Review

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# Investigation of epilepsy-related genes in a *Drosophila* model

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***From the Contents***



## Abstract

Complex genetic architecture is the major cause of heterogeneity in epilepsy, which poses challenges for accurate diagnosis and precise treatment. A large number of epilepsy candidate genes have been identified from clinical studies, particularly with the widespread use of next-generation sequencing. Validating these candidate genes is emerging as a valuable yet challenging task. *Drosophila* serves as an ideal animal model for validating candidate genes associated with neurogenetic disorders such as epilepsy, due to its rapid reproduction rate, powerful genetic tools, and efficient use of ethological and electrophysiological assays. Here, we systematically summarize the advantageous techniques of the *Drosophila* model used to investigate epilepsy genes, including genetic tools for manipulating target gene expression, ethological assays for seizure-like behaviors, electrophysiological techniques, and functional imaging for recording neural activity. We then introduce several typical strategies for identifying epilepsy genes and provide new insights into gene–gene interactions in epilepsy with polygenic causes. We summarize well-established precision medicine strategies for epilepsy and discuss prospective treatment options, including drug therapy and gene therapy for genetic epilepsy based on the *Drosophila* model. Finally, we also address genetic counseling and assisted reproductive technology as potential approaches for the prevention of genetic epilepsy.

**Key Words:** *Drosophila melanogaster*; electrophysiology; epilepsy; genetics; morphology; neurogenetic diseases; polygene; precision medicine; seizure behavior; UAS/GAL4 system

## Introduction

Epilepsy, a complex neurological disorder characterized by recurrent seizures, is a prevalent condition that affects approximately 50 million people worldwide, making it one of the most common neurological diseases and a significant public health issue (GBD 2016 Epilepsy Collaborators, 2019; Guekht et al., 2021). The etiology of epilepsy is multifaceted, with a multitude of gene variants identified as contributors to the disorder through extensive clinical and genetic studies (Wang et al., 2017; Zhang et al., 2024). Validating the candidate epilepsy-associated genes and investigating their functions and mechanisms have emerged as challenging and promising directions in epilepsy research. *Drosophila melanogaster*, commonly known as the fruit fly, has been established as an indispensable model organism for the study of epilepsy. The fruit fly's short lifespan, ease of genetic manipulation, and high degree of genetic homology with humans make it an ideal system for investigating the genetic basis of complex diseases such as epilepsy (Baraban, 2007; Cunliffe et al., 2015; Fischer et al., 2023; Tanaka and Chung, 2025). The use of *Drosophila* in epilepsy research has yielded significant insights into the molecular and cellular pathways that are disrupted in this disease. By replicating epilepsy-related phenotypes in the fruit fly, researchers can study the effects of specific gene mutations and test potential therapeutic interventions in a controlled environment. This model organism has proven instrumental in deciphering the complexities of epilepsy, from the identification of novel genes to the understanding of seizure susceptibility and epilepsy-associated comorbidities.

Moreover, the application of *Drosophila* in epilepsy research has the potential to revolutionize the field of precision medicine. As we gain a deeper understanding of the genetic architecture of epilepsy through this model, we can begin to tailor treatments to individual patients based on their genetic profiles, leading to more targeted and effective therapies (Dare et al., 2020; Silva-Cardoso and N'Gouemo, 2023). This review aims to highlight the pivotal role that *Drosophila* plays in epilepsy research, its contribution to validating genes linked to epilepsy, and the transformative implications that this research holds for the development of future therapeutic strategies.

## Search Strategy and Selection Criteria

In this narrative review, we searched the literature via terms, such as *Drosophila*, epilepsy, genetics, precision medicine in the title, and abstracts and keywords in PubMed and Google Scholar. Owing to the fast-growing field of epilepsy, we prioritized contemporary literature published within the last 10 years, with the exception of milestone papers. Online Mendelian inheritance in Man (OMIM, omim.org), GeneCards (genecards. org), the Human Gene Mutation Database (HGMD, hgmd. cf.ac.uk/) and FlyBase (http://flybase.org) were relevant tools/databases used in our study. OMIM and HGMD were used to search for relationships between genes/variants and human diseases. GeneCards was used to search for the basic information of the genes of interest. FlyBase was used to search for basic information and references related to fly genes. In summary, these databases can help us search for epilepsy-related causative genes and provide examples of how *Drosophila* can be used to validate epilepsy-causing genes.

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The major inclusion criteria were the literature on the link between epilepsy-associated genes and *Drosophila* and the validation of epilepsy-related genes through *Drosophila*. All the years were chosen for the search. Papers written in languages other than English were excluded.

## Epilepsy and Genetic Factors

### The intricacies of epilepsy as a neurological disorder

Epilepsy is a chronic condition of the nervous system characterized by recurrent, abrupt, and temporary disruptions in brain function due to excessive neuronal activity. The symptoms of epileptic seizures span a broad spectrum, from barely noticeable sensory anomalies to intense tonic–clonic movements, making epilepsy a multifaceted and heterogeneous condition (Fisher et al., 2014; Thijs et al., 2019; Jiao et al., 2025). Those with epilepsy also face a heightened risk of mortality, estimated to be two to three times higher than that of the general population, primarily due to sudden unexpected death in patients with epilepsy, prolonged seizures (status epilepticus), accidents, and suicide (Moshé et al., 2015; Rosenfeld et al., 2023).

Deciphering the causes of epilepsy is crucial for informing treatment approaches and prognostic evaluations. In 2017, the International League Against Epilepsy updated its classification system for epilepsy, categorizing its causes into six main types: structural, genetic, infectious, metabolic, immune-related, and unknown (Shorvon, 2011; Scheffer et al., 2017; Rastin et al., 2023). Current research suggests that genetic influences are significant in about 70% of epilepsy cases, underscoring the importance of genetic factors in the pathology of this condition (Hildebrand et al., 2013; Thomas and Berkovic, 2014; Perucca et al., 2020).

