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 *SLC2A14*, 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 ($p < 5 \times 10^{-8}$), 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 (Aß) 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 postmortem 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

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DOI 10.1016/j.ajhg.2011.01.006.

Table 1. GWAS Results and Functional Screening

SNP	Locus	Alleles	MAF	Beta (95% CI)	p Value	Human Gene(s)	Functional Screen		
							Fly Ortholog	LOF	GOF
rs393569	19q13	C/T	0.49	0.15 (0.09 to 0.21)	1.64×10^{-6}	***SPTBN4***	***B-spec***	**Enh**	-
						SHKBP1	*CG9467*	-	N/A
						LTBP4			
rs1941526	18q12	A/G	0.28	0.15 (0.09 to 0.22)	6.46×10^{-6}	*PIK3C3*	*Pi3K59F*	-	N/A
rs17468071	9p21	C/T	0.11	0.22 (0.12 to 0.31)	7.87×10^{-6}	***ELAVL2***	***fne***	**Sup**	**Enh**
rs2280861	8p21	C/T	0.25	−0.16 (−0.23 to −0.09)	1.40×10^{-5}	*ENTPD4*	*NTPase*	-	-
						SLC25A37	*mfrn*	-	-
rs10065260	5q14	C/A	0.49	0.13 (0.07 to 0.19)	2.38×10^{-5}	*SCAMP1*	*Scamp*	-	-
						LHFPL2	*CG3770*	-	N/A
rs1935502	10p12	A/G	0.30	0.15 (0.08 to 0.21)	2.66×10^{-5}	*SLC39A12*	*CG10006*	-	N/A
rs3824982	11p14	T/C	0.22	0.15 (0.08 to 0.22)	3.22×10^{-5}	*MPPED2*	*CG16717*	-	N/A
rs12378647	9q33	G/A	0.35	0.14 (0.08 to 0.21)	3.44×10^{-5}	*DBC1*			
rs16898	5q14	T/C	0.31	−0.13 (−0.19 to −0.07)	4.64×10^{-5}	*HAPLN1*			
rs2108720	7p14	T/C	0.22	−0.16 (−0.23 to −0.08)	5.23×10^{-5}	*POU6F2*	*pdm3*	-	N/A
rs527346	12p13	G/A	0.45	−0.12 (−0.18 to −0.06)	5.72×10^{-5}	*TSPAN9*	*tsp5D*	-	N/A
rs10845990	12p13	T/G	0.39	0.13 (0.06 to 0.19)	6.93×10^{-5}	***SLC2A14***	***Glut1***	**Enh**	**Sup**
						NANOG	*bsh*	-	-
rs9513122	13q32	G/A	0.43	−0.12 (−0.18 to −0.06)	1.70×10^{-4}	***HS6ST3***	***hs6st***	**Enh**	-
rs7591708	2p15	T/C	0.35	0.12 (0.06 to 0.18)	1.93×10^{-4}	*EHBP1*	*CG15609*	-	N/A
rs7128063	11q14	A/G	0.25	−0.13 (−0.20 to −0.06)	5.93×10^{-4}	***DLG2***	***dlg***	**Enh**	-
rs12634690	3p12	T/C	0.33	−0.11 (−0.17 to −0.04)	1.32×10^{-3}	*ROBO2*	*robo*	-	-
rs297808	5q35	G/A	0.36	0.09 (0.03 to 0.15)	2.60×10^{-3}	***SLIT3***	***slit***	**Enh**	**Sup**

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[50] within the annotated *Drosophila* genome. All orthologs had highly significant BLAST results: *E* value $< 10^{-10}$ and mean score = 398 (range: 67–1462). Fly genes with evidence of functional interactions with Tau toxicity are shown in boldface type.

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).[15,16] Linear regression was used to evaluate SNP associations with the continuous AD pathological trait, adjusting for both age at death and *APOE ε4* (MIM 107741) genotype. The top independently associated regions ($p < 1 \times 10^{-3}$) containing candidate genes are presented in Table 1 (for full results, see Table S2). Of note, the subjects in the study cohort were also part of a larger autopsy collection used for a recent candidate-based analysis of associations with AD pathology intermediate phenotypes;[11] however, none of the loci 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

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 Aß 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 screening[20] and (2) there is growing consensus that Tau is a downstream mediator of Aß 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 Tau^{V337M}, 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]

Tau^{V337M} 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 gene expression, allowing assessment for gain-of-function interactions for many loci.[33] Genetic modifier effects were scored with the use of a semiquantitative rating scale of rough-eye severity, allowing statistical comparison with Tau transgenic controls (Figure S4).

