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Drosophila and experimental neurology in the post-genomic era

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Abstract

For decades, the fruit fly, *Drosophila melanogaster*, has been among the premiere genetic model systems for probing fundamental neurobiology, including elucidation of mechanisms responsible for human neurologic disorders. Flies continue to offer virtually unparalleled versatility and speed for genetic manipulation, strong genomic conservation, and a nervous system that recapitulates a range of cellular and network properties relevant to human disease. I focus here on four critical challenges emerging from recent advances in our understanding of the genomic basis of human neurologic disorders where innovative experimental strategies are urgently needed: (1) pinpointing causal genes from associated genomic loci; (2) confirming the functional impact of allelic variants; (3) elucidating nervous system roles for novel or poorly studied genes; and (4) probing network interactions within implicated regulatory pathways. *Drosophila* genetic approaches are ideally suited to address each of these potential translational roadblocks, and will therefore contribute to mechanistic insights and potential breakthrough therapies for complex genetic disorders in the coming years. Strategic collaboration between neurologists, human geneticists, and the *Drosophila* research community holds great promise to accelerate progress in the post-genomic era.

Keywords

Drosophila melanogaster; Animal model; Genomics; Genome-wide association study; Exome sequencing; Epistasis; Neurology; Complex genetics; Mendelian disease

While experimentation in the fruit fly *Drosophila melanogaster* has contributed to many profound insights on heritability and neuroscience over the last hundred years (Bellen et al., 2010), that flies could serve as useful genetic models for human disease is a rather new concept. Based on PubMed search criteria, a 1971 study in *Acta Neuropathol* was among the first to highlight the potential for direct "modeling" of human nervous system injury in flies, based on observations of age-related changes in the fly brain similar to established human neuropathologic findings (Herman et al., 1971). It is striking that from this earliest conception of *Drosophila* disease models, applications were specifically imagined in experimental neurology. Indeed, numerous subsequent reports heralded fly models for Huntington's disease (Jackson et al., 1998), Spinocerebellar Ataxia (Fernandez-Funez et al., 2000), Alzheimer's disease (Finelli et al., 2004;Wittmann et al., 2001), and Parkinson's

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disease (Feany and Bender, 2000), among other applications (Shulman et al., 2003). This rapid progress was enabled by the relative ease of *Drosophila* transgenesis, the availability of versatile, targeted expression systems (Brand and Perrimon, 1993), and contemporaneous discoveries of human genes responsible for autosomal dominant, familial forms of neurodegeneration with toxic gain-of-function mechanisms. The resulting fly models have contributed enormously to our understanding of neurologic disorders and continue to spur mechanistic insights, as reviewed previously (Bellen et al., 2010; Jaiswal et al., 2012; Lessing and Bonini, 2009; Shulman et al., 2003) and discussed elsewhere in this special issue.

In recent years, however, powerful methods for gene manipulation have become available in mammalian models, including conditional knockout strategies, optogenetics, and genome-editing technology. Further, advances in induced-pluripotent stem (iPS) cell methods now permit modeling of disease biology directly in human patient-derived neurons. Therefore, for many applications in experimental neurology, flies no longer offer all the unique advantages they once did. Importantly, there has also been a paradigm shift from a simple to amore complex genetic framework for understanding common neurologic conditions. In contrast to Mendelian diseases characterized by single-gene etiologies, complex genetic disorders are defined by substantial heterogeneity and polygenicity. Although the precise genomic architectures remain to be fully elucidated, we now appreciate that most common neurologic diseases (e.g., migraine, stroke, epilepsies, multiple sclerosis, and neurodegenerative conditions) are likely influenced by a combination of many common and rare genomic variants with a range of effect sizes. Based on the current rapid rate of progress, we are beginning to have a glimpse of the “post-genomic era,” when the majority of genes or genomic loci responsible for most neurologic conditions are known. While this is an exciting prospect, it also presents a number of unprecedented challenges (Chakravarti et al., 2013) and is creating an urgent need for new experimental models and approaches. Thus, in this altered landscape, what will be the future role of *Drosophila* in experimental neurology? The primary goal of this review is to address this question, and I will argue that *Drosophila* is ideally suited to tackle many of the key emerging obstacles. I organize my discussion around four major problems arising from current human genomic studies, drawing on recent examples to illustrate how flies can offer potential solutions. Overcoming each of these roadblocks will be essential for moving from genomic discoveries to clinical applications. At the conclusion, I propose how multi-disciplinary teams including many in the *Drosophila* research community will ensure sustained momentum for effective translational research in neurogenomics.