### Unraveling the genetic foundations of epilepsy

Understanding the genetic architecture of epilepsy is critical for advancing our knowledge of this complex neurological disorder. Current research has identified four primary genetic factors contributing to epilepsy: monogenic mutations, polygenic mutations, epilepsy caused by genetic anomalies due to cellular (chromosomal) aberrations, and epilepsy associated with other genetic multisystem diseases (Ellis et al., 2020; Conboy et al., 2021; Johannesen et al., 2023).

Monogenic epilepsy refers to cases in which a single gene mutation is sufficient to trigger the epileptic phenotype. The advent of second-generation sequencing technology has revolutionized the field, leading to the discovery of numerous epilepsy-causing genes (Chung et al., 2020; Liu et al., 2021; He et al., 2023b; Li et al., 2024b; Luo et al., 2024). In 2017, researchers identified 977 genes associated with epilepsy by analyzing databases such as PubMed (https://pubmed.ncbi.nlm.nih.gov), OMIM (https://www.ncbi.nlm.nih.gov/omim/), HGMD (https://www.hgmd.cf.ac.uk/ac/index.php), and the Epilepsy Gene database (http://61.152.91.49/EpilepsyGene/), with 84 of these genes being directly linked to epilepsy (Wang et al., 2017). By July 2023, the OMIM database had listed 1506 genes connected to epilepsy, and an additional 1440 genes were identified as potential epilepsy-related candidates through a comprehensive review of the HGMD and PubMed databases (Zhang et al., 2024).

These pathogenic gene variations often follow Mendelian inheritance patterns, including autosomal dominant, autosomal recessive, and X-linked inheritance. The functions of these genes within the nervous system are diverse. The major gene categories linked to epilepsy include the following:

- Ion channels: These channels are crucial for maintaining neuronal function by regulating the movement of ions such as sodium, potassium, and calcium across cell membranes. Mutations in ion channels are significant contributors to idiopathic epilepsy (Guerrini et al., 2003; Meisler and Kearney, 2005; Wei et al., 2017; Oyrer et al., 2018; Brunklaus et al., 2020; Powell et al., 2025), as observed in Dravet syndrome, which is associated with SCN1A gene mutations (Escayg and Goldin, 2010; Dravet, 2011; Scheffer and Nabbout, 2019; Lagae, 2021; Brunklaus et al., 2022). Mutations in sodium-activated potassium channel subfamily T member 1 (*KCNT1*) have been associated with pediatric epilepsy disorders (Hinckley et al., 2023). Benign familial neonatal epilepsy (BFNE) is linked to *KCNQ2* and *KCNQ3* mutations (Mulley et al., 2003; Goto et al., 2019; Gribkoff and Winquist, 2023).

- Synaptic formation: Genes involved in synaptic formation and function can disrupt the balance of excitatory and inhibitory neurotransmission, potentially leading to seizures (Treiman, 2001). For example, mutations in *STXBP1* are associated with development (Saitsu et al., 2008; Hamdan et al., 2009; Stamberger et al., 2016; Lammertse et al., 2020; Xian et al., 2022), and epileptic encephalopathy and mutations in the *BSN* gene are associated with epilepsy (Ye et al., 2023).

- DNA repair: Genes involved in DNA repair processes, such as *MBD5* (Martins et al., 2023; Tang et al., 2023), *FAN1* (Deshmukh et al., 2021) and *PNKP* (Jilani et al., 1999; Shen et al., 2010; Furones García et al., 2021), can lead to genomic instability and increase susceptibility to epilepsy when mutated.

- Transcriptional regulation: Genes that control transcription and gene expression, such as *FOXG1* (Ariani et al., 2008) and *ARX* (Bienvenu et al., 2002), can influence neuron development and function, potentially causing epilepsy (Sun et al., 2022).

- Transporters within nerve cells: Mutations in transporter genes can disrupt neuronal function and cause seizures. Examples include *SLC2A1* mutations leading to GLUT1 deficiency syndrome (Brockmann, 2009; Arsov et al., 2012), UNC13B mutations associated with partial epilepsy (Wang et al., 2021), and *SLC6A1* mutations associated with epilepsy syndromes (Hirunsatit et al., 2009; Carvill et al., 2015; Stefanski et al., 2023).

Polygenic inheritance refers to scenarios where multiple gene variations, both rare and common, contribute to the risk of developing epilepsy (Leu et al., 2019; Gramm et al., 2020; Harris et al., 2023). Each variation may have a modest individual effect, but their combined impact significantly increases susceptibility. Environmental factors can also play a role in polygenic epilepsy, making the inheritance pattern complex and often difficult to trace within families.

As our understanding of epilepsy-related genes and pathogenesis deepens and as next-generation sequencing technology becomes more widespread in clinical settings, patients with epilepsy can expect faster and more precise genetic diagnoses. This, in turn, will lead to earlier interventions and more targeted treatments, ultimately improving the quality of life for those living with epilepsy (Beghi, 2016).

### The role of precision medicine in epilepsy care

The phenotypes of epilepsy patients with pathogenic variants are heterogeneous. Precision medicine is necessary and has been proven to be valuable for epilepsy treatment (Collins and Varmus, 2015; Reif et al., 2017; Ruiz-Reig et al., 2024). *GLUT1* deficiency syndrome, an autosomal dominant genetic disorder caused by mutations in the *SLC2A1* gene, serves as an example where precision medicine is applied (Pascual et al., 2004). Its typical clinical features include infantile onset of epileptic seizures, neurodevelopmental delay, acquired microcephaly, and complex movement disorders. Patients with *GLUT1* deficiency syndrome often respond well to a ketogenic diet, which provides alternative energy substrates for the brain (Wang et al., 2005; Pérez-Dueñas et al., 2009). Dravet syndrome, another autosomal dominant genetic disorder characterized primarily by prolonged febrile seizures or nonfebrile seizures, is primarily caused by loss-of-function mutations in the *SCN1A* gene, which encodes the α subunit of the voltage-gated sodium channel Nav1.1 (Myers, 2023). Traditional sodium channel-blocking drugs can exacerbate seizures in individuals with Dravet syndrome, highlighting the need for precision medicine to avoid these medications (Perucca and Perucca, 2019; He et al., 2022; Matricardi et al., 2023). Tuberous sclerosis complex (TSC), an autosomal dominant disorder, is characterized by epilepsy, intellectual disability, and other symptoms. This disease is caused by mutations in the *TSC1* and *TSC2* genes, which encode proteins involved in regulating the mammalian target of rapamycin (mTOR) signaling pathway. Inhibitors of this pathway have shown promise in precision treatment for TSC (Schubert-Bast et al., 2019; Łukawski and Czuczwar, 2021; Schubert-Bast and Strzelczyk, 2021).