Out of the 19 genes evaluated in the fly model, six genes show interactions with Tau toxicity in vivo (Table 1, Figure 1, and Figure S2), providing functional evidence that strengthens the validity of the GWAS results. In three notable cases, both loss- and gain-of-function experiments demonstrate reciprocal interactions. Specifically, *SLC2A14* (MIM 611039) was selected for evaluation on the basis of an associated intronic SNP (*rs10845990*), and a single ortholog (*glut1*) is present in the *Drosophila* genome.[34] A line predicted to increase *glut1* expression was a potent Tau suppressor, restoring the eye to nearly wild-type appearance (Figure 1C), and a *glut1* RNAi line had the opposite effect, enhancing Tau toxicity and leading to a worsened eye phenotype (Figure 1F). Similarly, *SLIT3* was selected for testing on the basis of an intronic SNP, *rs297808*. Increasing expression of the orthologous fly gene, *slit*, rescues the Tau-induced eye phenotype (Figure 1D), whereas *slit* RNAi increases Tau toxicity (Figure 1G). In addition, we find evidence to support functional validation of *ELAVL2* (MIM 601673), a gene found in the vicinity of *rs17468071*. Transgene-mediated expression of found in neurons (*fne*), an ortholog of *ELAVL2,* strongly increased Tau toxicity in the fly eye (Figure 1H), and at higher levels, *fne* caused pupal lethality when coexpressed with Tau. Reciprocally, an *fne* RNAi line attenuated 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 ($p_{DISC} = 6.9 \times 10^{-5}$, $p_{JOINT} = 8.1 \times 10^{-6}$). *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.[39] 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[41] 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