1. From susceptibility locus to causal gene

Over the last decade, genome-wide association studies (GWAS) have identified thousands of genomic loci (http://www.genome.gov/gwastudies/) that contribute to common and complex human genetic traits (Welter et al., 2014), including many neurologic and neuropsychiatric disorders. This successful strategy has begun to reveal genetic determinants for many conditions that long were relatively resistant to genetic dissection, including ischemic stroke (Kilarski et al., 2014), migraine (Anttila et al., 2013), Alzheimer’s disease (Lambert et al., 2013), Parkinson’s disease (Nalls et al., 2014), multiple sclerosis (International Multiple

Sclerosis Genetics Consortium IMSGC et al., 2013), and schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), among others. While the odds ratios for many such loci are modest, they are estimated to have broad population impact on disease risk since most are defined by common sites of genomic variation [single nucleotide polymorphisms (SNPs) with minor allele frequencies of >1%]. Most signals discovered by GWAS do not unambiguously define the responsible causal genes but rather implicate a broad genomic region tagged by associated SNPs (Fig. 1). Such regions usually contain a number of candidate genes. Thus, experimental confirmation is critical to move beyond an “educated guess” to a validated target for further mechanistic investigation.

Based on several studies, flies provide an outstanding platform for functional validation of genes from GWAS, and this approach has already shown success for neurologic (Chapuis et al., 2013;Macleod et al., 2013; Shulman et al., 2011, 2014) as well as non-neurologic disorders (Hoed et al., 2013; van der Harst et al., 2012). Given the strong conservation between the human and *Drosophila* genomes (Fortini et al., 2000; Rubin et al., 2000), the majority of candidate genes at associated loci can usually be tested in flies. In fact, evolutionary conserved genes appear more likely to be implicated in disease (Hu et al., 2011). Further, resources are immediately available to facilitate manipulation of virtually all such candidates (Matthews et al., 2005), including genome-wide RNA-interference (RNAi) transgenic stocks (Dietzl et al., 2007; Ni et al., 2011), classical mutant alleles including transposable element insertions from the gene disruption project (Bellen et al., 2011), and strains that facilitate gene overexpression in many cases (Bischof et al., 2013; Jenett et al., 2012). Coupled with the many established transgenic neurologic disease models (Jaiswal et al., 2012; Shulman et al., 2003), the array of available *Drosophila* genetic reagents is a potent combination for medium- to high-throughput functional validation of gene candidates nominated by GWAS. The underlying hypothesis for this strategy is that many susceptibility alleles identified by GWAS modulate mechanisms of pathogenesis recapitulated in fly models. In our recent study (Shulman et al., 2014), we examined whether genetic manipulation of fly homologs of candidate genes at susceptibility loci from Alzheimer’s disease GWAS (Hollingworth et al., 2011; Lambert et al., 2013; Naj et al., 2011; Seshadri et al., 2010) interact with the neurotoxicity of human Tau protein, responsible for the characteristic neurofibrillary tangle pathology. RNAi-mediated knockdown of fly homologs of *CD2AP*, *FERMT2*, and *CELF1* each enhanced retinal degeneration in transgenic models expressing human Tau, and in the case of *FERMT2* and *CELF1*, overexpression reciprocally suppressed Tau toxicity. At the *CELF1* locus, for example (Fig. 1), 8 out of 10 gene candidates within the implicated genomic region were conserved, and only manipulation of *aret*, the fly ortholog of *CELF1*, demonstrated modifier activity. Besides highlighting the most likely causal gene for GWAS signals, these results also provide important clues to potential mechanisms, suggesting that the implicated susceptibility loci may impact Tau-mediated neurodegeneration. Using similar experimental strategies, two other candidate-based studies recently provided support for functional validation of *BIN1* in Alzheimer’s disease (Chapuis et al., 2013) and the *RAB7L1* gene at the *PARK16* locus in Parkinson’s disease (Macleod et al., 2013) (discussed further below).

Despite the successes, there are some notable limitations to the generalizability of the described strategy. Only about 60–70% of candidate human disease genes are conserved,

based on cross-species comparisons (Fortini et al., 2000;Hu et al., 2011). At some genomic loci implicated in GWAS, there are no obvious gene candidates to test at all. In other cases, there can potentially be a very large number of candidates, especially if one accounts for the possibility of regulatory variants with long-range impact on gene expression. In addition, the described strategy relies on available fly disease models that can be deployed for modifier screening. Many but certainly not all neurologic disorders have useful transgenic models, and even where such models are available, they only permit probing the potential impact of candidate susceptibility genes on selected aspects of disease biology. Nevertheless, fly genetics shows great promise for accelerating the fine mapping of many susceptibility loci from GWAS, pinpointing likely causal genes and linking them to disease-relevant mechanisms.