Precision therapy for epilepsy involves tailoring treatments to the specific seizure type, syndrome, genetic makeup, and other individual factors. This approach includes gene therapy, antisense oligonucleotide therapy, and treatments that target specific protein functions. The range of precision therapies extends to diets, vitamins, cell signaling regulators, ion channel modulators, repurposed medications, molecular chaperones, and gene therapies (Kearney et al., 2019; Helbig and Ellis, 2020; Zimmern et al., 2022). **Table 1** presents a comprehensive summary of the current precision targeted therapies for epilepsy and epileptic syndromes.

Precision medicine is a transformative approach

**Table 1** | **Precision medicine based on the function of pathogenic genes**

| Gene | Phenotype | Animal model | Suggested precision medicine | Status |
|---|---|---|---|---|
| *ALDH7A1* | EPEO4 (OMIM #266100) | Mouse, zebrafish | -Pyridoxine<br>-Lysine-restricted diet<br>-Arginine supplementation | Established (Gospe, 1993; Yang et al., 2014; Mastrangelo and Cesario, 2019; Coughlin et al., 2021) |
| *CAD* | DEE50 (OMIM #616457) | Mouse | -Uridine | Established (Koch et al., 2017; Peng et al., 2022a) |
| *CHRNA4* | ENFL1 (OMIM #600513) | Mouse, zebrafish | -Nicotine | Established (Lossius et al., 2020) |
| *DHFR* | DHFR deficiency (OMIM #613839) | Mouse, rat, zebrafish, fruit fly | -Folinic acid | Established (Cario et al., 2011; Serrano et al., 2012) |
| *FOLR1* | NCFTD (OMIM #613068) | Mouse | -Folinic acid therapy<br>-Ketogenic diet | Established (Steinfeld et al., 2009; Molero-Luis et al., 2015; Potic et al., 2023)<br>Potential (Papadopoulou et al., 2021) |
| *GAMT* | CCDS2 (OMIM #612736) | Mouse | -Creatine monohydrate<br>-Supplementation of ornithine and dietary restriction of arginine or protein | Established (Mercimek-Andrews and Salomons, 1993; Schulze, 2013; Fernandes-Pires and Braissant, 2022) |
| *GATM* | CCDS3 (OMIM #612718) | Rat, mouse | -Creatine supplementation | Established (Edvardson et al., 2010; Verma, 2010; Ndika et al., 2012; Fernandes-Pires and Braissant, 2022) |
| *KCNH2* | LQT (OMIM #613688)<br>SQT (OMIM #609620) | Mouse | -Beta blockers<br>-Suppression and replacement gene therapy | Established (Itoh et al., 2001; Mazzanti et al., 2018)<br>Hypothetical (Bains et al., 2022) |
| *KCNQ2* | DEE7 (OMIM #613720) | Mouse | -Phenobarbital and sodium channel blockers (carbamazepine and fosphenytoin) | Established (Kuersten et al., 2020; Kazazian et al., 2022; Borggraefe and Wagner, 2023) |
| | BFNS1 (OMIM #121200) | | -Gabapentin, ezogabine, retigabine, pynegabine | Potential (Soldovieri et al., 2020; Knight et al., 2023; Yang et al., 2023) |
| *MTHFR* | MTHFR deficiency (OMIM #236250) | Mouse | -5-Methyltetrahydrofolate, folinic acid<br>-Betaine | Established (Molero-Luis et al., 2015)<br>Established (Huemer et al., 2017) |
| *POLG* | MTDPS4A (OMIM #203700) | Mouse, fruit fly | -VPA is absolutely contra-indicated in patients with POLG-related disease<br>-Ketogenic diet<br>-Perampanel | Established (Rahman and Copeland, 2019)<br>Potential (Pedersen et al., 2022)<br>Potential (Nissenkorn et al., 2023) |
| *PRRT2* | ICCA (OMIM #602066)<br>BFIS2 (OMIM #605751)<br>EKD1 (OMIM #128200) | Mouse, fruit fly | -Sodium channel blockers (carbamezepine and oxcarbazepine) | Established (Cao et al., 2021) |
| *CN1A* | DEE6B (OMIM #619317)<br>FEB3A (OMIM #604403)<br>GEFSP2 (OMIM #604403) | Mouse, fruit fly, rat, zebrafish | GOF: sodium channel blocker (carbamazepine, oxcarbazepine, phenytoin, lamotrigine, or lacosamide) | Potential (Brunklaus et al., 2022) |
| | DRVT (OMIM #607208) | | LOF: -avoid sodium channel blockers<br>-Valproate, clobazam and stiripentol<br>-Topiramate and bromide<br>-Cannabidiol and fenfluramine<br>-Perampanel<br>-Antisense oligonucleotide (ASO) | Established (Oyrer et al., 2018)<br>Established (Oyrer et al., 2018; Strzelczyk and Schubert-Bast, 2022; Wirrell et al., 2022)<br>Potential (Strzelczyk and Schubert-Bast, 2022)<br>Potential (Lagae, 2021; Strzelczyk and Schubert-Bast, 2022)<br>Potential (Nissenkorn et al., 2023)<br>Hypothetical (Yuan et al., 2024) |
| *SLC2A1* | GLUT1DS (OMIM #606777)<br>DYT9 (OMIM #601042)<br>EIG12 (OMIM #614847)<br>SDCHCN (OMIM #608885) | Mouse, zebrafish | -Ketogenic diet therapy | Established (Daci et al., 2018; Wang et al., 2024) |
| *SLC6A8* | CCDS1 (OMIM #300352) | Mouse, rat | -Creatine<br>-Arginine or/and glycine supplementation | Established (Dunbar et al., 2014; Li and Xu, 2023) |
| *STXBP1* | DEE4 (OMIM #612164) | Mouse, rat, zebrafish, fruit fly | -Levetiracetam<br>-Ketogenic diet or clobazam<br>-Chaperone proteins<br>-Clemizole and Trazodone | Established (Wang et al., 2022)<br>Established (Xian et al., 2022; Freibauer et al., 2023)<br>Hypothetical (Guiberson et al., 2018; Freibauer et al., 2023)<br>Hypothetical (Moog and Baraban, 2022) |
| *TPP1* | CLN2 (OMIM #204500)<br>SCAR7 (OMIM #609270) | Mouse, zebrafish, canine | -Cerliponase alpha<br>-Enzyme replacement therapy, small molecule therapy, neuroprotection, stem cell therapy<br>-AAV<br>-Flupirtine derivatives, gemfbrozil | Established (Kohlschütter et al., 2019; Specchio et al., 2021)<br>Potential (Kohlschütter et al., 2019; Specchio et al., 2021)<br>Hypothetical (Passini et al., 2006)<br>Hypothetical (Kim et al., 2017; Ghosh et al., 2018; Makoukji et al., 2018) |
| *TSC1/ TSC2* | TSC (OMIM #613254)<br>FCORD2 (OMIM #607341) | Mouse, fruit fly, rat, zebrafish | -mTOR inhibitors (sirolimus, everolimus)<br>-vigabatrin (VGB), cannabidiol and the ketogenic diet | Established (Lechuga and Franz, 2019)<br>Potential (Kossoff et al., 2005; Curatolo et al., 2018; Schubert-Bast and Strzelczyk, 2021) |

