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Validating Predicted Biological Effects of Alzheimer's Disease Associated SNPs Using CSF Biomarker Levels

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Abstract. Recent large-scale genetic studies of late-onset Alzheimer's disease have identified risk variants in *CALHM1*, *GAB2*, and *SORL1*. The mechanisms by which these genes might modulate risk are not definitively known. *CALHM1* and *SORL1* may alter amyloid-β (Aβ) levels and *GAB2* may influence phosphorylation of the tau protein. In this study we have analyzed disease associated genetic variants in each of these genes for association with cerebrospinal fluid (CSF) Aβ or tau levels in 602 samples from two independent CSF series. We failed to detect association between CSF Aβ_{42} levels and single nucleotide polymorphisms in *SORL1* despite substantial statistical power to detect association. While we also failed to detect association between variants in *GAB2* and CSF tau levels, power to detect this association was limited. Finally, our data suggest that the minor allele of rs2986017, in *CALHM1*, is marginally associated with CSF Aβ_{42} levels. This association is consistent with previous reports that this non-synonymous coding substitution results in increased Aβ levels *in vitro* and provides support for an Aβ-related mechanism for modulating risk for Alzheimer's disease.

Keywords: Alzheimer's disease, amyloid, association, *CALHM1*, endophenotypes, *GAB2*, genetics, *SORL1*, tau

INTRODUCTION

Although it has been demonstrated that late-onset Alzheimer's disease (LOAD) has a strong genetic component, until recently, only the ε4 allele of apolipoprotein E (*APOE ε4*) has been convincingly demonstrated to influence risk for LOAD [1]. Recent studies have identified several new and promising candidate genes [2–6]. In this manuscript we evaluate variation in GRB-associated binding protein 2 (*GAB2*), calcium homeostasis modulator 1 (*CALHM1*), and sortilin-related receptor (*SORL1*) for association with the cerebrospinal fluid (CSF) biomarkers that they are predicted

[1]Co-first authors with equal contributions.

[2]Some of the data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://www.loni.ucla.edu\ADNI). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. ADNI investigators include (complete listing available at http://www.loni.ucla.edu/ADNI/About/About_Investigators.shtml).

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to influence. As of February 24, 2009 single nucleotide polymorphisms (SNPs) from each of these genes are ranked in the top 27 hits on http://www.alzgene.org [1]. In 2007, Coon and colleagues identified *GAB2* (MIM#606203) as a genetic risk factor for LOAD especially in *APOE ε4* carriers [7]. GAB2 is the principal activator of phosphatidylinositol-3, which, through Akt, may regulate glycogen synthase kinase-3β, affecting tau phosphorylation. GAB2 is over-expressed in pathologically vulnerable neurons; and the GAB2 protein was detected in neurons, tangle-bearing neurons, and dystrophic neurites. Reiman et al. [6] postulated that GAB2 could contribute to Alzheimer's disease (AD) pathology through a tau-dependent mechanism, because inhibition of GAB2 expression with siRNA resulted in an increase in tau phosphorylation. Attempts to replicate these findings resulted in a mixture of negative and positive findings, with no present consensus [8–12]. The *CALHM1* gene (MIM#612234) was identified as a novel AD susceptibility gene using a multifaceted approach combining information from gene expression and genetic studies [4]. Functional studies have shown that the risk variant results in decreased permeability to calcium ions, lowering the intracellular calcium ion levels, ultimately leading to an increase in amyloid-β (Aβ) peptide [4]. This finding was initially replicated in independent datasets [4]. Subsequent studies, however, have failed to replicate the association of *CALHM1* with AD in case-control or in family-based analyses [11,13,13–16], and a recent study failed to detect association between rs2986017 in *CALHM1* for association with CSF biomarker levels in a small sample of LOAD and other dementia cases [17]. Genetic association between *SORL1* (MIM#602005) and AD was also identified because of its reduced expression in brain tissue from individuals with AD compared to healthy controls and its correlation between the SORL1 protein level and Aβ production [18]. Intronic variants in *SORL1* that regulate tissue-specific levels of SORL1 mRNA were later shown to be associated with risk for AD [5]. SORL1 can bind to APOE and, as a sorting and trafficking protein, guides amyloid-β protein precursor (AβPP) into the endosome recycling pathway, resulting in reduced Aβ production. Association of *SORL1* has been replicated in several different populations including Caucasians [19–21], Asians [22], Africans [23], and Hispanics [24,25]. Several other studies have failed to detect association between SNPs in *SORL1* and risk for AD [7,19,26,27]. Two studies have evaluated genetic variants in *SORL1* for association with CSF Aβ levels [25,28] with one failing to detect association [28] and the other detecting some evidence of association [25]. Lack of consistent replication for each of these genes is a common occurrence in the study of complex phenotypes and may be indicative of inadequate power resulting from small sample size and/or genetic and environmental heterogeneity.

