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Recent progress in using *Drosophila* as a platform for human genetic disease research

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As advanced sequencing technologies continue to uncover an increasing number of variants in genes associated with human genetic diseases, there is a growing demand for systematic approaches to assess the impact of these variants on human development, health, and disease. While in silico analyses have provided valuable insights, it is essential to complement these findings with model organism studies to determine the functional consequences of genetic variants *in vivo*. *Drosophila melanogaster* is an excellent genetic model for such functional studies due to its efficient genetic technologies, high gene conservation with humans, accessibility to mutant fly resources, short life cycles, and cost-effectiveness. The traditional *GAL4-UAS* system, allowing precise control of gene expression through binary regulation, is frequently employed to assess the effects of monoallelic variants. Recombinase medicated cassette exchange or CRISPR-Cas9-mediated *GAL4* insertion within coding introns or substitution of gene body with *Kozak-Gal4* result in the loss-of-function of the target gene. This *GAL4* insertion strategy also enables the expression of reference complementary DNA (cDNA) or cDNA carrying genetic variants under the control of endogenous regulatory cis elements. Furthermore, the CRISPR-Cas9-directed tissue-specific knockout and cDNA rescue system provides the flexibility to investigate candidate variants in a tissue-specific and/or developmental-timing dependent manner. In this review, we will delve into the diverse genetic techniques available in *Drosophila* and their applications in diagnosing and studying numerous undiagnosed diseases over the past decade.

Key words: Genetic variation, Genetic diseases, *Drosophila melanogaster*, Genetic techniques, Gene editing, Clustered regularly interspaced short palindromic repeats.

Introduction

Since the publication of Mendelian Inheritance in Man (MIM) by Dr. Victor A. McKusick in 1966, a landmark work from a former Johns Hopkins University School of Medicine professor, more than 25,000 human genetic diseases have been documented [1]. Initially released as a book, MIM evolved into an online database, the Online Mendelian Inheritance in Man (OMIM), made available in 1987 [2]. The past decade has seen a remarkable increase in the number of cataloged genetic diseases, primarily due to advancements in human genetic research and sequencing techniques. In the latter half of the 20th century, the study of human genetic diseases largely relied on extensive pedigree studies, linkage analyses, and association studies using single-nucleotide polymorphism markers [3]. Despite these efforts, numerous diseases caused by rare genetic variants remained unidentified. The introduction of advanced next-generation sequencing techniques, including whole-genome

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and whole-genome sequencing (WGS) in the last two decades, has significantly enhanced the identification of these genetic variants [4].

The inherent nature of rare variants prevents statistical analysis and patients often present multiple candidate variants across various genes. Computational analysis tools such as PolyPhen and CADD have been developed to predict the impact of the patient variants [5,6]. However, these prediction programs have limitations. Utilizing genetic model organisms for testing variants has proven to be an efficient and reliable method to ascertain the pathogenicity of variants found in undiagnosed patients [7]. These models include *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans*, *Danio rerio* (zebrafish), and *Drosophila melanogaster* (fruit flies). This review will focus on the utility of *Drosophila* model for studying human genetic diseases and recent advancements in *Drosophila* genetic technologies for determining the pathogenicity of genetic variants from rare diseases.

Advantages of Using *Drosophila* As a Genetic Model for Studying Human Diseases

Since Thomas Hunt Morgan pioneered the use of *Drosophila* in genetics in the early 1900s, studies using these fruit flies have revealed key principles of genetics and biology [8]. Efforts to validate Mendel's genetic findings with various organisms found success particularly with *Drosophila*, unlike early attempts using chickens.

