treatment was associated with a 1.70-fold increase in serine-262 phosphorylated tau. This increase was not attributable to a concomitant increase in total tau levels. Additional siRNA and protein validation studies are now being performed to determine the extent to which *GAB2* affects tau phosphorylation at additional relevant epitopes.

Immunohistochemical Validation

Gab2 immunohistochemistry was assessed in hippocampus and posterior cingulate cortex in LOAD cases. In hippocampus, Gab2 immunoreactivity was observed in structures with the morphology of dystrophic neurites or neuropil threads, neurons, and corpora amylacea. The putative neurons were almost entirely dystrophic in appearance (Figure 3A) or had cytoplasmic inclusions resembling neurofibrillary tangles (Figure 3B). Dystrophic neurons and neurites (Figure 3C) and neurofibrillary tangle-bearing cells (Figure 3D) were also revealed by the Gab2 antibody in posterior cingulate. Here, however, many relatively normal neurons were observed as well, with long stretches of immunoreactive apical dendrites ascending through the cortical layers (Figures 3C and 3D).

Discussion

In order to characterize and confirm associations between the *GAB2* gene and LOAD risk in *APOE* ε4 carriers, our studies capitalized on the genome-wide survey of more than 300,000 SNPs, two clinically characterized and neuropathologically verified cohorts of AD cases and controls, a third cohort of clinically well characterized subjects, and stratification of the samples with respect to carriers and noncarriers of a major LOAD susceptibility gene, *APOE*. Six SNPs that are part of a common haplotype block encompassing the entire *GAB2* gene were implicated in three independent cohorts. Maximal significance of the

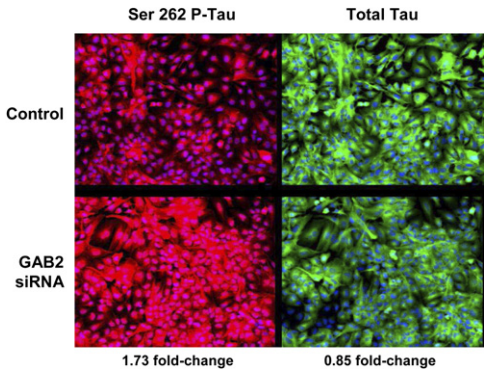


Figure 2. siRNA Knockdown of *GAB2* Increases Tau Phosphorylation

In comparison with vehicle treatment (red, upper left), *GAB2* siRNA treatment resulted in a 1.70-fold increase in tau phosphorylation at the serene-262 residue (red, lower left), which is phosphorylated in LOAD neurofibrillary tangle-bearing neurons. This fold-change was not attributable to an increase in total tau (green, upper and lower right).

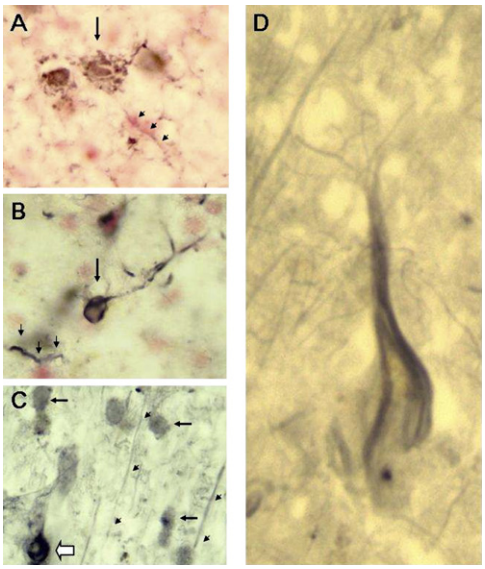


Figure 3. Gab2 Colocalizes with Dystrophic Neurons in the AD Brain

(A) LOAD hippocampus (neutral red counterstain) (40× objective). The arrow indicates a highly dystrophic cell with the size and morphology of a cortical pyramidal neuron. Arrowheads point to one of many structures in the sections that resemble dystrophic neurites or neuropil threads. (B) LOAD hippocampus (neutral red counterstain) (40×). The arrow denotes a putative neurofibrillary tangle-containing neuron. Arrowheads again indicate a dystrophic neurite. (C) LOAD posterior cingulate gyrus (40×). Filled arrows point to normal-appearing putative neurons. The open arrow points to a cell with the features of a neurofibrillary tangle-bearing neuron. Immunoreactive structures clearly resembling pyramidal cell apical dendrites were also observed ascending through the cortical layers (arrowheads). (D) LOAD posterior cingulate cortex (100× objective). Shown is a Gab2 immunoreactive cell with the flame-shaped cytoplasmic inclusion typical of the neurofibrillary tangle.

association was at SNP rs2373115 ($p = 9 \times 10^{-11}$) with an odds ratio of 4.06 (CI 2.81–14.69). An odds ratio of 24.64 (CI 7.44–116.79) for overall genetic risk is achieved when both ε4 and the *GAB2* rs2373115 risk alleles are present. Data from a microarray study of laser-capture microdissected neurons in LOAD cases and controls, immunohistochemistry, and an siRNA study provided converging evidence for the relevance of *GAB2* to the neuropathology of LOAD, raising testable hypotheses about the mechanisms by which *GAB2* could modify LOAD risk in ε4 carriers and provide targets at which to aim new treatments.

