Cell

No Association between *CALHM1* and Alzheimer's Disease Risk

In a recent paper in *Cell*, Dreses-Werringloer et al. (2008) reported the identification and functional characterization of a new Alzheimer's disease (AD) gene, *CALHM1*, encoding calcium homeostasis modulator 1. *CALHM1* (formerly annotated as *FAM26C*) represents a compelling candidate gene for late-onset AD as it is located on chromosome 10q24, a consistently replicated AD linkage region (Bertram et al., 2007), is highly expressed in the hippocampus, which is severely affected by AD-related pathology, and is involved in calcium ion homeostasis, which may be disrupted in AD. In view of these converging leads, Dreses-Werringloer et al. (2008) sequenced the open reading frame of *CALHM1* in a small sample of AD patients and healthy controls and identified a nonsynonymous polymorphism (Pro86Leu, rs2986017) whose minor leucine allele showed a higher frequency in the AD patients. Followup analyses in four additional independent samples of ~3400 DNAs revealed a consistent overrepresentation of the same allele in AD cases compared to controls in each dataset. From the combined analyses the authors estimated that inheritance of the leucine allele modestly, but significantly, elevated the risk for AD by ~40% (p value = 2×10^{-10}). Their genetics findings were supported by data generated in a large number of functional genomics and biochemical experiments showing evidence that the risk-associated leucine allele leads to a loss of protein function, including attenuated permeability to calcium ions and reduced cytosolic calcium ion levels, which, in turn, were associated with an increase in the pathogenic peptide, amyloid-β (Aβ). Here, we present an independent assessment of the potential association between AD and the Pro86Leu single-nucleotide polymorphism (SNP) in *CALHM1* in more than 8100 subjects from several independent datasets comprised of AD families—including those in which the original chromosome 10q24 linkage signal was identified (Bertram et al., 2000)—as well as unrelated cases and controls, and we find no evidence of a genetic association in these samples.

The family-based datasets (CAG, NIA, NIMH, NCRAD) tested in this project are of self-reported European (Caucasian) ancestry collected in the US for the study of genetic factors in AD (see Table S1, available online, for a summary of sample characteristics). All samples were primarily sibships and lacked parental genotypes. With the exception of the CAG sample, the majority of pedigrees analyzed here were nuclear families ascertained on the basis of multiple affected individuals. In addition to containing at least one affected relative pair, many pedigrees also had DNA available from additional affected or unaffected individuals (mostly siblings). The diagnosis of definite, probable, or possible AD was made according to NINCDS/ADRDA criteria for affected individuals in all four samples (McKhann et al., 1984). In addition to the family samples, we genotyped ~1300 unrelated AD cases and controls, which were collected at two sites in Northern Europe, Sweden, and Finland (Table S1). All subjects were Caucasian, and AD patients fulfilled NINCDS/ADRDA criteria for probable AD. The Swedish AD patients were ascertained at the Memory Disorder Unit at Uppsala University Hospital. Healthy control subjects were recruited from the same geographic region following advertisements in local newspapers and displayed no signs of dementia upon neuropsychological testing. The Finnish subjects were gathered from Eastern Finland and were examined in the Department of Neurology of Kuopio University Hospital. Control subjects had no signs of dementia following neuropsychological testing. Finally, we assessed the *CALHM1* locus in two previously published high-density genome-wide association studies (GWAS) (Li et al., 2008; Reiman et al., 2007) for which genotype data are publicly available. Together, these studies investigated 2900 unrelated AD cases and controls. Given that the rs2986017 (Pro86Leu) variant was not tested directly in either of the GWAS, we tested two SNPs showing strong and significant linkage disequilibrium with the rs2986017 (Pro86Leu) variant (rs2986030 [D' = 0.89, r2 = 0.71] and rs1555823 [D' = 0.89, r2 = 0.64]). Note that the power to detect the effect sizes described in the original report (Dreses-Werringloer et al., 2008) was still very high (>90%) due to the strong linkage disequilibrium between these variants (Table S2).