2. Confirming the functional impact of genetic variants

Next-generation sequencing technology has revolutionized the discovery of genes responsible for familial neurologic disorders with Mendelian inheritance (Bamshad et al., 2011) and is beginning to successfully define less common and rare variants that contribute to many neurologic diseases with complex genetic inheritance (Pittman and Hardy, 2013). In contrast to GWAS-defined susceptibility loci, sequencing-based approaches are capable of more precisely defining the most likely causal genes and variants when performed in sufficiently large family pedigrees or population samples (Goldstein et al., 2013; MacArthur et al., 2014). The discovery pipeline for sequencing studies incorporates analytic filters to distinguish variants with anticipated deleterious consequences from those that are benign. While some variants are obviously damaging (nonsense mutations, splicing mutations, or insertions/deletions that cause frameshifts), others cause non-synonymous amino acid changes of uncertain functional significance. Numerous bioinformatic algorithms (Goldstein et al., 2013; Liu et al., 2013) facilitate prediction of functional consequences for such variants. However, direct experimentation is critical for confirming the pathogenicity of identified mutations, understanding how such changes disrupt gene functions, and defining the broader role of the encoded protein in the nervous system context. For conserved genes, flies remain an outstanding animal model to explore the functions of genetic variants.

One recent study that illustrates the power of this approach began with an unusual, three-generation family pedigree with an autosomal dominant disorder consisting of variable limb weakness and electrophysiologic evidence of presynaptic neuromuscular junction failure (Herrmann et al., 2014). Whole-exome sequencing of three individuals followed by stringent analytic filters and segregation analysis in additional family members narrowed an initially unwieldy variant list to a missense mutation in the *synaptotagmin II* gene (*SYT2*), encoding a key mediator of synaptic vesicle exocytosis. The candidate variant fell within a conserved calcium-binding domain and was predicted to be deleterious to protein function using bioinformatics. To confirm this finding and reveal the potential mechanisms, the corresponding mutation was introduced in the single *Drosophila* synaptotagmin gene, *DSyt1*, and expression of the mutant transgene was directed throughout the nervous system. Interestingly, whereas mutant DSyt1 was unable to rescue $DSyt1^{-/-}$ null animals, consistent with reduced function, expression of the mutant protein in a wild-type genetic background also disrupted synaptic transmission. Overall, these data suggest a dominant-negative

mechanism for this human disorder and demonstrate how flies can be leveraged to rapidly dissect the functional consequences of missense variants in conserved genes. Indeed, understanding the mechanism of disease-associated mutations is often of critical importance to progress from a disease gene to potential therapies. For example, potential loss-of-function changes that reduce risk of disease (i.e., protective variants) may identify excellent candidates for potential drug targets.

With whole-exome sequencing becoming an increasingly available tool, both in the research (Bamshad et al., 2011) and clinical (Yaping Yang et al., 2013) settings, we can expect exponential growth in the pace of variant discovery underlying diverse neurologic disorders. In fact, large National Institutes of Health (NIH)-funded efforts are now under way with the goal of “solving” as many Mendelian disorders as possible (Bamshad et al., 2012). *Drosophila* is a powerful platform for accelerating the functional follow-up of promising variants emerging from such studies. As mentioned, genome-wide RNAi transgenic stocks (Dietzl et al., 2007; Ni et al., 2011) and large, publicly available collections of mapped transposon insertion alleles (Bellen et al., 2011) enable rapid genetic analysis of nearly all *Drosophila* genes. Other resources and technologies, including bacterial artificial chromosome libraries covering the fly genome (Venken et al., 2009) and the more recently developed CRISPR/Cas9 system (Bassett et al., 2013; Gratz et al., 2013; Kondo and Ueda, 2013; Yu et al., 2013), offer limitless flexibility for genomic manipulation in flies (Venken et al., 2011). One generalizable strategy for functional validation of a novel variant linked to human disease begins with characterization of the loss-of-function phenotype for the conserved gene homolog in flies. Importantly, any robust phenotype (e.g., lethality) can potentially serve as a substrate for variant functional analysis. Next, rescue experiments can be implemented comparing the activities of the wild-type gene or versions harboring disease-implicated variants. Such experiments can also be conducted using human cDNAs to demonstrate cross-species functional substitution. Where sufficient sequence conservation allows, corresponding mutations can additionally be engineered into the context of a fly cDNAor genomic rescue construct (or endogenous genomic locus using CRISPR). This cross-species strategy permits efficient characterization of variants of unknown significance, delineating those that alter protein function consistent with damaging, loss-of-function alleles versus other potential mechanisms (e.g., gain-of-function and/or dominant-negative). Such studies would also be a prelude to more detailed functional investigation in the relevant nervous system context.