Although only one genotyping platform was used in all three cohorts, our findings are unlikely to be attributable to any platform-related bias in genotyping calls because the observed association was not limited to a single SNP but was related to a large haplotype block in agreement with the LD structure of the HapMap CEPH population. Furthermore, all six of the implicated SNPs had high-quality SNiPer-HD scores (greater than 0.45), indicating that the data for each SNP clustered well (see Figure S2 for cluster diagrams).

Individual genotype data for all samples across >300,000 high-quality SNiPer-HD calls (see Experimental Procedures for QC metrics) is made available to the community as a .ped file at http://www.tgen.org/neurogenomics/data. This genome-wide scan data will enable replication of putative common LOAD risk alleles, and also enable further discovery of both independent and combinatorial genetic associations.

The *GAB2* haplotype block spans 189 kb and includes at least 614 known SNPs. Four of the six hundred and fourteen known SNPs in this locus are nonsynonymous coding SNPs, which are generally considered to be the best candidates for functional variation. However, all four of these SNPs are reported to have minor allele frequencies of 0.0% in the CEPH population (The International HapMap Consortium, 2005), and therefore are not candidates for the common functional variant on the *GAB2* risk haplotype.

GAB2 is a scaffolding protein involved in multiple signaling pathways, which could affect AD-related tau, amyloid, metabolic, or other aspects of AD pathology and cell survival in different ways (Koncz et al., 2002; Gu et al., 2001; Zompi, Gu and Colucci, 2004), and it has been found to be coexpressed with other putative AD-related genes (Li et al., 2004a). Discovery of this LOAD susceptibility gene, if further replicated, provides new opportunities to investigate LOAD pathogenesis, predisposition, treatment, and prevention. Genome-wide studies using even higher density platforms and compound genetic analyses in sufficiently large samples of well-characterized cases and controls promise to play increasingly important roles in the scientific understanding, evaluation, treatment, and prevention of AD and other common and genetically complex disorders. In the interim, public access to the raw genotyping data from our series will provide valuable information to assess the contribution of other putative risk loci to this devastating disease.

EXPERIMENTAL PROCEDURES

High-Density Genome-Wide Association Study

The 500K GeneChip (Affymetrix, Santa Clara, CA) was used to survey 502,267 SNPs in each subject as recently described (Coon et al., 2007). Genotypes were extracted using both SNiPer-HD (Hua et al., 2007) and BRLMM (Affymetrix) software. 312,316 SNPs were analyzed after excluding those that were monomorphic, did not cluster into three distinct Gaussian distributions, clustered poorly, had Hardy Weinberg equilibrium p values less than 0.01, had minor allele frequencies less than 2%, or exhibited less than 98% concordance between the SNiPer-HD and BRLMM calls. The software program STRUCTURE (Pritchard et al., 2000) was employed to test for underlying genetic stratification using 5000 randomly selected SNPs and including at least 100 SNPs per chromosome. The initial analysis yielded empirical evidence of three populations. Since 14 subjects belonged to a population far removed from the rest of the study population, they were eliminated from further analyses. STRUCTURE then was used to demonstrate a comparable admixture of the two populations in the cases and controls. After stratifying the LOAD cases and controls for presence or absence of the APOE ε4 allele, allelic χ^2 statistics were computed for each SNP. APOE genotypes were obtained in each subject

by either pyrosequencing (Ahmadian et al., 2000) or restriction fragment length polymorphism (RFLP) analysis (Lai et al., 1998).

LD mapping was performed by importing genotypes into the HaploView program v3.32. Pairwise LD values (as measured by *D'*) reflect the likelihood that two genetic markers are inherited together.

Neuronal Microarray Studies

Brain samples (mean post-mortem interval of 2.5 hr) from six brain regions that are either histopathologically or metabolically relevant to LOAD and aging were collected at the Sun Health Research Institute. Expression profiling was performed as described previously (Liang et al., 2007). Direct case-to-control comparisons were performed to analyze expression differences in each region.

Immunohistochemical Validation

Gab2 immunoreactivity in LOAD hippocampus and posterior cingulate cortex was examined using an affinity-purified goat polyclonal antibody directed against a C-terminal epitope of Gab2 (Santa Cruz Biotechnology, Santa Cruz, CA). Blocks were obtained from rapid autopsy LOAD cases (<3 hr postmortem) (n = 5). Hippocampus sections were derived from blocks that were fixed for 24 hr in 4% paraformaldehyde and sectioned at 40 μm on a freezing microtome. Posterior cingulate sections were derived from snap-frozen blocks that were sectioned at 6 μm on a cryostat. Immunohistochemical protocols were as previously described (Li et al., 2004b). Immunoreactivity was visualized with nickel-intensified diaminobenzidine.

Tau Phosphorylation siRNA Study

Neuroglioma cells overexpressing wild-type tau protein were grown in 96-well plates and transfected with siRNA directed at *GAB2* mRNA. Following 4 days of transfection, cells were fixed, permeablized, and immunostained with antibodies against total tau protein and tau protein phosphorylated on serine-262. A FITC- and Cy5-conjugated secondary antibody cocktail was then applied. After incubation and washing, images were captured and quantitated using the InCell imager 3000 (General Electric). The fold increase in serine-262 phosphorylated tau levels was calculated against control samples that had been transfected with a scrambled siRNA sequence.

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