BFNS1: Benign familial neonatal seizures-1; BFIS2: benign familial infantile seizures-2; CCDS1: cerebral creatine deficiency syndrome-1; CCDS2: cerebral creatine deficiency syndrome-2; CCDS3: cerebral creatine deficiency syndrome-3; CLN2: neuronal ceroid lipofuscinosis-2; DEE4: developmental and epileptic encephalopathy-4; DEE7: developmental and epileptic encephalopathy-7; DEE50: developmental and epileptic encephalopathy-50; DEE6B: developmental and epileptic encephalopathy-6B; DHFR deficiency: dihydrofolate reductase deficiency; DRVT: Dravet syndrome; DYT9: dystonia-9; EIG12: idiopathic generalized epilepsy-12; EKD1: episodic kinesigenic dyskinesia-1; ENFL1: nocturnal frontal lobe epilepsy-1; EPEO4: early-onset vitamin B6-dependent epilepsy-4; FCORD2: focal cortical dysplasia type II; FEB3A: familial febrile seizures-3A; GEFSP2: generalized epilepsy with febrile seizures plus, type 2; GLUT1DS1: GLUT1 deficiency syndrome-1; ICCA: familial infantile convulsions with paroxysmal choreoathetosis; LQT2: long QT syndrome-2; MTDPS4A: mitochondrial DNA (mtDNA) depletion syndrome-4A; MTHFR deficiency: methylenetetrahydrofolate reductase deficiency; NCFTD: neurodegeneration due to cerebral folate transport deficiency; SCAR7: spinocerebellar ataxia-7; SDCHCN: stomatin-deficient cryohydrocytosis with neurologic defects; SQT1: short QT syndrome-1; TSC: tuberous sclerosis.

for the treatment of epilepsy patients with definite pathogenic genes, offering personalized therapies that consider individual genetic, cellular, and metabolic characteristics (Sisodiya, 2021). Thus, identifying the disease-causing genes of patients is important in the design of precision medicine strategies. The identification of disease-causing genes often involves whole-exome sequencing in families or large cohorts of patients. However, interpreting the numerous rare variants with uncertain functional impacts remains challenging. Studies using model organisms, such as fruit flies, can provide complementary insights into disease mechanisms and contribute to a deeper understanding of pathogenic processes.

# *Drosophila Melanogaster*: a Versatile Model Organism for Epilepsy Research

*Drosophila melanogaster*, commonly known as the fruit fly, has been a pivotal model organism in genetic research for more than a century. Charles W. Woodworth's seminal proposal in 1901 recognized the fruit fly as an ideal model for genetic studies, and T. H. Morgan's discovery of white mutation and its linkage to the X chromosome further solidified its role in modern genetics in 1910. Since then, *Drosophila melanogaster* has become a cornerstone in various biological research areas, including genetics, physiology, microbial pathogenesis, and life history evolution (Rubin, 1988; Rieder and Larschan, 2014).

To date, six Nobel Prize-award studies have been conducted by *Drosophila melanogaster* in scientific exploration (St Johnston, 2002; Friedrich, 2011; Callaway and Ledford, 2017). As advancements in genetic tools and techniques have advanced, the utility of *Drosophila* has extended beyond genetics, with applications in evolutionary biology, developmental biology, cell biology, neurobiology, behavioral biology, immunology, etc. This model's broad and profound impact on biomedical research makes it an ideal candidate for investigating epilepsy-related genes and their functions, providing valuable insights that can inform precision medicine approaches in epilepsy treatment.

### *Drosophila melanogaster*: an efficient model for genetic research

The enduring prominence of *Drosophila melanogaster* as a model organism in genetic research can be attributed to its numerous advantages over other model organisms (Matthews et al., 2005; **Table 2**). These distinctive features contribute to its widespread utilization and make it a valuable asset in genetic studies.