3. Linking conserved genes to disease-relevant biology

Unbiased human genomic studies, whether GWAS or sequencing, often lead to candidate genes with little or no prior functional studies to support mechanistic hypotheses of disease pathogenesis. In the case of evolutionarily conserved genes, *Drosophila* remains a premiere system for efficient investigation of the consequences of gene loss-of function. In some cases, phenotypic consequences can reveal remarkable and unexpected parallels with the human disease. One illustrative example comes from a study of the *Drosophila dBTBD9* gene (Freeman et al., 2012), homologous to a candidate gene from human GWAS in Restless Legs Syndrome(RLS) (Stefánsson et al., 2007). RLS is a common neurologic condition characterized by bothersome nighttime, sensory symptoms of the lower extremities that

disrupt sleep, often forcing affected individuals out of bed to pace about for relief. Besides the strong association of an intronic *BTBD9* SNP with RLS in humans, little was previously known to connect this gene to informative, disease biology. Based on public databases, *BTBD9* showed fairly widespread tissue expression in mammals and sequence analysis revealed only a conserved, BTB protein interaction domain. To learn more, Freeman et al. (2012) generated a null allele of *dBTBD9* in *Drosophila* through imprecise excision of an available transposon insertion stock, and characterized the resulting loss-of-function phenotype. Remarkably, $dBTBD9^{-/-}$ flies exhibited disrupted and fragmented sleep and a hyperlocomotor phenotype that resembles restlessness. In further studies, the authors found that loss-of-*dBTBD9* function is associated with reduced brain dopamine levels and altered iron homeostasis, both of which have been implicated in the pathophysiology of human RLS (e.g., iron supplementation and dopamine agonists are both employed as effective therapies).

The *BTBD9* story exemplifies the potential power of "reverse genetic analyses" in flies—the targeted characterization of loss-of-function phenotypes for genes initially implicated by other studies (i.e., in humans). The diversity of available reagents and methods for targeted gene knockdown makes this an attractive strategy for probing functions of genes implicated in disease. In at least some cases, such manipulations unexpectedly lead to phenotypes that recapitulate key features of disease, as in RLS. Moreover, the reduced genetic redundancy within *Drosophila*, that is the number of gene paralogs that can potentially functionally substitute for one another, often accelerates investigation of gene functions in this system. Therefore, a single genetic knockdown can frequently provide answers where more time-consuming double- or triple-knockdown would be required in vertebrate models. Finally, a variety of experimental strategies are available to facilitate tissue-specific and/or conditional knockdown. Indeed, many genes with adult nervous system functions are expected to have earlier, developmental roles or to mediate similar essential functions in non-neuronal tissues. In such cases, fly experimental approaches enable the genetic analysis of nervous system phenotypes, including within the adult, aging animal, which is the most relevant context for many adult-onset, progressive neurologic disorders. Available strategies include classical approaches such as the generation of genetic mosaic tissues, consisting of labeled homozygous mutant cell clones—including in the brain (Lee and Luo, 2001)—in an otherwise heterozygous animal. Besides RNAi transgenic lines, newer strategies targeting GFP-tagged transcripts (Neumüller et al., 2012; Pastor-Pareja and Xu, 2011) or proteins (Caussinus et al., 2011) allow precise spatial and temporal control as well as reversible knockdown. Importantly, the *Drosophila* gene disruption project (Jaiswal et al., in press) will generate GFP-tagged stocks for thousands of genes in the coming years, including prioritization of human disease gene homologs.

Despite the ease and potential power of reverse genetic approaches, *Drosophila* has historically excelled at "forward genetic" investigations (St Johnston, 2002; Venken and Bellen, 2014)—the identification of genes based on unbiased screening for selected mutant phenotypes. Indeed, genetic screens in flies have generated innumerable insights with relevance to diverse neurologic disorders (Bellen et al., 2010; Lessing and Bonini, 2009). In the post-genomic era, there is great potential for forward genetics to even more directly inform the discovery and functional elucidation of genes responsible for human disease. For example, based on the results of a large-scale, chemical mutagenesis screen, Yamamoto et