The use of CSF biomarker levels for genetic studies of AD may provide increased statistical power and important insight into the biological mechanisms by which these variants modulate risk for disease. Our previous results suggest that both 42 amino acid Aβ(Aβ_{42}) and tau phosphorylated at threonine 181 (ptau$_{181}$) levels can be effectively used as endophenotypes, or intermediate traits, to study genetic risk for AD [29–31]. Based on previously reported functional studies, we have tested for association of SNPs in *CALHM1* and *SORL1* with CSF Aβ_{42} levels and SNPs in *GAB2* with CSF ptau$_{181}$ levels in a total of 602 samples from two independent CSF series.

MATERIALS AND METHODS

Samples

CSF for the Washington University in St. Louis (WU) series was collected from 345 individuals by lumbar puncture after overnight fasting. CSF collection, processing, and CSF biomarker measurements were performed as described previously [32]. Sample characteristics, including age, clinical dementia rating, gender, *APOE ε4* status, and mean and standard deviation of the CSF biomarkers can be found in Table 1. Qualified scientists may request these data through the Alzheimer's Disease Genetics Consortium (http://alois.med.upenn.edu/adgc/) or by direct request to the authors.

Data from 257 samples with biomarker data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) were also used. Data used in the preparation of this article were obtained from the ADNI database (http://www.loni.ucla.edu/ADNI). The Principal Investigator of this initiative is Michael W. Weiner, M.D., VA Medical Center and University of California–San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research – approximately 200 cognitively normal older individuals to be followed

Table 1
Sample characteristics. Sample size (n), mean and standard deviation for age, Clinical Dementia Ratings (CDR), the percentage of females in the sample (% female), percentage of the sample that carries at least one *APOE ε4* allele (% ε4pos) and the mean and standard deviation for Aβ_{42}, tau and ptau$_{181}$ for the complete Washington University CSF sample (WU: All), cases and controls and the complete Alzheimer's Disease Neuroimaging Initiative (ADNI: All), cases and controls are shown. CSF biomarker values are in pg/mL

	WU All	Cases	Controls	ADNI All	Cases	Controls
n	345	97	248	257	154	103
age (SD)	68 (11)	74 (8)	66 (11)	76 (7)	75 (8)	77 (5)
CDR	$0 = 72\%$ $0.5 = 20\%$ $1 = 7\%$ $2 = 1\%$	All > 0	All $= 0$	$0 = 40\%$ $0.5 = 27\%$ $1 = 28\%$ $2 = 3\%$	All > 0	All $= 0$
% female	60	46	66	56	59	50
% ε4pos	39	51	35	47	64	23
Aβ_{42} (SD)	941 (548)	676 (392)	1041 (568)	173 (58)	149 (46)	208 (55)
ptau$_{181}$ (SD)	62 (33)	82 (44)	54 (23)	34 (19)	40 (19)	24 (14)

Table 2
CSF biomarkers and covariates. Associations between CSF Aβ_{42} and ptau181 and age, *APOE ε4* genotype (ε4) and Clinical Dementia Rating (CDR). Associations in the WU sample were calculated using ANCOVA. Associations in the ADNI sample were calculated using the SAS macro npar

		age	ε4	CDR
WU	Aβ_{42}	0.015	< 0.0001	< 0.0001
	ptau$_{181}$	< 0.0001	0.0006	< 0.0001
ADNI	Aβ_{42}	0.79	< 0.0001	< 0.0001
	ptau$_{181}$	0.84	< 0.0001	< 0.0001

for 3 years, 400 people with mild cognitive impairment (MCI) to be followed for 3 years, and 200 people with early AD to be followed for 2 years. For up-to-date information, see http://www.adni-info.org. Sample characteristics, including age, clinical dementia rating, gender, *APOE ε4* status, and mean and standard deviation of the CSF biomarkers can be found in Table 1. ADNI phenotype and GWAS data are publically available (http://www.loni.ucla.edu/ADNI). The genotypes from this study will be provided upon request to the authors.