#### *Short lifespan and rapid reproduction*

Fruit flies have a remarkably short generation time, with their entire life cycle spanning only 50–60 days. This rapid reproduction allows researchers to observe multiple generations in a relatively short period, making it easier to study genetic traits, mutations, and inheritance patterns. Each female fruit fly can lay hundreds of eggs, resulting in a large number of offspring in a single generation. This high reproductive rate provides ample opportunities for genetic experiments and statistical analysis, increasing the reliability of research findings (Ogienko et al., 2022).

#### *Simple genome and genetic tools*

*Drosophila* has a relatively small and well-characterized genome consisting of approximately 14,000 genes. This simplicity makes it easier to study individual genes and their functions, as researchers can easily manipulate and analyze the fly genome (Celniker and Rubin, 2003). Additionally, an array of extensive genetic tools and resources are available for *Drosophila* research. These include a wide range of mutant strains, genetic markers, and genetic mapping techniques, such as transgenic lines and RNA interference (RNAi), which facilitate the identification and analysis of specific genes and mutations (Huang et al., 2016).

#### *Conserved genes and genetic mechanisms*

Many fundamental genetic and molecular processes are conserved between fruit flies and humans. This conservation allows researchers to make inferences about human genetics based on *Drosophila* studies. Discoveries in *Drosophila* often hold relevance to human genetics and diseases.

#### *Ease of genetic manipulation*

*Drosophila* is amenable to genetic manipulation, allowing researchers to introduce, delete, or modify genes for the investigation of their roles in development, behavior, and disease.

#### *Visible phenotypes and well-characterized development*

Fruit fly genetics often result in readily observable phenotypes, such as eye color, wing shape, or body size. These visible traits make it easy to track and analyze genetic inheritance patterns and the effects of specific genes or mutations. Moreover, *Drosophila* development is well understood and highly stereotyped. The fly undergoes distinct stages of development, and the fate of individual cells is known with precision. This makes it an excellent model for studying developmental biology and the genetic control of development (Xie et al., 2023).

#### *Cost-effective research and ethical considerations*

*Drosophila* research is cost-effective and ethical, reducing concerns associated with experiments on vertebrate animals. Fruit flies lack complex pain perceptions and consciousness, minimizing harm during research (Staats et al., 2018).

#### *Well-organized databases and stock centers*

The *Drosophila* research community has a rich history spanning over a century, resulting in a vast body of knowledge and a wealth of genetic tools. FlyBase (http://flybase.org), which functions as an online "encyclopedia" of *Drosophila*, offers comprehensive information on genes and genomes (Drysdale and FlyBase Consortium, 2008; Rey et al., 2018; Larkin et al., 2021). Collaborative efforts and a spirit of generosity among researchers have facilitated the establishment of stock centers worldwide. These centers provide global access to essential genetic resources, including mutants and transgenic lines (**Table 3**).

These databases and stock centers are pivotal in advancing epilepsy research using *Drosophila* as a model organism, providing researchers with the necessary genetic tools and resources to investigate epilepsy-related genes and their functions.

**Table 2** | **Comparison of different model organisms**

| Species | Genome size | Human disease gene conservative rate | Neuron | Generation time | Database resource |
|---|---|---|---|---|---|
| *Saccharomyces cerevisiae* (brewer's yeast) | 0.12 Gbp | N/A | N/A | 90 min | SGD (https://www.yeastgenome.org/) |
| *Caenorhabditis elegans* (roundworm) | 0.10 Gbp | 65% | 302 | 2–3 d | WormBase (http://www.wormbase.org/) |
| *Drosophila melanogaster* (fruit fly) | 0.14 Gbp | 75% | 150000 | 10–14 d | FlyBase (http://flybase.org) |
| *Danio rerio* (zebrafish) | 1.4 Gbp | 82% | ~1000000 | 3–4 mon | ZFIN (http://zfin.org/) |
| *Mus musculus* (mice) | 2.8 Gbp | 99% | ~70000000 | 6–12 wk | MGI (https://www.informatics.jax.org) |
| *Rattus norvegicus* (rat) | 2.75 Gbp | 99% | ~200000000 | 3–4 wk | RGD (https://rgd.mcw.edu) |

N/A: Not applicable.

**Table 3** | **Fly resource centers**

| Name | Content | Website |
|---|---|---|
| FlyBase | Integrated database of *Drosophila* information | http://flybase.org |
| Bloomington *Drosophila* Stocks Center, BDSC | Comprehensive collection of *Drosophila* stocks | https://flystocks.bio.indiana.edu |
| Vienna *Drosophila* Resource Center, VDRC | Comprehensive collection of *Drosophila* stocks | http://stockcenter.vdrc.at/ |
| *Drosophila* Genetic Resource Center, DGRC | Comprehensive collection of *Drosophila* stocks | https://www.dgrc.kit.ac.jp |
| National *Drosophila* Resource Center of China, NDRCC | Comprehensive collection of *Drosophila* stocks | http://ndrcc.sibcb.ac.cn/ndrcc/ |
| Tsinghua Fly Center | Comprehensive collection of *Drosophila* stocks | https://thfc.zzbd.org |
| Core Facility of *Drosophila* Resource and Technology, CEMCS, CAS | Comprehensive collection of *Drosophila* stocks | http://sjzx.sibcb.ac.cn/Cn/Index/pageView/catid/105.html |
| DRSC/TRiP Functional Genomics Resources & DRSC-BTRR | *Drosophila* RNAi Screening Center (DRSC), Transgenic RNAi Project (TRiP) and *Drosophila* Research & Screening Center-Biomedical Technology Research Resource (DRSC-BTRR) | https://fgr.hms.harvard.edu |

***Drosophila melanogaster*: a compelling model for neurogenetic disorders and epilepsy research**

Understanding the intricate molecular and physiological underpinnings of neurogenetic diseases, such as epilepsy, demands comprehensive and innovative research approaches. In this context, *Drosophila melanogaster* has emerged as a formidable model organism, offering unique advantages that propel investigations beyond conventional boundaries. The following discussion delves into the multifaceted strengths of *Drosophila* as a tool for unraveling the complexities of neurogenetic diseases, focusing on conserved neural mechanisms, neuroanatomy and neuronal imaging, and diverse behavioral manifestations.

