Functional Screening of Alzheimer Pathology Genome-wide Association Signals in Drosophila

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We have leveraged a Drosophila model relevant to Alzheimer disease (AD) for functional screening of findings from a genome-wide scan for loci associated with a quantitative measure of AD pathology in humans. In six of the 15 genomic regions evaluated, we successfully identified a causal gene for the association, on the basis of in vivo interactions with the neurotoxicity of Tau, which forms neurofibrillary tangles in AD. Among the top results, rs10845990 within SLCA2A14, encoding a glucose transporter, showed evidence of replication for association with AD pathology, and gain and loss of function in glut1, the Drosophila ortholog, was associated with suppression and enhancement of Tau toxicity, respectively. Our strategy of coupling genome-wide association in humans with functional screening in a model organism is likely to be a powerful approach for gene discovery in AD and other complex genetic disorders.

Genome-wide association studies (GWAS) have emerged as powerful tools for the dissection of complex genetic traits, such as susceptibility to Alzheimer disease (AD, MIM 104300);1 however, efficient methods are needed to enhance follow-up of association signals in order to accelerate the identification and functional validation of genes affected by causal variants.2 On the basis of recent analyses, the top of GWAS-results distributions (10−3 < p < 10−7), though falling short of genome-wide significance (p < 5 × 10−8), are likely enriched for true associations, but these signals are obscured by a substantial number of chance observations with comparable statistical evidence.3–5 New strategies are therefore needed, not only to validate associations with the best evidence, but also to facilitate identification of true signals of association in circumstances where statistical power is limited and increased sample size is not feasible. One potential solution is to couple the GWAS with a functional screen that evaluates candidate genes for participation in a relevant pathological cascade, a two-stage strategy that might effectively increase overall study power. Here, we leverage a model system relevant to AD in the fruit fly, Drosophila melanogaster, to perform functional testing of 19 genes from 15 distinct genomic regions identified in a GWAS for loci influencing the burden of AD pathology in humans.

AD is the most common cause of dementia, and it is characterized at autopsy by widespread neuronal loss in association with extracellular amyloid plaques and intracellular neurofibrillary tangles, predominantly comprising the amyloid-β peptide (Aβ) and Tau, respectively.6 Both rare mutations and common polymorphisms have been found to influence susceptibility for AD, and GWAS have recently been successful at discovering such loci.1,7–9 Most GWAS conducted to date have relied on the dichotomous outcome of AD clinical diagnosis; however, this study design is potentially confounded by genetic heterogeneity of dementia in cases and subclinical disease in controls. In a complementary approach, we have based our analysis on a relevant AD intermediate phenotype: a quantitative measure of global AD pathology from post-mortem counts of amyloid plaques and neurofibrillary tangles. Although this approach potentially offers more statistical power than a case-control study of comparable size,10,11 it is limited by the difficulty in obtaining neuropathologic data on large numbers of older individuals. Thus, we anticipated a challenge in meeting the statistical burden of proof for gene discovery, and therefore we coupled our association analysis with a functional screening paradigm in order to validate our results.

A GWAS was performed in an autopsy cohort consisting of 227 participants from the Religious Orders Study and the Rush Memory and Aging Project, two longitudinal, epidemiologic studies of aging and AD that include brain donation at death.12–14 Written informed consent was given and an Anatomic Gift Act signed by all study participants after the procedures were fully explained, and both studies were approved by the institutional review board of Rush University Medical Center. Subjects were nondemented at recruitment and were followed prospectively with annual clinical evaluations. Proximate to death, 40% of subjects had normal cognition, 22% had mild...
cognitive impairment, and 38% met clinical criteria for AD (Table S1 available online). After quality control, 334,575 SNP genotypes were available for analysis (Figure S1). The outcome was a continuous measure of global AD pathology, based on averaged counts of neuritic plaques, diffuse plaques, and neurofibrillary tangles on silver-stained tissue sections from five brain regions (midfrontal, middle temporal, inferior parietal, and entorhinal cortices and the hippocampal CA1 sector). Linear regression was used to evaluate SNP associations with the continuous AD pathological trait, adjusting for both age at death and APOE ε4 genotype. The top independently associated regions (p < 1 × 10^{-3}) containing candidate genes are presented in Table 1 (for full results, see Table S2). Of note, the subjects in the study cohort were also examined in that study exceeded the significance threshold applied here, and many of those SNPs were not captured by the Illumina genotyping platform used in this genome scan.