al. (2014) recently reported on 165 essential genes with secondary requirements for neuronal development, function, and/or maintenance, many of which had never before been examined. Importantly, these genes were highly enriched for evolutionarily conserved loci, a third of which were previously linked to human disease in the Online Mendelian Inheritance in Man database (http://omim.org/). In order to determine if the screen may have also identified novel disease genes, the investigators teamed with the Baylor-Hopkins Centers for Mendelian Genomics, a large-scale effort to use next-generation sequencing technology to identify unsolved Mendelian genetic disorders (Bamshad et al., 2012). In an innovative cross-species analysis, the nervous system functional annotation of genes from the *Drosophila* screen were integrated with data from whole-exome sequencing in nearly 2,000 human subjects. Remarkably, the results of this joint analysis helped define likely causal genes/variants in several families (Yamamoto et al., 2014). For example, three cases of bull's eye maculopathy, a late-onset, progressive retinal disorder, were associated with dominant mutations in the human *CRX* gene, based in part on the complementary discovery that mutations in the fly *CRX* homolog, *ocelliless*, led to age-dependent disruptions in the electroretinogram. In another compelling case, loss-of-function of a previously uncharacterized fly gene, *l(1)G0222* (*dANKLE2*), disrupted development of thoracic sensory bristles, and mutations in the human homolog, *ANKLE2*, were associated with a recessively-inherited microcephaly syndrome. Subsequent detailed phenotypic characterization further demonstrated that loss-of-*dANKLE2* function leads to diminished neuroblast numbers and concomitantly decreased larval brain size. In prior related work (Bayat et al., 2012; Neely et al., 2010), *Drosophila* forward genetic screening identified mutant phenotypes (*straightjacket* and *Aats-met*) prompting follow-up human genetic studies that highlighted conserved roles of the homologous loci (*MARS2* and *CACNA2D3*) in neuronal maintenance and nociception, respectively.

These studies highlight the urgent need for comprehensive and systematic functional annotation of evolutionary conserved genes for possible roles in the nervous system, perhaps using a combination of reverse and forward genetic strategies in *Drosophila*. With such a valuable community resource, any gene implicated from a human genomic study could be instantly cross-referenced in a convenient online database, such as FlyBase (St Pierre et al., 2014). Such functional annotation might provide critical clues to support fine mapping of an associated locus from GWAS (From Susceptibility Locus to Causal Gene section) or to enhance confidence in a gene with potential loss-of-function variants from a family or population-based sequencing study (Confirming the Functional Impact of Genetic Variants section). But what phenotypes should such large-scale screening efforts focus on? One potential strategy is to generate flies with nervous system phenotypes that mimic human neurologic disorders. While reflexively satisfying, this approach is based on a potentially misguided assumption that most genes, including those highly conserved throughout evolution, will necessarily generate similar phenotypes when disrupted in flies as in humans. With this problem in mind, Marcotte and colleagues proposed the powerful concept of "phenologs," or homologous phenotypes (McGary et al., 2010; Woods et al., 2013). Using bioinformatic approaches incorporating available model organism data, these investigators mapped human disease-associated orthologous gene sets with less obvious, homologous phenotypes in other species. For example, a statistically significant overlap was discovered

between genes causing breast and ovarian cancer in humans and the high incidence of male progeny in the nematode, *Caenorhabditis elegans,* among many other similar and striking comparisons. While this study did not consider *Drosophila*, an extension of this successful approach has the potential to redefine the nature of fly disease models in the future. Rather than emphasizing the particular clinical and pathologic manifestations characteristic of human disease, this provocative work teaches us to embrace the species-specific consequences emerging from dysfunction in conserved molecular modules. Besides providing a potential path to new "disease models," the phenolog conceptual framework provides an attractive approach to implicate new disease genes. Specifically, once the phenolog for a given human disease is defined, one can mount screens for mutants causing similar phenotypes, thereby identifying additional genes that may participate in common functional pathways.