***Conserved neural mechanisms***

*Drosophila* shares a surprising degree of genetic conservation with humans in key pathways associated with neurogenesis, synaptogenesis, and neurotransmission (Donelson and Sanyal, 2015; Yildirim et al., 2019). Notably, conserved signaling pathways, such as the Notch, Wnt, and BMP pathways, play crucial roles in orchestrating neural development (Bray, 1998; Raftery and Umulis, 2012; Swarup and Verheyen, 2012). Manipulating these pathways in *Drosophila* via state-of-the-art genetic tools allows researchers to discern the functional consequences of specific genetic alterations. For instance, the clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR-Cas9) system, with its ability to edit genes with unparalleled precision, enables the creation of *Drosophila* models that faithfully recapitulate genetic variations associated with epilepsy in humans. This approach allows researchers to dissect the role of individual genes and their interactions in the context of seizure susceptibility (Bassett and Liu, 2014). Furthermore, the advent of single-cell RNA sequencing technology has enabled the comprehensive profiling of gene expression in specific neuronal populations, providing a nuanced understanding of how genetic alterations impact the molecular landscape of the *Drosophila* brain during epileptogenesis (Davie et al., 2018). Advances in functional genomics have significantly enhanced our ability to unravel the conserved neural mechanisms underlying neurogenetic diseases. Techniques like RNAi screening in *Drosophila* have proven invaluable in systematically identifying genes that influence neural processes relevant to epilepsy. By silencing specific genes and observing resulting phenotypes, researchers can discern the functional impact of individual genes on neural development and function. This approach, coupled with high-throughput technologies, allows for the rapid identification of candidate genes involved in epileptogenesis (Liu et al., 2023).

Beyond understanding individual gene functions, researchers have employed cutting-edge technologies like optogenetics to dissect the neural circuits involved in epilepsy. Optogenetic tools enable precise control of neuronal activity using light, allowing for the targeted activation or inhibition of specific neurons in *Drosophila*. This level of control is instrumental in delineating the contributions of distinct neural circuits to seizure susceptibility. By combining optogenetics with sophisticated behavioral assays, researchers can link alterations in specific neural circuits to observable epileptic phenotypes in fruit flies (Lim et al., 2021). These approaches provide a nuanced understanding of how evolutionarily conserved genes and pathways contribute to the complex landscape of neurogenetic diseases, shedding light on potential therapeutic targets for epilepsy and related disorders.

***Neuronal imaging and neuroanatomy***

The *Drosophila* brain is located in the cephalic shell, with the esophagus running through its center, and consists of two main parts: the suprapharyngeal and hypopharyngeal nerve regions. The supraesophageal zone primarily contains the jaw ganglion, which controls the activity of the mouthparts. It also includes three regions: the forebrain, midbrain, and hindbrain. The forebrain functions as the higher sensory center and includes structures like the optic lobes, formants, and central complex. The midbrain mainly contains the antennal lobes, whereas the hindbrain is located beneath the midbrain and fused with the jaw ganglia, making it challenging to identify. Through various neural labeling techniques, the *Drosophila* brain has been subdivided into 12 major sections, 47 neuromedullary structures, and 75 substructures (Peng et al., 2011; Ito et al., 2014). Although relevant simplicity of organization is shown in the fruit fly brain, many critical homologous brain substructures of humans can be found in the fruit fly. Previous studies have found that the mushroom body is indirectly connected to the fan-shaped body through excitatory neural circuits via the superior medial protocerebrum (Ito et al., 1998; Liu et al., 2006), transmitting visual information, learning, and emotional information. Therefore, the prefrontal cortex should correspond to the superior medial protocerebrum, while the hippocampus and amygdala correspond to the mushroom body (Raup and Seilacher, 1969). The details about homologous correspondence between the human and *Drosophila* brains are shown in **Table 4** (Strausfeld and Hirth, 2013).

*Neuronal imaging techniques*

Confocal microscopy allows for high-resolution imaging of neuronal structures by the use of fluorescent markers to label specific neurons or proteins. This technique enables the study of neuronal morphology, synaptic connections, and structural changes over time (Li et al., 2021). Two-photon microscopy provides deeper tissue imaging with reduced phototoxicity, making it suitable for live imaging of neuronal activity and long-term observations. It allows the visualization of dynamic processes such as synaptic plasticity and neuronal signaling in real time (Seelig et al., 2010). Light-sheet microscopy offers rapid imaging of large volumes with minimal photodamage, making it useful for whole-brain imaging and tracking of neuronal circuits. It supports detailed analysis of brain development and disease progression (Chen et al., 2014). Electron microscopy delivers ultra-high resolution images of neuronal ultrastructures, which are essential for examining synaptic architecture and subcellular components. This technique complements light microscopy by providing detailed anatomical information at the nanometer scale (Eckstein et al., 2024).

*Neuroanatomical mapping*

The GAL4/UAS (Gal4/upstream activating sequence) system facilitates the targeted expression of fluorescent proteins in specific neuronal populations. The LexA/LexAop and QF/QUAS systems provide additional layers of control for labeling and manipulating neurons (Wendler et al., 2022). The Split-GAL4 technique enhances specificity by combining two independent GAL4 lines to label a more restricted set of neurons (Meissner et al., 2023; Rubin and Aso, 2024). Connectomics involves the reconstruction of neuronal circuits through serial electron microscopy and image analysis (Shih et al., 2015; Lo and Chiang, 2016). This approach maps synaptic connections to understand network dynamics and information processing, integrating functional imaging data to correlate anatomical and physiological properties (Lin et al., 2015). Linking neuroanatomical data with behavioral outputs helps identify the neural substrates of specific behaviors.

The combination of sophisticated neuronal imaging techniques and comprehensive neuroanatomical mapping in fruit flies enables researchers to dissect the complex mechanisms underlying neurogenetic disorders and epilepsy, which offers insights into the mechanisms underlying epilepsy and potential targets for therapeutic interventions. This model organism offers unparalleled advantages in visualizing and manipulating neuronal circuits, providing insights that are translatable to higher organisms, including humans. This approach offers insights into the mechanisms underlying epilepsy and potential targets for therapeutic interventions.