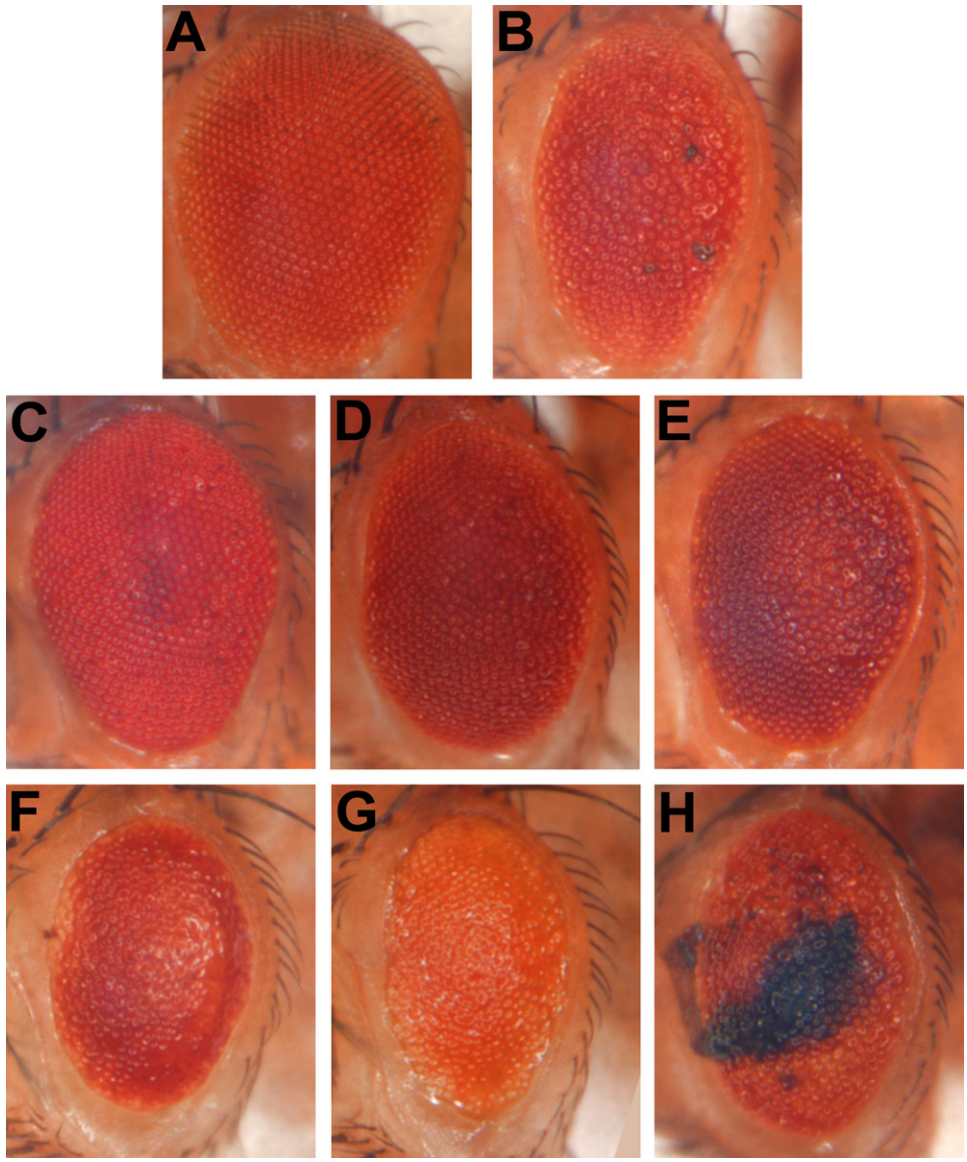

Figure 1. Functional Screening of GWAS Results, Based on Interactions of Gene Orthologs with Tau Toxicity In Vivo
Compared to control animals (A, *GMR-Gal4/+*), expression of human Tau generates a reduced eye size and moderate roughened appearance (B, *UAS-Tau^{V337M}/+; GMR-Gal4/+*).[19] Lines predicted to increase the expression of *glut1* (C, *UAS-Tau^{V337M}/+; GMR-Gal4/+; Glut1^{d05758}/+*)[33] and *slit* (D, *UAS-Tau^{V337M}/+; GMR-Gal4/+;UAS-sli.B/+*)[47] or RNAi directed against *fne* (E, *UAS-Tau^{V337M}/+; GMR-Gal4/UAS-fne.IR.v101508*) suppressed Tau toxicity, restoring a near-wild-type eye. Reciprocally, RNAi directed against *glut1* (F, *UAS-Tau^{V337M}/+; GMR-Gal4,UAS-Dcr2/UAS-glut1.IR.v13326*) and *slit* (G, *UAS-Tau^{V337M}/+; GMR-Gal4,UAS-Dcr2/UAS-slit.IR.v38233*) or increasing expression of *fne* (H, *UAS-Tau^{V337M}/+; GMR-Gal4/+;UAS-fne.4-10B/+*)[48] enhanced Tau toxicity, exacerbating the rough-eye phenotype. Spatially and temporally defined expression of the yeast GAL4 transcription factor within the *Drosophila* retina, via the *GMR-GAL4* driver line, directs Tau transgene expression from upstream activating sequence (UAS) sites. In the case of activating gain-of-function and RNAi lines for candidate genes, coexpression is also directed to the eye via the GAL4/UAS system.[49] All photographed animals are female so as to facilitate comparisons, but consistent modifier effects were observed in both sexes. All crosses used a *w^{1118}* genetic background and were conducted at 25°C, with the exception of *UAS-fne.4-10B*, which was lethal in combination with *UAS-Tau^{V337M}* at this temperature and was therefore tested at 23°C. All genetic enhancer lines were also tested in the absence of Tau to confirm that there was no significant toxicity in isolation (Figure S3). Immunoblot analysis was performed to confirm that reagents identified as modifiers of the Tau eye phenotype did not alter Tau expression levels. All genetic modifier effects were scored with the use of a semi-quantitative scale and were shown to be significantly different ($p < 0.0001$) from Tau controls (Figure S4).

about the mechanisms of AD pathogenesis, supported by a large body of work, Tau-induced neurotoxicity defines a key pathway mediating the effects of Aß.[6,21–23] Therefore, our functional screen may be relevant to many susceptibility loci that influence downstream mechanisms of Aß toxicity. Nevertheless, our approach would not be expected to detect genes that directly influence the processing of amyloid precursor protein (APP), Aß 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 Aß transgenic flies or Aß/Tau dual transgenic flies.[17,24,45]

Additional strengths of our approach include the substantial genomic conservation between flies and mammals[46] 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