As expected for our small study, no variant achieved genome-wide significance, and we therefore implemented our functional screening strategy. Candidate genes in the vicinity of top-scoring SNPs were identified on the basis of linkage disequilibrium criteria (Table 1 and Table S2), and in each case, all such genes were included for further evaluation in an unbiased fashion. In nine out of 24 cases, no candidate genes were identified in the target genomic region around an index SNP, and these association signals were not pursued further. We additionally chose to evaluate two genomic regions that were identified by SNP associations of more modest significance but contained genes (SLIT3 [MIM 603745] and ROBO2 [MIM 602431]) that function as ligand and receptor, respectively, in a common

<table>
<thead>
<tr>
<th>SNP</th>
<th>Locus</th>
<th>Alleles</th>
<th>MAF</th>
<th>Beta (95% CI)</th>
<th>p Value</th>
<th>Human Gene(s)</th>
<th>Functional Screen</th>
</tr>
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<tbody>
<tr>
<td>rs193569</td>
<td>19q13</td>
<td>C/T</td>
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<td>0.15 (0.09 to 0.21)</td>
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<td>SPTBN4</td>
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<td>C/T</td>
<td>0.11</td>
<td>0.22 (0.12 to 0.31)</td>
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<td>PIK3C3</td>
<td>P135K9F N/A</td>
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<tr>
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<td>SLIT3</td>
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</tbody>
</table>

Alleles are denoted as minor/major. Beta is calculated per copy of minor allele under the additive genetic model with adjustment for age at death and APOE ε4 genotype. CI, confidence interval. Functional Screen shows screening results based on testing of gain or loss of function (GOF and LOF, respectively) in orthologous fly genes for enhancement (Enh) or suppression (Sup) of Tau toxicity. MAF, minor allele frequency; -, no interaction observed; N/A, genetic reagent not available. Fly orthologs were identified on the basis of implementation of the tBLASTn algorithm within the annotated Drosophila genome. All orthologs had highly significant BLAST results: e value < 10^{-18} and mean score = 398 (range: 67–1462). Fly genes with evidence of functional interactions with Tau toxicity are shown in boldface type.
neuronal signaling pathway. Nineteen out of the 22 candidate genes had conserved orthologs in Drosophila and were promoted to functional testing.

A variety of Drosophila experimental models relevant to AD have been developed, including transgenic systems based on the neurotoxicity of both Aβ and Tau.17–19 For functional screening of GWAS results, we selected the Tau transgenic model because (1) it has previously been successfully employed for rapid genetic screening20 and (2) there is growing consensus that Tau is a downstream mediator of Aβ toxicity in AD.6,21–23 Expression of human Tau (MAPT [MIM 157140]) in the Drosophila nervous system recapitulates several features of AD, including age-dependent neurodegeneration, decreased lifespan, and abnormally phosphorylated and misfolded Tau.19 We used transgenic animals, allowing tissue-specific expression of TauV337M, a mutant form of Tau associated with familial frontotemporal dementia (FTD [MIM 600274]). Importantly, wild-type and mutant forms of human Tau demonstrate similar mechanisms of toxicity when expressed in the Drosophila nervous system and show consistent interactions with known genetic modifiers.19,24,25 Therefore, similar to transgenic mouse models based on FTD mutant Tau,26,27 the fly model selected for our study is relevant to understanding the mechanisms of Tau toxicity in AD.28

TauV337M expression in the fly eye causes a moderately reduced eye size and roughened surface (Figure 1B), a phenotype that is amenable to rapid screening for second-site genetic modifiers.20 Specifically, by scoring for lines that either exacerbate or rescue the eye phenotype, genes can be characterized as enhancers or suppressors of Tau toxicity, respectively. For loss-of-function analysis, transgenic RNA-interference (RNAi) lines were tested for all 19 target genes,29,30 and classical Drosophila mutant alleles were also available in most cases.31,32 In addition, we evaluated lines known or predicted to activate Tau transgenic controls (Figure S4). TauV337M expression was a potent enhancer of Tau toxicity (Figure 1E). The Drosophila genome contains two other ELAVL2 orthologs, including the founding family member, elav, and Rbp9; however, manipulating the expression of these genes in the absence of Tau was associated with substantial toxicity, limiting further evaluation using our screening strategy. Finally, RNAi directed against three other fly genes, β-spectrin, heparan sulfate 6-O-sulfotransferase, and discs large 1, each enhance Tau toxicity, supporting functional validation of the orthologous loci implicated by our GWAS (Table 1 and Figure S2).