**Table 4** | **Homologous correspondence between the human and *Drosophila* brains**

| | Human | Drosophila |
|---|---|---|
| Based on genetic and embryonic perspectives | Basal ganglia | Central complex |
| | Striatum | Fan-shaped body, protocerebral bridge |
| | GPe and GPi | Ellipsoid Body |
| Based on projection and neural function | Frontal cortex | SMP |
| | Amygdala, hippocampus | Mushroom body |
| | Somatosensory cortex | IMP, ILP |
| | Subthalamic nucleus | PPM1 and PPM3 neurons |
| | Thalamus | Lateral accessory lobe |
| | Motor cortex | ILP, VLP |
| | Spinal cord | ventral cord and ganglia |

GPe: External globus pallidus; GPi: internal globus pallidus; ILP: inferior lateral protocerebrum; IMP: intermediate lateral protocerebrum; PPM1/3: paired posterior medial 1/3; SMP: superior medial protocerebrum; VLP: ventrolateral protocerebrum.

***Diverse behavioral manifestations***

Behavior represents one of the most complex and dynamic aspects of an organism, reflecting its interactions with and adaptations to a constantly changing external environment. In fruit flies, a wide array of behaviors—including locomotion, grooming, feeding, communication, flight, migration, learning, reproduction, and responses to environmental stimuli—can be observed and systematically analyzed (Benzer, 1971; Maier, 1984; Vosshall, 2007; Garber et al., 2012; Yang et al., 2013; Anderson and Adolphs, 2014). This diversity highlights the complexity of the fruit fly's nervous system and shows its utility as a model organism for studying the intricate relationship between genetics and behavior.

*Drosophila* exhibit both innate and learned behaviors crucial for survival and reproduction, which are often observed and quantified in controlled laboratory settings. Movement assays provide insights into motor coordination, grooming patterns help examine the interplay between sensory inputs and motor outputs, and feeding behavior can be analyzed to understand appetitive and aversive responses. The ability of *Drosophila* to exhibit both innate and learned responses to environmental cues makes it an ideal model for studying behavioral plasticity. Researchers leverage paradigms like habituation, sensitization, and associative learning to investigate how organisms adapt to repeated stimuli and respond to novel or significant stimuli. By manipulating specific genes or neural circuits, researchers can dissect the mechanisms underlying these forms of learning and memory (Qiao et al., 2022). *Drosophila*'s responsiveness to environmental stressors such as temperature fluctuations and toxin exposure allows for exploring gene-environment interactions. Studies in this domain provide valuable insights into how genetic predispositions interact with external factors to influence behavior (Wang et al., 2018). Advanced behavioral assays, combined with sophisticated genetic tools like optogenetics and thermogenetics, provide a powerful framework for understanding the neural and genetic basis of behavior. These tools allow precise control of neuronal activity, enabling researchers to probe the function of specific neural circuits in real time. High-throughput behavioral assays facilitate large-scale screening of genetic mutants to identify novel genes involved in behavior.

Fruit fly's diverse behavioral manifestations, coupled with its amenability to genetic manipulation and advanced behavioral assays, make it a premier model organism for unraveling the complex interplay between genetics and behavior. This comprehensive approach enhances our understanding of normal and abnormal behaviors and offers critical insights into the mechanisms underlying neurogenetic disorders, paving the way for innovative therapeutic strategies.

## Methods for Epilepsy Research Utilizing *Drosophila*

In light of the aforementioned benefits of *Drosophila* as a model organism, an increasing array of genetic manipulation tools has been developed. Moreover, diverse methods encompassing behavioral analyses, neurobiological approaches, and memristor-clamp utilization have been employed (Takai et al., 2020). These advancements have collectively contributed to the establishment of a variety of research strategies.

### Genetic manipulation for inducing the *Drosophila* model

To explore gene function, two primary types of manipulation methods are commonly employed. Loss-of-function (LOF) approaches aim to either partially or completely eliminate gene functions, whereas gain-of-function (GOF) approaches seek to acquire functional information by creating conditions where the gene is excessively or ectopically expressed or whose function is exaggerated. Some common methods used for gene manipulation and their applications in *Drosophila* research are as follows:

***Mutant alleles***

LOF mutant alleles can be induced through various techniques, including chemical mutagenesis, radiation-induced mutagenesis, or more precise methodologies like CRISPR/Cas9 genome editing (Obe et al., 1971; Bassett and Liu, 2014; Sekelsky, 2017; Koreman et al., 2021). These alleles typically lead to a partial or complete loss of gene function, enabling the study of the effects resulting from the absence or reduction of gene expression. Conversely, GOF mutant alleles can be engineered to confer novel or enhanced functions to the gene product, leading to altered protein properties, such as increased activity or modified substrate specificity.

CRISPR/Cas9 technology has revolutionized the field of genetics by allowing precise and targeted the modification of genes (Doudna and Charpentier, 2014). This method involves designing guide RNAs that specifically bind to a target gene, directing the Cas9 enzyme to induce double-strand breaks. In the repair process, errors may occur, leading to the creation of LOF or GOF mutant alleles. By controlling the design of guide RNAs, researchers can achieve gene knockout (LOF) or introduce specific mutations for GOF studies. In *Drosophila*, this approach facilitates the creation of LOF or GOF mutant alleles, providing a powerful tool for dissecting gene functions. Whether through gene knockout for LOF or introducing specific mutations for GOF studies, CRISPR/Cas9 allows for fine-tuned genetic manipulations in the fruit fly.