The advantages of *Drosophila* as a genetic model are notable. Its four chromosomes simplify genetic studies, and its short life cycle, coupled with high fecundity, enhances efficiency. The low cost of fruit fly cultures, requiring basic ingredients like corn meal, sugar, yeast extract, and agar, makes it more economical than other systems. These attributes make *Drosophila* valuable for human genetic research, especially in identifying numerous variants/genes in rare diseases through WGS and exome sequencing. While mouse models are feasible, especially with CRISPR-Cas9 genome editing, testing multiple variants is impractical due to cost, life cycle length, and progeny number for analysis. Opposite to intuition, *Drosophila* shares many genetic similarities with humans; recent studies show that fruit flies share 57.9% of human genes and 73.1% of genes linked to rare diseases [9,10].

The accumulation of genetic technologies over the last century, including balancers, various mutagenesis methods (e.g., diverse transposable elements), and binary gene expression systems (such as *GAL4-UAS*), has made it easier to introduce patient variants into *Drosophila*. This positions the fruit fly as a preferred model for studying human genetic diseases. In the past decade, there have been significant advancements in *Drosophila* genetic technologies applicable to human genetic disease research. The following sections will discuss the challenges and troubleshooting strategies in selecting optimal genetic tools for studying various cases of human pathogenic variants.

Determine the Impact of Monoallelic Variants Using GAL4-UAS System

To determine the effects of heterozygous variants from patient in *Drosophila*, the *GAL4-UAS* system would be the ideal choice [11]. This approach, as exemplified by our investigation into *ATAD3A* (*ATPase family, AAA domain containing 3A*), provides a framework for analyzing heterozygous variants. The *de novo* p.Arg528Trp variant in *ATAD3A*, identified in five unrelated patients, is associated with developmental delay, peripheral neuropathy, and/or optic atrophy and cardiomyopathy [12]. Dominance in heterozygous variants is known to arise from haploinsufficiency, a dominant-negative function, or gain of function (hypermorph or neomorph). Haploinsufficiency was ruled out, as the individuals with a heterozygous deletion of *ATAD3A* are normal. Similarly, flies that are deficient for one copy of the gene do not display obvious phenotype.

For investigating dominant-negative function and gain of function, we took advantage of *GAL4-UAS* system [11], a binary gene expression genetic tool in *Drosophila*. Using the system we can express transgenes, including complementary DNA (cDNA), under the control of GAL4-UAS. Numerous tissue/cells or developmental timing specific Gal4 drivers have been available [13,14]. Ubiquitous overexpression of wild-type copy of the gene (*Tub or Act-Gal4>UAS-dAtad3a* [*WT*]) did not affect viability but led to expanded mitochondria, indicating that the gain of function phenotype is mild and promotes mitochondrial fusion. In contrast, ubiquitous or neuronal expression of *dAtad3a* carrying the homologous mutation to the patient variant (*UAS-dAtad3a* [*R534W*]) causes lethality and mitophagy and reduced numbers of mitochondria, suggesting that this variant is dominant-negative mutation [12]. These data are also consistent with the observation that ATAD3 proteins have been proposed to form oligomers [15]. Collectively, this study on *ATAD3A* variant identified a new neurological syndrome, later named Harel-Yoon syndrome (HYOS, MIM: 617183), but also demonstrated the effectiveness of the *GAL4-UAS* system in studying dominant

diseases.

Use of Null Alleles Created by Chemical and Transposable Element-Mediated Mutagenesis

Over the past century, *Drosophila* researchers have generated a vast collection of mutant flies, many of which are well-characterized and available at public stock centers like the Bloomington *Drosophila* Stock Center (https://bdsc.indiana.edu) [16]. This resource allows researchers to access well-defined null mutant alleles for studying biallelic and recessive aspects of rare diseases. Traditional practice in *Drosophila* research involves performing rescue experiments to confirm if observed mutant phenotypes result from the loss of the gene of interest. These rescue experiments typically involve introduction of genomic DNA covering the gene [17] or *UAS-cDNA* (*Drosophila* reference cDNA) for rescue. For human disease studies, *UAS-cDNA* expression is preferred for its ease in testing multiple variants and the tissue specificity offered by the *GAL4-UAS* system [18].