4. From genes to pathways and networks

Many complex genetic disorders are believed to be polygenic, potentially arising from gene–gene (and gene–environment) interactions among a large number of susceptibility loci. This model contrasts with the single-gene etiologies underlying many simple Mendelian disorders. Grouping genes into functional pathways and networks, and probing the logic of their interactions, is therefore a major goal for understanding the pathophysiology of many common neurologic diseases with complex genetic architectures (Chakravarti et al., 2013). A major advantage of *Drosophila* is the capability to efficiently map epistatic, additive, synergistic, or antagonistic interactions by examining the phenotypic consequences of multiple gene manipulations in combination. Such pairwise genetic interaction experiments facilitate the reconstruction of linear models for serial gene action. In one notable example relevant to autosomal recessive juvenile-onset parkinsonism, studies of fly genes homologous to human *PARK2/parkin* and *PARK6/pink1* demonstrated that the encoded proteins function in a sequential regulatory pathway impinging on mitochondrial dynamics (Clark et al., 2006; Deng et al., 2008; Greene et al., 2003; Park et al., 2006; Poole et al., 2008; Yufeng Yang et al., 2008). In more recent work also related to Parkinson's disease, MacLeod et al. (2013) used flies to validate interactions between *LRRK2* and the *PARK16* locus, initially suggested from analyses of large-scale human genomic and transcriptomic data sets. In *Drosophila*, the expression of the Parkinson's disease-associated LRRK2^{G2019S} mutant protein led to reduced survival and loss of dopaminergic neurons, and both phenotypes were suppressed by co-expression of a constitutively active form of RAB7L1 protein, encoded by a candidate gene at the *PARK16* locus identified in GWAS (Nalls et al., 2014). Loss-of-function mutations in *lightoid*, the fly homologue of *RAB7L1*, also caused reduced dopaminergic neuron counts. Interestingly, additional experiments further suggested that the LRRK2-RAB7L1 pathway impinges on another Parkinson's disease gene product, VPS35 (Vilariño-Güell et al., 2011; Zimprich et al., 2011), which regulates the sorting of lysosomal substrates. In flies, MacLeod et al. found that knockdown or overexpression of the *VPS35* homolog reciprocally enhanced or suppressed mutant LRRK2-induced dopaminergic neuron toxicity, respectively. These elegant experiments integrate human genomic data and studies in *Drosophila* to interlink susceptibility genes from GWAS

(PARK16/RAB7L1) and sequencing studies (LRRK2, VPS35) into a unified functional pathway.

Despite the power of relatively simple pathway models for informing hypothesis-based investigation, such a conceptual framework may be ill-equipped to fully explain the pathophysiologic mechanisms underlying most complex genetic diseases, where widespread gene and allelic heterogeneity, incomplete penetrance, and polygenicity predominate (Chakravarti et al., 2013). The potential challenge is illustrated by a recent study focused on Hereditary Spastic Paraplegias (HSPs), a heterogeneous group of disorders characterized in part by corticospinal tract dysfunction and progressive lower limb weakness. Although mutations in greater than 20 distinct genes have been implicated to cause HSP, the majority of cases remain unexplained. Novarino et al. (2014) performed whole-exome sequencing on more than 90 individuals from 60 families with autosomal recessive HSP, identifying candidate mutations in 15 new genes. For confirmation, many of the genes were replicated in an independent HSP cohort, and functional validation of several other loci was pursued using zebrafish models. The investigators then developed a protein interaction network based on all previously established and newly identified HSP genes (Fig. 2). This “interactome” model identifies several highly conserved subnetworks in HSP pathogenesis, including proteins involved in endoplasmic reticulum biology, endosomal/membrane trafficking, and purine metabolism. Similar large-scale sequencing projects are now in progress for many other neurologic disorders, including Alzheimer’s disease, Parkinson’s disease, and epilepsies, among others. Due to the large number of new candidate genes likely to arise from these studies, there is a pressing need for new experimental strategies to test the predictions emerging from the resulting disease networks. Even more intricate models are on the horizon, as systems biology approaches (Civelek and Lusis, 2014) are applied toward integration of large-scale genomic data sets with complementary transcriptomic, proteomic, metabolomic, and epigenomic surveys conducted in neurologic patients. Unlike genomic variation, changes in other “-omic” data (e.g., the transcriptome) can be either a proximate cause of disease or a more distal, secondary effect (e.g., a biomarker). Fly models may be useful for pinpointing network features representing primary causes and decoding whether observed changes are pathogenic or rather compensatory, and potentially protective.

Currently prevailing approaches in flies and most other experimental systems predominantly investigate the consequences of isolated gene manipulations, ignoring the likelihood that majority of genetic variants probably act in combinations. In budding yeast, for example, a systematic study of more than 5 million pairwise interactions among ~1,700 genes identified ~170,000 interactions affecting cellular fitness (Costanzo et al., 2010). This study highlights the pervasive role that genetic interactions play in the heritability and expressivity of complex phenotypes. While many of the *Drosophila* experimental methodologies introduced earlier may be adaptable for probing gene–gene interactions, further innovations will also be needed to enable similar genome-wide combinatorial analyses that are currently feasible only in yeast or cell-based models. Even experimental strategies for pairwise testing of gene interactions within implicated functional pathways constitute an oversimplification. The phenotypic consequences of many genetic variants are probably influenced by higher-order interactions within large-scale disease regulatory networks comprising the “genetic background.” One innovative approach that may lead to advances in functional dissection of