While both studies measured biomarker values in a rigorous manner with internal standards and controls that ensure consistent and reliable measurements [32, 33], there are differences between the measured values in the WU and ADNI samples. This is due to differences in the antibodies and measurement technologies employed to measure the samples (e.g., standard ELISA with Innotest in the WU samples, Luminex with AlzInnoBio3 in the ADNI samples). In addition, differences in ascertainment (more AD cases and older individuals in the ADNI sample) or handling of the CSF after collection (e.g., number of freeze-thaw cycles prior to analysis) could account for some of the variation in the biomarker measurements between the two samples. CSF biomarkers in the two samples show association with similar covariates (Table 2). Age is not a significant covariate in the ADNI samples, but this is likely due to the low variability in age in the ADNI samples.

SNP selection

SNPs in *CALHM1* and *GAB2* were selected directly from the initial reports of association. For *CALHM1* we genotyped rs2986017 which was reported to show association with risk for AD and shown to influence Aβ levels [4]. For *GAB2* we genotyped three SNPs (rs2373115, rs7115850, and rs4291702) that show significant association with risk for AD [6]. For *SORL1* a large number of SNPs were genotyped in the original report, and several of these variants have been reported to show association with AD in various reports [5,19–23,27,34–38]. We genotyped seven SNPs (rs668387, rs689021, rs641120, rs2070045, rs2282649, rs3824968, and rs661057) that showed significant association with AD in the AlzGene meta-analyses of published studies AD on October 27, 2008 [1].

Analysis

We used stepwise discriminant analysis to identify the significant covariates for CSF Aβ_{42} and ptau$_{181}$ levels. CDR and *APOE ε4* genotype were significantly associated with CSF Aβ_{42} and ptau$_{181}$ in both the WU and ADNI series (Table 2). In the WU sample age also showed association with CSF Aβ_{42} and ptau$_{181}$ (Table 2). In the WU CSF series Aβ_{42} levels were tested for association with the additive genetic model of each SNP after adjustment for the appropriate covariates (age, APOE ε4 genotype, and CDR). In the ADNI series CSF Aβ_{42} levels are not normally distributed and simple transformations failed to approximate a normal distribution. For analysis of this variable in the ADNI sample we used the SAS macro npar, a nonparametric

method to include covariates (APOE ε4 genotype and CDR) in the test for association [39]. For the Aβ_{42} combined analysis the WU and ADNI series were each standardized to zero, then combined and analyzed using site, age, APOE ε4 genotype, and CDR as covariates in the SAS macro npar [39]. For the analyses in the ADNI and combined samples we report the ANOVA test statistic with covariates from npar.

CSF ptau$_{181}$ was log-log transformed to approximate a normal distribution. Analysis of the covariance (ANCOVA) was used to test for association between genotypes and CSF ptau$_{181}$ levels after adjustment for the appropriate covariates. Because the CSF ptau$_{181}$ levels in the WU and ADNI samples were measured using different platforms (Innotest plate ELISA and the AlzBia3 bead-based ELISA, respectively) we were not able to combine the raw data, rather we combined the residual values of the CSF ptau$_{181}$ obtained after correcting for the covariates then analyzed the data using ANCOVA with site as a covariate.

The evaluations of CSF biomarker associations for each gene are based on specific predictions from published papers concerning the SNP(s) in the report and the published biological data. Based on these gene specific hypotheses we applied a Bonferroni correction for multiple tests within each gene. This yielded a Bonferroni corrected alpha of 0.05 for *CALHM1*, 0.0083 for *GAB2*, and 0.0071 for *SORL1*.

Power analyses

We used proc power in SAS to perform power analyses for the overall F test in a one-way, three group analysis of variance. Power was calculated separately for each SNP using genotype counts from the combined sample. All power analyses were performed specifying an alpha of 0.05 and the effect size we observed for rs2986017 (a 1.05 fold difference in mean biomarker levels). This effect size is equivalent to a delta (difference between the group means in units of standard deviation) of approximately 0.6. We also calculated the effect size (delta) at which there was 80% power to detect an association for each SNP using proc power in SAS.