One case in point is the study of the *NRD1* (*Nardilysin*) gene, identified from a forward genetic screen (EMS mutagenesis on X chromosome) to isolate genes essential for neuronal maintenance and neurodevelopment [10,19]. From this screen, four null mutant alleles of *Drosophila Nrd1* (*dNrd1*) were isolated, all resulting in late-onset neurodegeneration in photoreceptor neurons [19]. To assess the gene's relevance to human genetic diseases, collaboration was sought with human geneticists via GeneMatcher (https://genematcher.org), a web tool for connecting physicians, geneticists, and researchers [20]. The UCLA Clinical Genomics Center identified an individual with a homozygous truncating variant in *NRD1*. The patient exhibited severe global developmental delay, ataxia, and seizures. To assess the functionality of this variant, we first tested whether expression of human wild-type *NRD1* cDNA could rescue *dNrd1* mutant phenotypes. Subsequently, we tested the patient's *NRD1* variant in the same context. The results demonstrated that ubiquitous expression of the mutant *NRD1* cDNA with the variant failed to rescue the loss of *dNrd1*, suggesting that the variant is a loss-of-function (LoF) allele [19]. Hence, this study demonstrated that combination of classical null alleles with *GAL4-UAS* system enables functional studies of recessive variants identified in human patients.

Combination of RNA Interference with UAS-Human cDNA Strategy

In situations where mutant stocks are not available in public stock centers, RNA interference (RNAi) serves as a robust method to study LoF of a target gene [21]. However, a common limitation of RNAi is its tendency for off-target effects, meaning it may inadvertently knockdown mRNAs other than its intended target. Therefore, it's crucial to validate the phenotypes resulting from RNAi expression by rescuing them with a reference sequence, such as cDNA from another species. *Drosophila* cDNA is typically unsuitable for rescue because RNAi can target both endogenous mRNA and mRNA transcribed from UAS transgenes. Thus, while using RNAi is straightforward, it requires that human cDNA be available to counteract the loss of gene function or that cDNAs from other *Drosophila* species (e.g., *Drosophila persimilis*) are accessible [22].

An illustrative example is the study of *ATP5F1D* (*ATP synthase, H^+ transporting, mitochondrial F1 complex, δ subunit*), where two patients with homozygous missense variants presented with episodic lethargy, metabolic acidosis, 3-methylglutaconic aciduria, and hyperammonemia [23]. Knockdown of *Drosophila ATPsynδ*, the homolog of human *ATP5F1D*, in the developing brain and eyes led to complete loss of the fly head. This phenotype was fully rescued by expressing wild type human *ATP5F1D* cDNA. However, expression of cDNA with the patient variants only rescued head-size phenotypes, resulting in abnormal eyes and antennae, akin to defects seen in other mutants affecting mitochondrial electron transport chain components [23]. This study demonstrated the importance of RNAi-based genetic studies and the crucial role of cross-species genetic tools in understanding the functional impact of human genetic variants.

Generation of T2A-Gal4 Null Mutant Allele Using Recombinase Medicated Cassette Exchange (RMCE)

As previously discussed, using null mutants or RNAi reagents can be simple and effective for studying biallelic variants in human genes. However, these strategies are not universally applicable, as some genes require specific expression in certain cells and tissues, and existing *GAL4* drivers, like ubiquitous and pan-neuronal Gal4, may not accurately replicate the expression patterns of the gene of interest. Furthermore, the expression of some human wild type cDNAs using existing *GAL4* drivers can be lethal. This necessitates a different approach to study human

variants.

To illustrate, I would like to share an episode from my research on the *OGDH* (*2-oxoglutarate dehydrogenase*) and *OGDHL* (*OGDH-like*) genes [19]. OGDH is a crucial enzyme in the Krebs cycle within mitochondria. In spring 2015, human genetic collaborators informed me a patient with a recessive missense variant in *OGDHL* presented with severe developmental delays and other symptoms [24]. Initial attempts using RNAi for *Drosophila Ogdh* (*dOgdh*) were reliable for metabolic phenotypes, but expressing human *OGDH* or *OGDHL* cDNA did not rescue the lethality or metabolic issues caused by *dOgdh* knockdown. In fact, ubiquitous or tissue-specific expression of *OGDH* cDNA itself induced developmental lethality [19], suggesting that OGDH expression could be toxic if its expression is not correctly regulated temporally and spatially.