For the six loci highlighted by the Drosophila functional screen, we genotyped the index SNP in an additional 305 deceased study participants with completed neuropathological evaluation (Table S3). rs10845990, within the SLC2A14 locus, showed suggestive evidence of replication (p = 0.03), and the association was improved in a pooled analysis of 532 subjects, including both the discovery and the replication cohorts (pDISC = 6.9 × 10⁻⁵, pJOINT = 8.1 × 10⁻⁶). SLC2A14, encoding a glucose transporter (GLUT14), is an attractive biological candidate given the well-known dysregulation of glucose metabolism in the AD brain and likely pathogenic role of oxidative stress.6 Although predominantly expressed in the testes,35 less abundant SLC2A14 transcripts are also detected in the central nervous system, on the basis of publically available transcriptome data (see Web Resources).36–38 Glucose transporter expression has been reported to be reduced in brains affected by AD, correlated with both Tau phosphorylation and neurofibrillary tangle burden.40 Interestingly, genetic and pharmacological manipulation of oxidative stress has previously been shown to modulate Tau-induced toxicity in flies,40 potentially consistent with this mechanism of action for the observed interaction with glut1.

In summary, on the basis of genetic association in humans and functional screening in a pertinent model organism, we have identified six candidate loci that influence the accumulation of AD neuropathology. Our strategy of integrating human GWAS with a Drosophila genetic screen builds on similar successful cross-species studies in which fly models of neurodegenerative disease enabled secondary screens to reinforce findings from mammalian systems, including transcriptome analysis and drug discovery.42 The Drosophila Tau transgenic model selected for our functional screening pipeline has been used in prior successful genetic screens and numerous other investigations,20,24,25,43 and many results have been consistent with findings in mouse models and other AD experimental paradigms.28,44 In current hypotheses...
about the mechanisms of AD pathogenesis, supported by a large body of work, Tau-induced neurotoxicity defines a key pathway mediating the effects of Aβ. Therefore, our functional screen may be relevant to many susceptibility loci that influence downstream mechanisms of Aβ toxicity. Nevertheless, our approach would not be expected to detect genes that directly influence the processing of amyloid precursor protein (APP), Aβ aggregation, or other proximal events in the pathologic cascade. In the future, such loci might be functionally screened with
the use of either APP or Aβ transgenic flies or Aβ/Tau dual transgenic flies.17,24,45

Additional strengths of our approach include the substantial genomic conservation between flies and mammals46 and the availability of reagents to manipulate the function of nearly all Drosophila genes.31 The success rate of our strategy exceeds the returns of unbiased Drosophila genetic screens using the same transgenic model,20 suggesting that the list of 19 loci tested was enriched for genes influencing the development of AD pathology. Although a negative result in our screen does not exclude a gene as potentially associated with AD, the six validated loci highlight pathways of potential relevance to disease pathogenesis. Future functional investigation in Drosophila, and in other experimental systems, may reveal the mechanisms by which these genes modulate Tau-induced neurodegeneration, and these loci are also excellent targets for further replication analysis in human cohorts. Importantly, our functional screening strategy highlights genes that are likely responsible for association signals, and in two cases, rs393569 and rs10845990, we are able to nominate causal genes (SPTBN4 and SLC2A14, respectively) for which more than one candidate was initially found on the basis of linkage disequilibrium with the index SNP, a commonly encountered problem in following up GWAS results.

The association signals uncovered in our GWAS are comparable to that of numerous published reports in larger case-control cohorts that have identified candidate risk loci with suggestive but not definitive statistical evidence of association to AD or other relevant intermediate traits.1 Evidence is emerging in support of a polygenic model of inheritance for complex genetic disorders, particularly neuropsychiatric diseases, in which hundreds or even thousands of common variants collectively contribute to disease risk.3–5 Given the very small effect sizes, it is unrealistic that the majority of such loci can be validated individually by statistical evidence alone. Our strategy of coupling GWAS in humans to functional genetic screening in a model organism will therefore likely be a powerful strategy for follow-up of such signals in the future for the prioritization of genes and pathways for further investigation.

Supplemental Data

Supplemental Data include four figures and three tables and can be found with this article online at http://www.cell.com/AJHG.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

FlyBase, http://flybase.org/
Harvard Transgenic RNAi Project (TRiP), http://www.flyrna.org/TRiP-HOME.html
UCSC Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway
Vienna Drosophila RNAi Center (VDRC), http://stockcenter.vdrc.at/control/main

References