RESULTS

Analyses of rs2986017 in *CALHM1* detected marginally significant association with CSF Aβ_{42} levels. The minor allele, T, showed a correlation with increased CSF Aβ_{42} levels in the WU sample ($p = 0.042$) and in the combined sample ($p = 0.0095$; Fig. 1). This correlation was mainly driven by homozygous carriers of the "T" allele. The increase in sample size, and therefore statistical power, combined with the fact that the ADNI data shows a trend in the same direction in which significant association was observed in the WU series results in a more significant effect in the combined series than in the WU or ADNI series alone. We failed to detect significant associations in the SNPs from *SORL1* and *GAB2* in both the individual and combined samples (Table 3; Fig. 2). We had greater than 90% power to detect a 1.05 fold difference in biomarker levels for rs661057, rs668387, rs689021, rs1010159, rs2282649, and rs3824968 from *SORL1* by our power calculations. For these variants we would have 80% power with delta values of 0.33-0.52. Due to differences in allele frequency, statistical power for rs2070045 was 75%. Power at delta of 0.6 in the *GAB2* SNPs was approximately 75% in the total sample but only 0.33 in the *APOE ε4* carriers. Power values for all SNPs are shown in Table 3.

DISCUSSION

Our data suggest that rs2986017 is significantly associated with CSF Aβ_{42} levels but failed to detect association between CSF Aβ_{42} levels and SNPs in *SORL1* or CSF ptau$_{181}$ levels and SNPs in *GAB2*. Giedraitis and colleagues failed to detect association between rs2986017 and CSF biomarker levels in a sample of 186 individuals with AD or other cognitive disorders [17]. This study differs from our study in several important ways. First, Giedraitis and collaborators did not include covariates, such as *APOE ε4* genotype and CDR, which show strong association with CSF Aβ_{42} levels in our study (Table 2) and other studies [40–42]. Second, power to detect a 1.2-fold increase in CSF Aβ_{42} levels in the Giedraitis et al. study was reported to be 0.84. In contrast our study, which included more than three times as many samples, has power of 0.94 to detect a 1.05-fold difference in CSF Aβ_{42} levels (and power greater than 0.99 to detect a 1.20 fold difference). Finally, CSF Aβ_{42} levels are decreased in AD cases but not necessarily with other cognitive impairments due to other diseases or disorders. A sample of AD cases is likely to have lower overall CSF Aβ_{42} levels and less variance in CSF Aβ_{42} levels than a sample of controls (see Table 1).

Table 3
Association with CSF Aβ_{42} levels in the WU, ADNI, and Combined samples. For each SNP the gene, p-value in the Washington University sample (WU), p-value in the Alzheimer's Disease Neuroimaging Initiative sample (ADNI), p-value in the combined WU and ADNI samples (COMB), Power in the combined sample when delta equals 0.6 (1.05 fold difference in biomarker levels) and delta level at which power is 80% shown. *Results are for association with CSF ptau$_{181}$ levels in the total sample. **Results are for association with CSF ptau$_{181}$ levels in *APOE ε4* positive subgroup. ***Results for association between this SNP and CSF biomarker levels from a subset of the WU sample were previously reported in Kauwe et al., 2009 [29]

SNP	Gene	WU	ADNI	COMB	Power (delta = 0.6, 1.05 fold diff)	Delta (power = 80%)
rs2986017	CALHM1	0.042	0.68	0.0095	0.93	0.53
*rs2373115	*GAB2*	0.45	0.46	0.88	0.76	0.63
**rs2373115	*GAB2*	0.25	0.43	0.30	0.33	1.07
*rs4291702	*GAB2*	0.50	0.38	0.99	0.75	0.64
**rs4291702	*GAB2*	0.21	0.17	0.16	0.33	1.07
*rs7115850	*GAB2*	0.28	0.38	0.82	0.76	0.63
**rs7115850	*GAB2*	0.31	0.32	0.29	0.33	1.06
rs661057	*SORL1*	0.75	0.53	0.21	$>$ 0.99	0.34
rs668387	*SORL1*	0.23	0.65	0.32	$>$ 0.99	0.33
rs689021	*SORL1*	0.21	0.53	0.34	$>$ 0.99	0.34
***rs1010159	*SORL1*	0.90	0.17	0.09	0.98	0.43
rs2070045	*SORL1*	0.33	0.43	0.29	0.75	0.64
rs2282649	*SORL1*	0.79	0.16	0.10	0.91	0.52
rs3824968	*SORL1*	0.80	0.18	0.23	0.95	0.48