A breakthrough idea emerged from a different project on which I was collaborating with Dr. Pei-Tseng Lee, a lab mate who was researching learning and memory. We were creating a *dOgdh-T2A-GAL4* line to define the neural circuit where *dOgdh* is expressed. The T2A-Gal4 toolkit, developed by Ben White's group, allows for efficient substitution of a *MiMIC* (*Minos* mediated integration cassette) transposable element located in a coding intron (an intron flanked by two coding exons) with an artificial exon containing *SA* (*splicing acceptor*)-*T2A-GAL4-polyA* (abbreviated as *T2A-Gal4*) [25]. *MiMIC* is a versatile tool for functional gene annotation in *Drosophila* and has a Swappable Integration Cassette (SIC) [26]. MiMIC SICs located between two *attP* sites can be exchanged with any DNA sequence flanked with *attB* sites through RMCE by ϕC31 integrase [26]. We took advantage of the presence of a MiMIC transposon inserted in a coding intron of *dOgdh* and created *dOgdh-T2A-Gal4* line through RMCE.

This *T2A-Gal4* insertion into the coding intron of *dOgdh* genomic locus results in truncating the protein at the insertion site, leading to the generation of a null mutant. In the other hand, the viral T2A sequence causes the truncation of the nascent *dOgdh* polypeptide and reinitiating of translation of the downstream GAL4 as an independent protein. The expression of *Gal4* is under the control of the endogenous *dOgdh* regulatory control [19]. During the challenging period with the *dOgdh* RNAi, the generation of the *dOgdh-T2A-GAL4* line happened concurrently. I realized that I had already created a null mutant for *dOgdh* that could express *Gal4* under endogenous control. Using the *dOgdh-T2A-GAL4* line, I could test null mutant phenotypes and simultaneously express *UAS-human OGDH* cDNA for rescue. Eureka!!! I ran to Dr. Hugo Bellen, my postdoctoral mentor, and shared this idea with him, who encouraged the approach and suggested using deficiency flies lacking the *dOgdh* genomic region to confirm the LoF phenotypes. This approach proved successful. Homozygous *dOgdh-T2A-GAL4* flies exhibited embryonic lethality, which was rescued by expression of human *OGDH* cDNA, but not by the cDNA with the patient's variant [19]. Hence, the results indicate that the missense variant in *OGDHL* is a LoF allele and underlie the manifestation in patient. This study highlighted the utility of the *T2A-Gal4* approach for studying human diseases. Furthermore, this study exemplified an approach of humanizing *Drosophila* to determine variants' function *in vivo* [7].

Generation of *T2A-Gal4* Alleles Using CRIMIC

MiMIC is a versatile tool for functional gene annotation in *Drosophila* and features a SIC located between two *attP* sites [26]. These SICs can be exchanged with any DNA sequence flanked with *attB* sites through RMCE by ϕC31 integrase [26]. In the previous section, we mentioned that *Drosophila* mutant alleles created by replacing ISC in MiMICs with an artificial exon encoding a *SA-T2A-GAL4-polyA signal* (*T2A-Gal4*) are valuable for studying patient variant studies, as it enables replacing the gene of interest in *Drosophila* with *Drosophila* or *human UAS-cDNA*. Hence, MiMIC alleles of *Drosophila* are invaluable resources for human disease studies.