large-scale gene and protein interaction networks comes from studies of the "*Drosophila* Genetic Reference Panel" (DGRP) (Huang et al., 2012; Mackay et al., 2012). This remarkable resource includes 205 distinct inbred *Drosophila* strains that have been comprehensively sequenced to generate a reference map of naturally occurring genomic variation. There is already evidence to support a shared genomic architecture between flies and humans for selected phenotypes; for example, an analysis of fly sleep using the DGRP identified gene candidates homologous to loci implicated in human sleep disorders (Harbison et al., 2013). In the future, it is possible that similar population genetic experimental approaches might elucidate large-scale genomic regulatory networks with pleiotropic effects on nervous system traits and potentially conserved roles from flies to humans.

5. Conclusions

In sum, there is tremendous potential for *Drosophila* experimentation to confront challenges in the post-genomic era. However, no single experimental model species is likely to provide the complete solution. Indeed, there are some dimensions to neurologic disease, where cross-species genomic and biologic conservation is more limited. For example, besides autoimmune disorders, such as multiple sclerosis, there is now increasing evidence that immune and inflammatory mechanisms play important roles in primary neurodegenerative conditions, including Alzheimer's and Parkinson's disease. Because *Drosophila* lack adaptive immunity, modeling T- or B-cell related processes is not possible. However, flies do possess an innate immune system with rich conservation of many immune-related signaling systems, including the Toll-NFkB pathway that was originally elucidated in flies (Lemaitre, 2004). Moreover, recent studies of fly brain glial subpopulations have defined a type of ensheathing glia that can mediate phagocytic responses to neuronal injury similar to mammalian microglia (Doherty et al., 2009). Another potential limitation of fly models relate to diseases or selected disease manifestations that arise from brain network properties that are found only in higher vertebrates. For example, many of the defining features of movement disorders, including basal ganglionic (tremor, dyskinesia) or cerebellar (ataxia) dysfunction, relate to emergent properties of neural circuits that are simply not present in flies. In fact, many neurologic and neuropsychiatric disorders remain "functional disorders," in which the neuroanatomic and pathologic substrates remain poorly defined. Examples include dystonia, essential tremor, autism spectrum disorders, major depression, and schizophrenia. As discussed earlier however, many of the implicated genes and functional pathways underlying such conditions may in fact be evolutionary conserved; therefore mapping the corresponding homologous loss-of-function phenotypes in flies may be a successful strategy for probing relevant mechanisms [see earlier discussion of "phenologs" (McGary et al., 2010; Woods et al., 2013)].

Given the scope of the challenges outlined above, a successful functional genomics strategy with broad applicability to diverse neurologic disorders will likely require contributions from numerous complementary experimental models. For first-pass screening of large lists of candidate genes/variants, truly high-throughput approaches, including *in silico*, *in vitro*, or cell-based genetic systems (e.g., yeast, iPS cells, etc.), are attractive. Importantly, while human iPS cell strategies show remarkable promise for disease modeling, many critical

aspects of neurologic disorders, including properties of neural networks, interactions between neurons and glia, systemic influences on nervous system function (e.g., from the endocrine or cardiovascular system), as well as the essential role of aging, may be difficult if not impossible to recapitulate in cell-based systems. Thus, *Drosophila* along with the nematode, *C. elegans*, and zebrafish offer an intermediate platform, bridging *in vitro* and *ex vivo* systems to more time-intensive mammalian genetic models, such as mouse, rat, or primates. Ultimately, the confirmation and functional elucidation of genes and genomic variants responsible for neurologic disease, including dissection of the gene–gene interaction networks, will require unprecedented cooperation among investigators with varied skills, including neurologists, computational/systems biologists, and both human and model organism geneticists, among others. Many of the cross-disciplinary studies discussed above point to examples for how such collaborations can be leveraged effectively. In recent years, large human genomics consortia have formed to successfully pursue gene discovery in neurology, and it stands to reason that such organizations might naturally evolve next to grapple with the urgently needed functional investigations. Funders, including the NIH, will likely also serve an important organizing and enabling role. Compared with the deep investment in technology and resources for gene discovery, the field still needs to be effectively mobilized for the essential subsequent translational research. Reason for hope comes from the Undiagnosed Disease Network (http://commonfund.nih.gov/Diseases/), which began as an NIH intramural initiative to help patients with unknown diseases, and was recently expanded to include partnerships among several academic medical centers. This effort draws together diverse clinical and genomics expertise, given the expectation that many rare/undiagnosed conditions will have genetic underpinnings. Importantly, a model organism screening component for functional follow-up studies was also recently announced, including a pivotal role for flies. Similar national and worldwide efforts, including deep engagement of the *Drosophila* research community, will likely be needed to sustain the current momentum in our progress toward understanding the mechanisms responsible for neurologic disorders in the post-genomic era.