We are grateful to our colleagues, Lei Yu and Sue Leurgans, for assistance with statistical analyses. We thank Christian Klambt, Jimena Sierralta, and Hiroshi Nakato for generously providing *Drosophila* stocks. We are grateful Dr. Bradley Hyman and Chris Cotsapas for comments on the manuscript and valuable discussion. We also thank the Bloomington *Drosophila* stock center, the Vienna *Drosophila* RNAi Center (VDRC), and the Harvard Transgenic RNAi Project (TRiP, NIH/NIGMS R01GM084947) for providing fly stocks. J.M.S. is supported by NIH grant K08AG034290 and by the Clinical Investigator Training Program: Beth Israel Deaconess Medical Center – Harvard/MIT Health Sciences and Technology, in collaboration with Pfizer Inc. and Merck & Co. M.B.F. is supported by the Ellison Medical Foundation. The authors also thank the participants of the Religious Orders Study and the Rush Memory and Aging Project, which were supported by NIH grants P30AG10161, R01AG15819, and R01AG17917.

Received: July 5, 2010
Revised: January 8, 2011
Accepted: January 13, 2011
Published online: February 3, 2011

Web Resources

The URLs for data presented herein are as follows:

BioGPS, http://biogps.gnf.org
FlyBase, http://flybase.org/
Harvard Transgenic RNAi Project (TRiP), http://www.flyrnai.org/TRiP-HOME.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
UCSC Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway
Vienna *Drosophila* RNAi Center (VDRC), http://stockcenter.vdrc.at/control/main

References

1. Bertram, L., and Tanzi, R.E. (2009). Genome-wide association studies in Alzheimer's disease. Hum. Mol. Genet. *18*, R137–R145.
2. Ioannidis, J.P.A., Thomas, G., and Daly, M.J. (2009). Validating, augmenting and refining genome-wide association signals. Nat. Rev. Genet. *10*, 318–329.
3. International Schizophrenia Consortium, Purcell, S.M., Wray, N.R., Stone, J.L., Visscher, P.M., O'Donovan, M.C., Sullivan, P.F., and Sklar, P. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature *460*, 748–752.
4. Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Nyholt, D.R., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W., et al. (2010). Common SNPs explain a large proportion of the heritability for human height. Nat. Genet. *42*, 565–569.
5. International Multiple Sclerosis Genetics Consortium, Bush, W.S., Sawcer, S.J., de Jager, P.L., Oksenberg, J.R., McCauley, J.L., Pericak-Vance, M.A., and Haines, J.L. (2010). Evidence for polygenic susceptibility to multiple sclerosis–the shape of things to come. Am. J. Hum. Genet. *86*, 621–625.
6. Querfurth, H.W., and LaFerla, F.M. (2010). Alzheimer's disease. N. Engl. J. Med. *362*, 329–344.
7. Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M.L., Pahwa, J.S., Moskvina, V., Dowzell, K., Williams, A., et al. (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat. Genet. *41*, 1088–1093.
8. Lambert, J.-C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M., Combarros, O., Zelenika, D., Bullido, M.J., Tavernier, B., et al. (2009). Genome-wide association study

identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat. Genet. *41*, 1094–1099.
9. Seshadri, S., Fitzpatrick, A.L., Ikram, M.A., DeStefano, A.L., Gudnason, V., Boada, M., Bis, J.C., Smith, A.V., Carassquillo, M.M., Lambert, J.C., et al. (2010). Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA *303*, 1832–1840.
10. Bennett, D.A., De Jager, P.L., Leurgans, S.E., and Schneider, J.A. (2009). Neuropathologic intermediate phenotypes enhance association to Alzheimer susceptibility alleles. Neurology *72*, 1495–1503.
11. Shulman, J.M., Chibnik, L.B., Aubin, C., Schneider, J., De Jager, P., and Bennett, D. (2010). Intermediate phenotypes identify divergent pathways to Alzheimer's disease. PLoS ONE *5*, e1124.
12. Bennett, D.A., Schneider, J., Arvanitakis, Z., Kelly, J., Aggarwal, N., Shah, R., and Wilson, R. (2006). Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology *66*, 1837–1844.
13. Bennett, D.A., Wilson, R., Schneider, J., Evans, D., Beckett, L., Aggarwal, N., Barnes, L., Fox, J., and Bach, J. (2002). Natural history of mild cognitive impairment in older persons. Neurology *59*, 198–205.
14. Bennett, D.A., Schneider, J.A., Buchman, A.S., Mendes de Leon, C., Bienias, J.L., and Wilson, R.S. (2005). The Rush Memory and Aging Project: study design and baseline characteristics of the study cohort. Neuroepidemiology *25*, 163–175.
15. Bennett, D.A., Schneider, J.A., Tang, Y., Arnold, S.E., and Wilson, R.S. (2006). The effect of social networks on the relation between Alzheimer's disease pathology and level of cognitive function in old people: a longitudinal cohort study. Lancet Neurol. *5*, 406–412.
16. Bennett, D.A., Wilson, R.S., Schneider, J.A., Evans, D.A., Aggarwal, N.T., Arnold, S.E., Cochran, E.J., Berry-Kravis, E., and Bienias, J.L. (2003). Apolipoprotein E epsilon4 allele, AD pathology, and the clinical expression of Alzheimer's disease. Neurology *60*, 246–252.
17. Finelli, A., Kelkar, A., Song, H.J., Yang, H., and Konsolaki, M. (2004). A model for studying Alzheimer's Abeta42-induced toxicity in Drosophila melanogaster. Mol. Cell. Neurosci. *26*, 365–375.
18. Moloney, A., Sattelle, D.B., Lomas, D.A., and Crowther, D.C. (2010). Alzheimer's disease: insights from Drosophila melanogaster models. Trends Biochem. Sci. *35*, 228–235.
19. Wittmann, C.W., Wszolek, M., Shulman, J., Salvaterra, P., Lewis, J., Hutton, M., and Feany, M. (2001). Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles. Science *293*, 711–714.
20. Shulman, J.M., and Feany, M. (2003). Genetic modifiers of tauopathy in Drosophila. Genetics *165*, 1233–1242.
21. Ittner, L.M., Ke, Y.D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wölfing, H., Chieng, B.C., Christie, M.J., Napier, I.A., et al. (2010). Dendritic Function of Tau Mediates Amyloid-beta Toxicity in Alzheimer's Disease Mouse Models. Cell *142*, 387–397.
22. Roberson, E.D., Scearce-Levie, K., Palop, J., Yan, F., Cheng, I., Wu, T., Gerstein, H., Yu, G., and Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science *316*, 750–754.
23. Vossel, K.A., Zhang, K., Brodbeck, J., Daub, A.C., Sharma, P., Finkbeiner, S., Cui, B., and Mucke, L. (2010). Tau Reduction Prevents Abeta-Induced Defects in Axonal Transport. Science *330*, 198.
24. Fulga, T.A., Elson-Schwab, I., Khurana, V., Steinhilb, M.L., Spires, T.L., Hyman, B.T., and Feany, M.B. (2007). Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration in vivo. Nat. Cell Biol. *9*, 139–148.
25. Khurana, V., Lu, Y., Steinhilb, M., Oldham, S., Shulman, J., and Feany, M. (2006). TOR-mediated cell-cycle activation causes neurodegeneration in a Drosophila tauopathy model. Curr. Biol. *16*, 230–241.
26. Gotz, J., Chen, F., van Dorpe, J., and Nitsch, R. (2001). Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science *293*, 1491–1495.
27. Lewis, J., McGowan, E., Rockwood, J., Melrose, H., Nacharaju, P., Van Slegtenhorst, M., Gwinn-Hardy, K., Paul Murphy, M., Baker, M., Yu, X., et al. (2000). Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. Nat. Genet. *25*, 402–405.
28. Götz, J., and Ittner, L.M. (2008). Animal models of Alzheimer's disease and frontotemporal dementia. Nat. Rev. Neurosci. *9*, 532–544.
29. Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature *448*, 151–156.
30. Ni, J.Q., Markstein, M., Binari, R., Pfeiffer, B., Liu, L.P., Villalta, C., Booker, M., Perkins, L., and Perrimon, N. (2008). Vector and parameters for targeted transgenic RNA interference in Drosophila melanogaster. Nat. Methods *5*, 49–51.
31. Matthews, K.A., Kaufman, T.C., and Gelbart, W.M. (2005). Research resources for Drosophila: the expanding universe. Nat. Rev. Genet. *6*, 179–193.
32. Tweedie, S., Ashburner, M., Falls, K., Leyland, P., McQuilton, P., Marygold, S., Millburn, G., Osumi-Sutherland, D., Schroeder, A., Seal, R., et al. (2009). FlyBase: enhancing Drosophila Gene Ontology annotations. Nucleic Acids Res. *37*, D555–D559.
33. Bellen, H.J., Levis, R.W., Liao, G., He, Y., Carlson, J.W., Tsang, G., Evans-Holm, M., Hiesinger, P.R., Schulze, K.L., Rubin, G.M., et al. (2004). The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. Genetics *167*, 761–781.
34. Escher, S.A., and Rasmuson-Lestander, A. (1999). The Drosophila glucose transporter gene: cDNA sequence, phylogenetic comparisons, analysis of functional sites and secondary structures. Hereditas *130*, 95–103.
35. Wu, X., and Freeze, H.H. (2002). GLUT14, a duplicon of GLUT3, is specifically expressed in testis as alternative splice forms. Genomics *80*, 553–557.
36. Rhead, B., Karolchik, D., Kuhn, R.M., Hinrichs, A.S., Zweig, A.S., Fujita, P.A., Diekhans, M., Smith, K.E., Rosenbloom, K.R., Raney, B.J., et al. (2010). The UCSC Genome Browser database: update 2010. Nucleic Acids Res. *38*, D613–D619.
37. Su, A.I., Cooke, M.P., Ching, K.A., Hakak, Y., Walker, J.R., Wiltshire, T., Orth, A.P., Vega, R.G., Sapinoso, L.M., Moqrich, A., et al. (2002). Large-scale analysis of the human and mouse transcriptomes. Proc. Natl. Acad. Sci. USA *99*, 4465–4470.
38. Wu, C., Orozco, C., Boyer, J., Leglise, M., Goodale, J., Batalov, S., Hodge, C.L., Haase, J., Janes, J., Huss, J.W., et al. (2009). BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol. *10*, R130.
39. Liu, Y., Liu, F., Iqbal, K., Grundke-Iqbal, I., and Gong, C.-X. (2008). Decreased glucose transporters correlate to abnormal