The *Drosophila* Gene Disruption project (http://flypush.imgen.bcm.tmc.edu/pscreen), led by Dr. Hugo Bellen laboratory at Baylor College of Medicine, generated 17,500 *MiMIC* insertion lines [26,27]. Of these, approximately 1,860 genes have MiMIC insertions within introns, which can be converted into a *T2A-GAL4* or *GFP-tagged protein traps* [28]. However, with around 13,600 *Drosophila* genes [29], a complementary strategy was needed to generate *Drosophila* alleles for genes that do not have a *MiMIC* insertion within a coding intron. Bellen laboratory developed a CRIMIC (CRISPR mediated integration cassette) strategy. This involves integrating a modified *SIC* (*attP-FRT-SA-T2A-GAL4-polyA-FRT-attP*) into a coding intron of gene of interest via CRISPR/Cas9-mediated homology directed repair (HDR). This strategy expanded the number of genes with MiMICs from 1,860 to approximately 6,000 [30], significant increasing *Drosophila* genetic resources and thus providing useful resources for human genetic studies.

Kozak-Gal4 Null Mutant Generation via CRISPR-Cas9 Editing

The *MiMIC* and *CRIMIC*-based *T2A-Gal4* resources have been instrumental for numerous human disease studies [18,19,31-35]. However, their application is limited as a large coding intron, typically over 100 nt, is required for the genomic integration of an artificial exon, leaving over half of *Drosophila* genes untargetable by *CRIMIC* [28]. About 58% of *Drosophila* genes lack an optimal coding intron for the integration of artificial exons in all annotated splicing isoforms.

To address this gap, an alternative and efficient strategy is needed to create null mutant alleles that express *GAL4* under the control of endogenous regulatory elements. Traditionally, *Drosophila* researchers have created *GAL4* knock-in lines using the pw35GAL4 construct-mediated transgenesis and homologous recombination [36]. This process involves cloning a 3 kb homologous sequence for both arms into the vector. However, this cloning method is notoriously inefficient, and the selection of *GAL4* knock-in flies typically requires an extended period of 4 to 5 months.

CRISPR/Cas9-mediated genome editing, which has been proven effective in *Drosophila*, offers a solution [30,37]. One approach involves using CRISPR/Cas9 to integrate a *KozakGAL4* cassette that replaces the entire gene body [38]. This strategy employs creating double-stranded breaks at the 5' and 3'UTR of the target gene, using two sgRNAs, and then replaces the coding sequence of genes with a *Kozak sequence-GAL4-polyA-FRT-3xP3EGFP-polyA-FRT* (*KozakGal4*) cassette [38]. In addition, for HDR-mediated integration, it uses short homology arms (100-200 bps) for HDR-mediated integration, effectively inserting large DNA segments (>5 kb) into the genome [39]. Kanca et al. [38] successfully applied this method to approximately 200 genes and found that the efficiency of transgenesis is about 80%. The *KozakGal4* method is expected to be highly useful for studying human genetic diseases in genes where *MiMIC* and *CRIMIC* are not applicable.

CRISPR/Cas9-Mediated Tissue-Specific Knockout and cDNA Rescue

CRISPR/Cas9 genome editing significantly expanded the tool kit for *Drosophila* genetics [40]. It particularly when combined with classical genetic tools like the *GAL4-UAS* system, enabling tissue or cell-specific knockout of target genes [41]. Port et al. [41,42] created a large-scale transgenic short guide (sg) RNA library that enabled efficient CRISPR/Cas9-based disruption of target genes in a constitutive or conditional manner. This technique also allows for the introduction of pathogenic variants into the *Drosophila* genome.

Functional studies of variants from human patients in *Drosophila* often require a tissue-specific approaches. In cases where human genes have multiple paralogs with tissue-specific expression, while *Drosophila* has a single, ubiquitously expressed orthologs, tissue-specific expression of *UAS-Cas9* along with ubiquitous sgRNA expression can be an effective strategy. However, the majority of sgRNAs in the Transgenic RNAi project and the Weizmann Knock Out project target common coding exons [41,42], limiting their use in studies requiring genomic knockout alongside rescue by *GAL4*-directed *UAS-wild type cDNA* expression.