Genotyping of the rs2986017 (Pro86Leu) variant in the US family samples and the Northern European case-control datasets was based on an individually optimized single-base extension reaction detected by high-efficiency fluorescent polarization (HEFP; described in Bertram et al., 2005; protocols available on request). Overall, genotyping efficiency was 97.6%, and the average error rate was below 0.2%. Resequencing of the Pro86Leu variant in 20 individuals revealed 100% concordance with the HEFP genotype results. None of the markers deviated significantly from Hardy-Weinberg equilibrium. To test for association with AD risk in the family-based samples we used PBAT (http://www.biostat.harvard.edu/~clange/default.htm) applying an additive model. Odds ratios (ORs) for the family samples were calculated by fitting a conditional logistic regression model to each dataset, where family defines the stratum. To test for association in the case-control samples (including the markers extracted from the GWAS) we calculated allele-based study-specific crude ORs, 95% confidence intervals, and p values for each marker (Bertram et al., 2007). To combine the effect size estimates obtained in this study with those estimated in the original publication, summary ORs across all samples were calculated using the DerSimonian and Laird random effects model, in line with the analyses routinely performed for the AlzGene database (Bertram et al., 2007). Power calculations (performed in PBAT) suggested that we had sufficient (i.e., 70% or greater) power to detect the genetic effect size estimated in the original study in each of the samples, with the exception of the CAG dataset (Table S2).

As shown in Figure S1 and Table S2, none of the eight samples we investigated showed evidence for significant association between the rs2986017 (Pro86Leu)

variant in *CALHM1* and AD risk (p values ranging from 0.15 to 0.84). Stratification by age of AD onset (using 65 years as cutoff) or *APOE* ε4-genotype did not appreciably change these results (data not shown; stratified analyses were not possible in the GWAS samples as no onset age or *APOE* ε4 data were supplied). Effect size estimates indicated insignificant ORs that were opposite in direction to those reported by Dreses-Werringloer et al. (2008) in six of the eight samples (see Figure S1). Accordingly, summary ORs calculated across the newly genotyped samples in our study (labeled "This study" in Figure S1) were insignificant (OR = 0.94 [95% CI: 0.83–1.07], p = 0.4) and tended toward null when combined with the published GWAS genotype data ("All follow-up"; OR = 0.99 [95% CI: 0.91–1.09], p = 0.9). Upon combining these data with the results of the original study ("All studies"), that is, generating a meta-analysis on all ~11,700 currently available subjects, the overall summary OR became insignificant as well (OR = 1.13 [95% CI: 0.99–1.27], p = 0.06). Using rs1555823 (instead of rs2986030) as proxy for the rs2986017 (Pro86Leu) variant in the GWAS samples revealed even less pronounced and less significant overall effects (data not shown).

Thus, we have independently assessed the potential association between AD risk and the rs2986017 (Pro86Leu) variant in the *CALHM1* gene in a large number of independent datasets, including AD families in which the original chromosome 10q24 linkage signal was identified (Bertram et al., 2000). Despite good to excellent power to detect genetic effect sizes on the order described by Dreses-Werringloer et al. (2008), no association between *CALHM1* and AD was observed, either in the individual samples or in the combined analyses of more than 8100 subjects. Based on these negative data, it is doubtful that *CALHM1* represents more than a minor genetic determinant of AD risk.

Supplemental Data

Supplemental Data include two tables and one figure and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(08)01505-5.

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Response

CALHM1 Association with Alzheimer's Disease Risk

We recently reported the association of the *CALHM1* gene with late-onset Alzheimer's disease (LOAD) risk in four independent populations of more than 3000 participants (Dreses-Werringloer et al., 2008). We showed that the rare allele of the nonsynonymous SNP rs2986017 in *CALHM1* increases LOAD risk by about 40% (p = 2×10^{-10}). Importantly, we also demonstrated the functional significance of the *CALHM1* rs2986017 SNP by showing that the corresponding Pro86Leu polymorphism increased levels of the pathogenic peptide, Aβ (Dreses-Werringloer et al., 2008). In their Correspondence, Bertram et al. independently assess the association between LOAD risk and *CALHM1* and report an inability to replicate the association in more than 5000 individuals. Here, we comment on several important methodological points in the study of Bertram et al., and we present new evidence that supports the association of *CALHM1* with both LOAD risk and age of AD onset.

Family-Based Association Studies

The main contribution of the Bertram et al. study lies in the analysis of the *CALHM1* rs2986017 SNP on LOAD risk in four independent family-based populations. The authors observed no significant genetic association. They combined these family-