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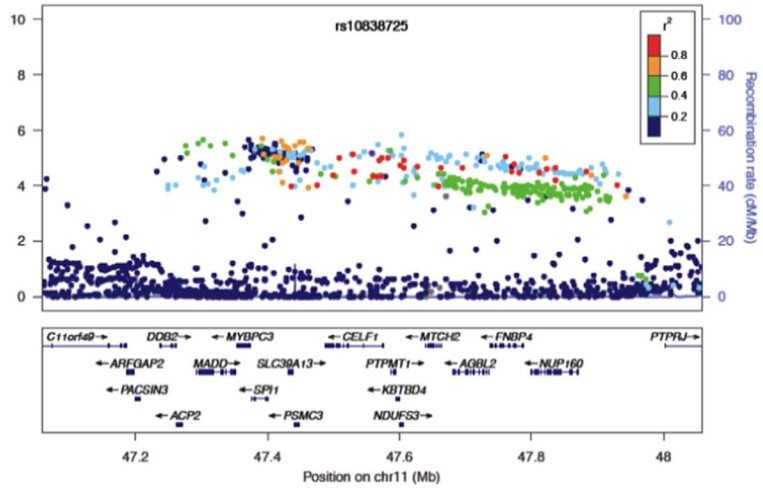

Fig. 1.

Multiple gene candidates implicated at an Alzheimer's disease susceptibility locus. Regional association plot is shown from the GWAS discovery phase, with −log(P-value) for each SNP plotted on the left (Lambert et al., 2013). Following joining with replication phase data (not shown), the *rs10838725* SNP exhibited genome-wide significant evidence of association with disease susceptibility; however, numerous genes fall under the association peak, based on regional linkage-disequilibrium (measured in r^2). Independently, functional screening in *Drosophila* highlighted *CELF1* as a potential causal gene for this locus (Shulman et al., 2014). Figure adapted by permission from Macmillan Publishers Ltd: Lambert et al. *Nature Genetics* 45(12):1452–1458 (2013), copyright 2013.

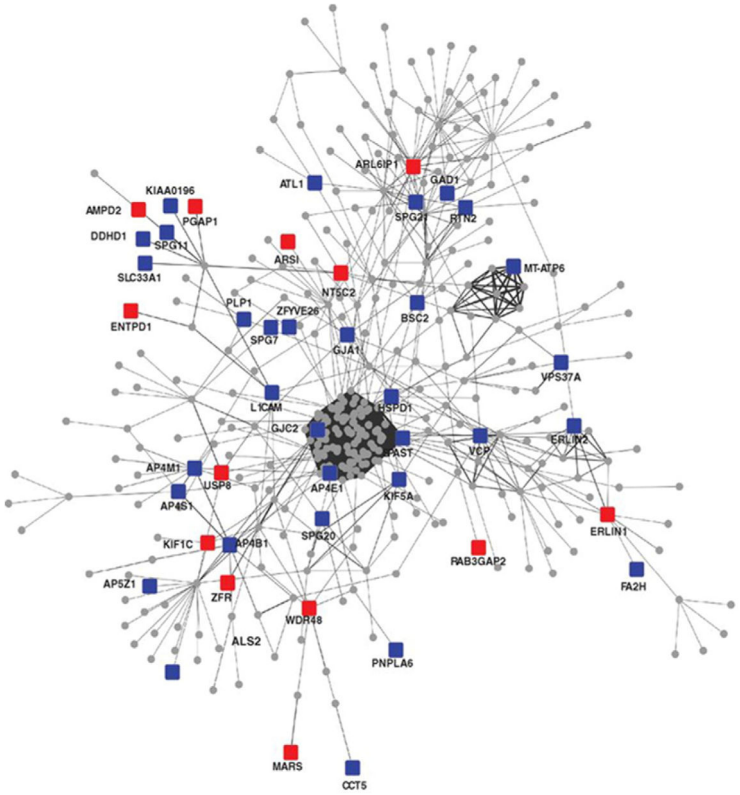

Fig. 2.

A hereditary spastic paraplegia (HSP) protein interaction network. In combination with proteins previously known to harbor mutations in HSP (blue), newly discovered gene candidates from whole-exome sequencing (red) populate a complex network of protein interactions with evidence of significant connectivity. From Novarino et al. *Science* 346 (6170): 506–511. Reprinted with permission from AAAS.