To address this, a novel strategy was developed involving sgRNAs targeting exon-intron junctions, allowing selective targeting of genomic loci while sparing *UAS-cDNA* transgenes [31,35,43]. This approach, termed "CRISPR/Cas9-mediated tissue-specific knockout and cDNA rescue," was exemplified in a study of *OGDHL* variants. In our study of *OGDHL* variants, we identified nine individuals from eight unrelated families with biallelic variants presenting diverse neurodevelopmental symptoms, including epilepsy and microcephaly [31]. The variants include nine missense mutations. Using the *dOgdh-T2A-Gal4* allele, we found that *dOgdh* cDNA carrying mutations analogous to the human variants failed to rescue lethality resulting from *dOgdh* loss. However, this finding has limitations, as *OGDHL* is brain-specific in humans [44,45], whereas *dOgdh* is ubiquitously expressed in *Drosophila* [46]. Employing the new CRISPR knockout with cDNA rescue system, we demonstrated that neuron-specific knockout of *dOgdh* led to lethality, which was fully rescued by wild type *dOgdh* cDNA expression. Interestingly, while using the *dOgdh-T2A-Gal4* allele showed all nine variants failing to rescue lethality, the new CRISPR system revealed that three variants failed to rescue, whereas the other six did, albeit with behavioral defects observed in the flies [31]. This result from the CRISPR-Cas9-mediated knockout with cDNA rescue approach allowed us to differentiate the allelic strengths of *OGDHL* variants in a tissue-specific manner, thereby enhancing our understanding of their impact and expanding the capabilities of *Drosophila* genetic tools. This approach is expected to be widely used for in-depth studies of human disease-associated variants and their specific roles in tissues or cells, as well as for broader genetic research in *Drosophila*.

Conclusion

The recent progress in *Drosophila* genetic technologies, particularly through the integration of CRISPR/Cas9 genome editing and classic genetic tools, has markedly enhanced the study of human genetic diseases. The development of innovative methods such as *T2A-Gal4* alleles using *CRIMIC, Kozak-Gal4* via CRISPR-Cas9 editing, and tissue-specific knockout and cDNA rescue strategies, has expanded our ability to model and understand complex genetic disorders. These advancements underscore *Drosophila*'s value in functional genomics, enabling precise dissection of variant pathogenicity and providing novel insights into disease mechanisms. This progress not only enhances our understanding of disease biology but also opens up potential therapeutic avenues, highlighting *Drosophila*'s continuing relevance in the era of personalized medicine.