hyperphosphorylation of tau in Alzheimer disease. FEBS Lett. *582*, 359–364.
40. Dias-Santagata, D., Fulga, T.A., Duttaroy, A., and Feany, M.B. (2007). Oxidative stress mediates tau-induced neurodegeneration in Drosophila. J. Clin. Invest. *117*, 236–245.
41. Desai, U.A., Pallos, J., Ma, A.A.K., Stockwell, B.R., Thompson, L.M., Marsh, J.L., and Diamond, M.I. (2006). Biologically active molecules that reduce polyglutamine aggregation and toxicity. Hum. Mol. Genet. *15*, 2114–2124.
42. Karsten, S.L., Sang, T.-K., Gehman, L.T., Chatterjee, S., Liu, J., Lawless, G.M., Sengupta, S., Berry, R.W., Pomakian, J., Oh, H.S., et al. (2006). A genomic screen for modifiers of tauopathy identifies puromycin-sensitive aminopeptidase as an inhibitor of tau-induced neurodegeneration. Neuron *51*, 549–560.
43. Blard, O., Feuillette, S., Bou, J., Chaumette, B., Frebourg, T., Campion, D., and Lecourtois, M. (2007). Cytoskeleton proteins are modulators of mutant tau-induced neurodegeneration in Drosophila. Hum. Mol. Genet. *16*, 555–566.
44. Moloney, A., Sattelle, D.B., Lomas, D.A., and Crowther, D.C. (2009). Alzheimer's disease: insights from Drosophila melanogaster models. Trends Biochem Sci. *35*, 228–235.
45. Fossgreen, A., Bruckner, B., Czech, C., Masters, C., Beyreuther, K., and Paro, R. (1998). Transgenic Drosophila expressing human amyloid precursor protein show gamma-secretase activity and a blistered-wing phenotype. Proc. Natl. Acad. Sci. USA *95*, 13703–13708.
46. Rubin, G.M., Yandell, M., Wortman, J., Gabor Miklos, G., Nelson, C., Hariharan, I., Fortini, M., Li, P., Apweiler, R., Fleischmann, W., et al. (2000). Comparative genomics of the eukaryotes. Science *287*, 2204–2215.
47. Battye, R., Stevens, A., and Jacobs, J.R. (1999). Axon repulsion from the midline of the Drosophila CNS requires slit function. Development *126*, 2475–2481.
48. Samson, M.L., and Chalvet, F. (2003). found in neurons, a third member of the Drosophila elav gene family, encodes a neuronal protein and interacts with elav. Mech. Dev. *120*, 373–383.
49. Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.
50. Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. *25*, 3389–3402.