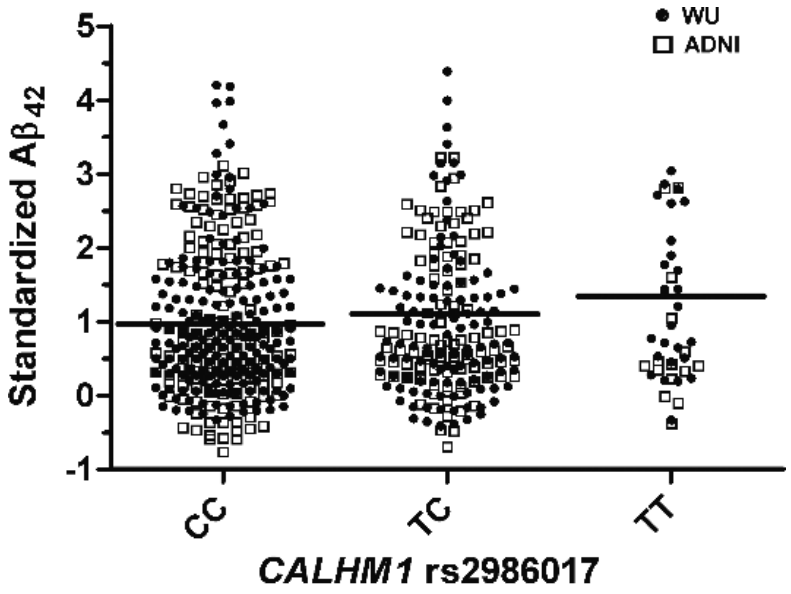

Fig. 1. Standardized CSF Aβ_{42} levels and rs2986017 genotypes. Standardized CSF Aβ_{42} levels by rs2986017 genotype for the WU CSF and ADNI CSF samples. Horizontal lines show the mean from the *standardized, covariate adjusted* Aβ_{42} levels for the combined samples for each genotype (CC = 0.95, TC = 1.03, TT = 1.31).

Several recent reports failed to detect evidence replicating the initial report of association between risk for LOAD and rs2986017. While our data do not directly address the association with AD risk, they do suggest that the minor allele, of rs2986017 is significantly associated with higher CSF Aβ_{42}. This finding is consistent with previous data suggesting that *in vitro* the "T" allele, which also increases risk for AD, results in increased Aβ_{42} levels [4]. The direction of this association is different from that of the *APOE ε4* allele, where carriers show a strong and highly significant decrease in CSF Aβ_{42} levels, but similar to the effect observed for the PSEN1 A79V Familial AD mutation, where CSF data from a living mutation carrier (clinically unaffect-