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References

1. Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 2005;33:D514-7.
2. McKusick VA. Mendelian Inheritance in Man and its online version, OMIM. Am J Hum Genet 2007;80:588-604.
3. Ott J, Wang J, Leal SM. Genetic linkage analysis in the age of whole-genome sequencing. Nat Rev Genet 2015;16:275-84.
4. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, et al. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proc Natl Acad Sci U S A 2009;106:19096-101.
5. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet 2013;Chapter 7:Unit7.20.
6. Rentzsch P, Schubach M, Shendure J, Kircher M. CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. Genome Med 2021;13:31.
7. Bellen HJ, Wangler MF, Yamamoto S. The fruit fly at the interface of diagnosis and pathogenic mechanisms of rare and common human diseases. Hum Mol Genet 2019;28:R207-14.
8. St Johnston D. The art and design of genetic screens: drosophila melanogaster. Nat Rev Genet 2002;3:176-88.
9. Wang J, Al-Ouran R, Hu Y, Kim SY, Wan YW, Wangler MF, et al. MARRVEL: integration of human and model organism genetic resources to facilitate functional annotation of the human genome. Am J Hum Genet 2017;100:843-53.
10. Yamamoto S, Jaiswal M, Charng WL, Gambin T, Karaca E, Mirzaa G, et al. A drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. Cell 2014;159:200-14.
11. Brand AH, Perrimon N. Raf acts downstream of the EGF receptor to determine dorsoventral polarity during Drosophila oogenesis. Genes Dev 1994;8:629-39.
12. Harel T, Yoon WH, Garone C, Gu S, Coban-Akdemir Z, Eldomery MK, et al. Recurrent de novo and biallelic variation of ATAD3A, encoding a mitochondrial membrane protein, results in distinct neurological syndromes. Am J Hum Genet 2016;99:831-45.
13. Jenett A, Rubin GM, Ngo TT, Shepherd D, Murphy C, Dionne H, et al. A GAL4-driver line resource for Drosophila neurobiology. Cell Rep 2012;2:991-1001.
14. Li HH, Kroll JR, Lennox SM, Ogundeyi O, Jeter J, Depasquale G, et al. A GAL4 driver resource for developmental and behavioral studies on the larval CNS of Drosophila. Cell Rep 2014;8:897-908.
15. Gilquin B, Taillebourg E, Cherradi N, Hubstenberger A, Gay O, Merle N, et al. The AAA+ ATPase ATAD3A controls mitochondrial dynamics at the interface of the inner and outer membranes. Mol Cell Biol 2010;30:1984-96.
16. Cook KR, Parks AL, Jacobus LM, Kaufman TC, Matthews KA. New research resources at the Bloomington Drosophila Stock Center. Fly (Austin) 2010;4:88-91.
17. Venken KJ, He Y, Hoskins RA, Bellen HJ. P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in D. melanogaster. Science 2006;314:1747-51.
18. Yap ZY, Park YH, Wortmann SB, Gunning AC, Ezer S, Lee S, et al. Functional interpretation of ATAD3A variants in neuro-mitochondrial phenotypes. Genome Med 2021;13:55.
19. Yoon WH, Sandoval H, Nagarkar-Jaiswal S, Jaiswal M, Yamamoto S, Haelterman NA, et al. Loss of nardilysin, a mitochondrial co-chaperone for α-ketoglutarate dehydrogenase, promotes mTORC1 activation and neurodegeneration. Neuron 2017;93:115-31.
20. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat 2015;36:928-30.

21. Matranga C, Tomari Y, Shin C, Bartel DP, Zamore PD. Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. Cell 2005;123:607-20.
22. Kondo S, Booker M, Perrimon N. Cross-species RNAi rescue platform in Drosophila melanogaster. Genetics 2009;183:1165-73.
23. Oláhová M, Yoon WH, Thompson K, Jangam S, Fernandez L, Davidson JM, et al. Biallelic mutations in ATP5F1D, which encodes a subunit of ATP synthase, cause a metabolic disorder. Am J Hum Genet 2018;102:494-504.
24. Karaca E, Harel T, Pehlivan D, Jhangiani SN, Gambin T, Coban Akdemir Z, et al. Genes that affect brain structure and function identified by rare variant analyses of Mendelian neurologic disease. Neuron 2015;88:499-513.
25. Diao F, Ironfield H, Luan H, Diao F, Shropshire WC, Ewer J, et al. Plug-and-play genetic access to drosophila cell types using exchangeable exon cassettes. Cell Rep 2015;10:1410-21.
26. Venken KJ, Schulze KL, Haelterman NA, Pan H, He Y, Evans-Holm M, et al. MiMIC: a highly versatile transposon insertion resource for engineering Drosophila melanogaster genes. Nat Methods 2011;8:737-43.
27. Nagarkar-Jaiswal S, Lee PT, Campbell ME, Chen K, Anguiano-Zarate S, Gutierrez MC, et al. A library of MiMICs allows tagging of genes and reversible, spatial and temporal knockdown of proteins in Drosophila. Elife 2015;4:e05338.
28. Lee PT, Zirin J, Kanca O, Lin WW, Schulze KL, Li-Kroeger D, et al. A gene-specific T2A-GAL4 library for Drosophila. Elife 2018;7:e35574.
29. Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, et al. The genome sequence of Drosophila melanogaster. Science 2000;287:2185-95.
30. Li-Kroeger D, Kanca O, Lee PT, Cowan S, Lee MT, Jaiswal M, et al. An expanded toolkit for gene tagging based on MiMIC and scarless CRISPR tagging in Drosophila. Elife 2018;7:e38709.
31. Yap ZY, Efthymiou S, Seiffert S, Vargas Parra K, Lee S, Nasca A, et al. Bi-allelic variants in OGDHL cause a neurodevelopmental spectrum disease featuring epilepsy, hearing loss, visual impairment, and ataxia. Am J Hum Genet 2021;108:2368-84.
32. Marcogliese PC, Shashi V, Spillmann RC, Stong N, Rosenfeld JA, Koenig MK, et al. IRF2BPL is associated with neurological phenotypes. Am J Hum Genet 2018;103:456. Erratum for: Am J Hum Genet 2018;103:245-60.
33. Wang J, Rousseau J, Kim E, Ehresmann S, Cheng YT, Duraine L, et al. Loss of oxidation resistance 1, OXR1, is associated with an autosomal-recessive neurological disease with cerebellar atrophy and lysosomal dysfunction. Am J Hum Genet 2019;105:1237-53.
34. Chung HL, Wangler MF, Marcogliese PC, Jo J, Ravenscroft TA, Zuo Z, et al. Loss- or gain-of-function mutations in ACOX1 cause axonal loss via different mechanisms. Neuron 2020;106:589-606.e6.
35. Whittle EF, Chilian M, Karimiani EG, Progri H, Buhas D, Kose M, et al. Biallelic variants in OGDH encoding oxoglutarate dehydrogenase lead to a neurodevelopmental disorder characterized by global developmental delay, movement disorder, and metabolic abnormalities. Genet Med 2023;25:100332.
36. Moon SJ, Lee Y, Jiao Y, Montell C. A Drosophila gustatory receptor essential for aversive taste and inhibiting male-to-male courtship. Curr Biol 2009;19:1623-7.
37. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 2014;346:1258096.
38. Kanca O, Zirin J, Hu Y, Tepe B, Dutta D, Lin WW, et al. An expanded toolkit for Drosophila gene tagging using synthesized homology donor constructs for CRISPR-mediated homologous recombination. Elife 2022;11:e76077.
39. Kanca O, Zirin J, Garcia-Marques J, Knight SM, Yang-Zhou D, Amador G, et a. An efficient CRISPR-based strategy to insert small and large fragments of DNA using short homology arms. Elife 2019;8:e51539.
40. Port F, Bullock SL. Augmenting CRISPR applications in Drosophila with tRNA-flanked sgRNAs. Nat Methods 2016;13:852-4.
41. Port F, Strein C, Stricker M, Rauscher B, Heigwer F, Zhou J, et al. A large-scale resource for tissue-specific CRISPR mutagenesis in Drosophila. Elife 2020;9:e53865.
42. Port F, Chen HM, Lee T, Bullock SL. Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in Drosophila. Proc Natl Acad Sci U S A 2014;111:E2967-76.
43. Chilian M, Vargas Parra K, Sandoval A, Ramirez J, Yoon WH. CRISPR/Cas9-mediated tissue-specific knockout and cDNA rescue using sgRNAs that target exon-intron junctions in Drosophila melanogaster. STAR Protoc 2022;3:101465.
44. GTEx Consortium. The genotype-tissue expression (GTEx) project. Nat Genet 2013;45:580-5.
45. GTEx Consortium; Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis Working Group; Statistical Methods groups—Analysis Working Group; Enhancing GTEx (eGTEx) Groups; NIH Common Fund; NIH/NCI; et al. Genetic effects on gene expression across human tissues. Nature 2017;550:204-13. Erratum for: Nature 2018;553:530.
46. Yap ZY, Strucinska K, Matsuzaki S, Lee S, Si Y, Humphries K, et al. A bi-allelic pathogenic variant in the OGDH gene results in a neurological disorder with features of a mitochondrial disease. J Inherit Metab Dis 2021;44:388-400.