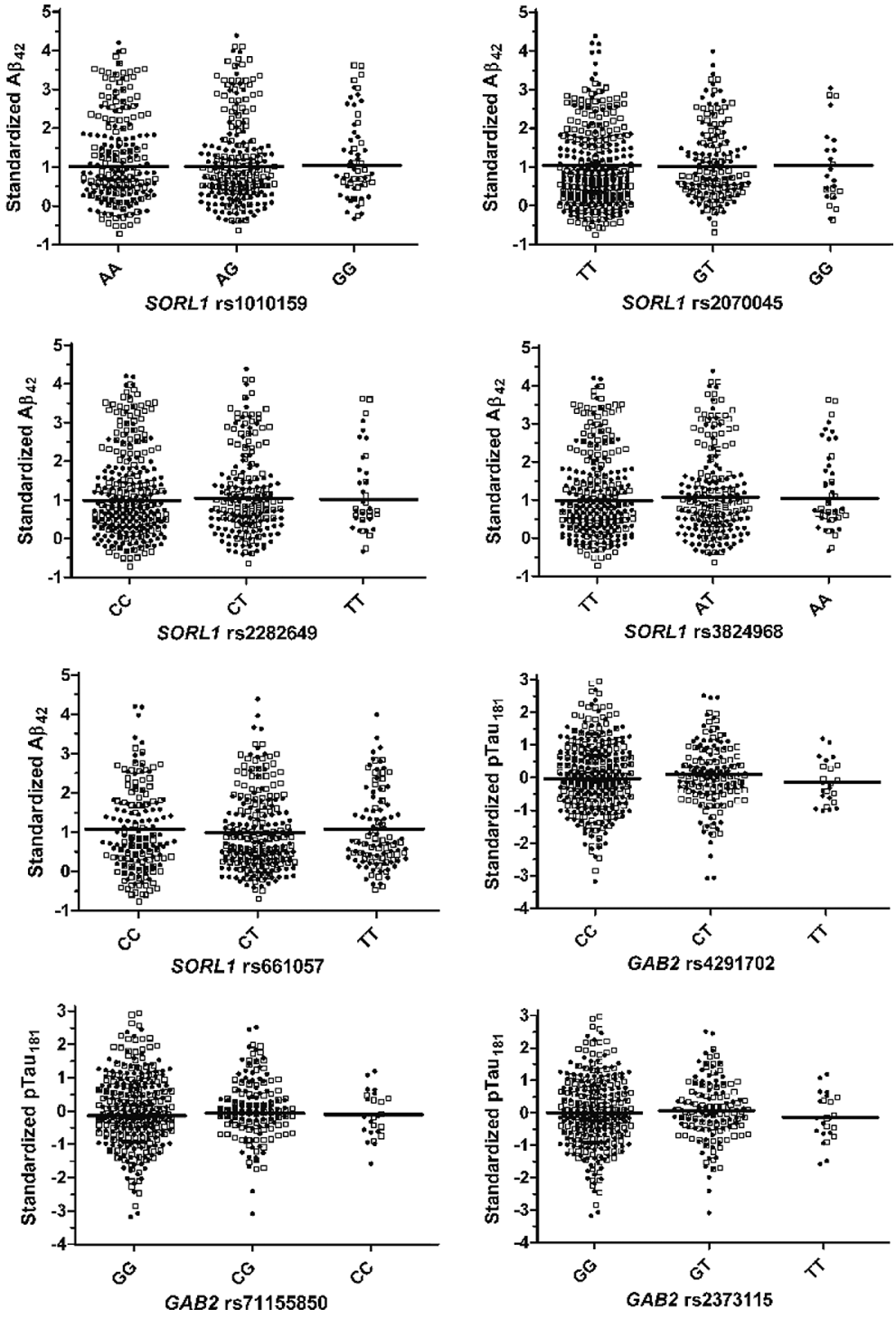

Fig. 2. Scatter plots for each of the SNPs in *GAB2* and *SORL1* from this study. Each graph is labeled with the Gene and SNP. The y-axis is the standardized biomarker levels that were tested for each SNP (pTau_{181} for *GAB2*, $\mathrm{A}\beta_{42}$ for *SORL1*). The x-axis is the genotype groups for each SNP. Lines represent the grand means for the combined sample sets for each genotype. The WU samples are represented by filled circles, the ADNI samples are represented by open squares.

ed) and findings from *in vitro* experiments showed significantly increased CSF Aβ_{42} levels in the presence of the mutation [30]. At present it is difficult to predict the expected direction of a genetic association with CSF Aβ levels as it appears that those changes may be specific to the risk mechanism of the variant and the point of progression through the disease process.

In 2008, a small study using data from 153 AD cases reported association between CSF Aβ_{42} levels and SNPs in *SORL1* [25]. A more recent report using CSF from approximately 700 AD cases failed to detect association between CSF Aβ_{42} and 6 SNPs in *SORL1*. Our study, which included CSF from 602 cases and controls, failed to detect evidence for association between SNPs in *SORL1* and Aβ_{42}. Power was excellent for an effect of the magnitude detected in *CALHM1* (delta = 0.60) and 80% power extended down to delta of about half that size. In addition, the inclusion of a large number of controls in our analysis provides much greater variance in Aβ_{42} levels than a cases-only sample. These calculations suggest it is likely that we would have detected effects of variants in *SORL1* on CSF Aβ_{42}. Unfortunately we did not have measurements of other Aβ species, such as Aβ_{40} in the ADNI series and therefore could not address the possibility of an effect on other Aβ fragments.

We failed to detect evidence of association between SNPs in *GAB2* and ptau$_{181}$. Power was sufficient (80%) to detect a 1.05 fold difference in the total sample but is poor (0.33) in the *APOE ε4* positive subgroup, making it unlikely that we would have detected any effects in this sub-group.

In summary, we have detected marginally significant association between rs2986017, a putative functional SNP within *CALMH1*, and CSF Aβ_{42} levels. Our result does not directly address association with risk for LOAD but is consistent with previous reports suggesting that this non-synonymous coding substitution results in increased Aβ levels *in vitro*. While the signal appears consistent in the combined WU and ADNI datasets, the association is modest and it remains possible that it represents a false positive association. We failed to detect association between SNPs in *SORL1* and CSF Aβ_{42} levels despite substantial statistical power. Both our findings and those of another report from a large CSF sample fail to detect association between *SORL1* and CSF Aβ_{42} levels. This report, along with our previous work further illustrates the possible utility of using CSF endophenotypes to evaluate and understand the biological mechanisms by which variants might modulate risk for AD.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=424).

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