***RNA interference***

RNAi technology has been a cornerstone in genetic research, especially in model organisms like fruit flies. This powerful tool allows for the specific silencing of genes through the introduction of double-stranded RNA, triggering sequence-specific degradation of homologous mRNA molecules. In *Drosophila*, RNAi has been extensively utilized to investigate gene function, dissect signaling pathways, and study various biological processes. The simplicity, efficiency, and specificity of RNAi make it invaluable for both large-scale genetic screens and targeted gene knockdown experiments. Moreover, the advent of RNAi libraries and transgenic RNAi lines has facilitated high-throughput functional genomics studies in *Drosophila*, enabling researchers to systematically interrogate the role of individual genes in diverse biological contexts. RNAi technology continues to play a central role in advancing our understanding of gene function and regulation in *Drosophila*, serving as a foundation for numerous discoveries in developmental biology, neurobiology, and beyond (Mohr, 2014; Heigwer et al., 2018).

***NanoTag nanobody tools***

Nanobodies are special proteins derived from camelids and sharks that have been transformed to study protein functions in *Drosophila*. These tiny proteins, about one-tenth the size of regular antibodies, can access hidden spots on proteins that larger antibodies cannot access. They are incredibly stable and precise, making them ideal for experiments. With detailed protocols established for nanobody production and expression in *Drosophila* S2 cells and bacterial cells, researchers can efficiently generate and purify nanobodies for a variety of experimental applications, including immunostaining, immunoblotting, and immunoprecipitation (Kim et al., 2022). Functionally engineered nanobodies, such as chromobodies and NanoTag traps, enable the visualization and alteration of protein localization *in vivo* (Xu et al., 2022). Moreover, CRISPR-mediated gene targeting facilitates the tagging of endogenous proteins with NanoTags, simplifying the study of protein dynamics and function (Lepeta et al., 2022).

NanoTags are small sequences of amino acids that can be joined to proteins that the investigators want to study. When a nanobody that recognizes a specific NanoTag is added, it sticks to the protein with great accuracy. This allows us to see and manipulate the protein without disturbing it. Nanobody-based methods for *in vivo* cell labeling, such as the NaNuTrap, allow for early and specific labeling of cell nuclei, overcoming limitations of fluorescent protein maturation time and expanding the utility of fluorescent proteins for live imaging studies in fast or early developmental processes.

Overall, nanobodies provide versatile and validated tools for a wide array of applications in *Drosophila* research, contributing to a deeper understanding of protein function and dynamics in multicellular organisms.

***Inducible expression systems***

Inducible expression systems offer a versatile approach to modulating gene expression levels in fruit flies, providing researchers with precise control over the timing and magnitude of gene activation or repression. Among these systems, the GAL4/UAS system stands out as one of the most widely used and effective tools. This system leverages the yeast transcription factor GAL4, which binds to the UAS element to drive the expression of target genes when activated. By employing tissue-specific GAL4 drivers, researchers can restrict gene expression to particular cell types or tissues, allowing for spatial control over gene activity. GAL80 protein can prevent GAL4-dependent transcription at 18°C, but it is inactivated at 29°C. Thus, by combining GAL4/UAS with GAL80 at specific temperatures, researchers can restrict gene expression to

particular developmental stages, allowing for temporal control over gene activity (**Figure 1**). This temporal regulation is particularly advantageous for studying dynamic processes such as embryonic development, tissue regeneration, or neuronal activity patterning. Moreover, inducible expression systems enable the manipulation of gene expression levels in a reversible manner, facilitating the investigation of both the short- and long-term effects of gene perturbations. By modulating the timing and duration of gene activation or silencing, researchers can uncover intricate gene regulatory networks, elucidate gene function in specific contexts, and dissect the molecular mechanisms underlying complex biological processes.

### Behavioral analysis of seizures in *Drosophila melanogaster*

Behavioral analysis of seizures and seizure-like characteristics in *Drosophila melanogaster* has been instrumental in elucidating the intricate genetic and molecular mechanisms underlying epilepsy. Despite substantial differences between the nervous systems of fruit flies and mammals, researchers have devised a plethora of assays to observe and analyze behaviors associated with seizures or altered neuronal activity. This comprehensive approach has yielded invaluable insights into the nature of epileptic phenomena and has provided a robust platform for investigating epilepsy-related disorders.

Since the groundbreaking identification of bang-sensitive mutants by Benzer in 1971, *Drosophila* has served as a pivotal model organism in epilepsy research. These mutants display characteristic seizure-like behavior and paralysis triggered by mechanical shocks, providing a tangible manifestation of epileptic events (Parker et al., 2011). The behavioral phenotype of these mutants can be delineated into distinct stages, ranging from the shock-induced initial seizure to subsequent phases of paralysis, tonic–clonic activity, and recovery, mirroring the complex nature of epileptic seizures in humans. One notable class of *Drosophila* mutants, exemplified by the bang senseless (*parabss1*) mutant, has shed light on the role of voltage-gated sodium channels in epilepsy. The identification of GOF mutations in the paralytic (*para*) gene, which encodes the sole *Drosophila* voltage-gated sodium channel α-subunit, underscores the relevance of *Drosophila* models in studying intractable epilepsy (Pavlidis and Tanouye, 1995). Moreover, diverse classes of mutants, such as temperature-sensitive paralytic mutants and leg-shaking mutants, encoding various potassium channel subunits, have contributed significantly to our understanding of epilepsy pathogenesis.

Recent research has identified several novel seizure behaviors that further highlight *Drosophila*'s utility in epilepsy studies (Liu et al., 2023). Knockdown of *Tango14* results in hyperactive seizure behavior, characterized by increased movement and excitement, similar to hyperesthesia and consistent with the hyperactive-automatism subtype in complex partial epilepsy. Conversely, knockdown of *Klp3A* results in late-phase seizure behavior, which may correlate with focal epilepsy originating from the sensory cortex. These findings were validated by the demonstration of hyperactivity seizure behavior in the knockdown of the *Ywhaz* gene (Wan et al., 2023), suggesting that this novel behavior could be used to verify other epilepsy candidate genes. These results imply that the loss of function in different candidate genes can result in distinct phenotypes, necessitating further investigation into the genotype–phenotype relationship in *Drosophila*. The novel seizure behaviors are summarized in **Figure 2**.

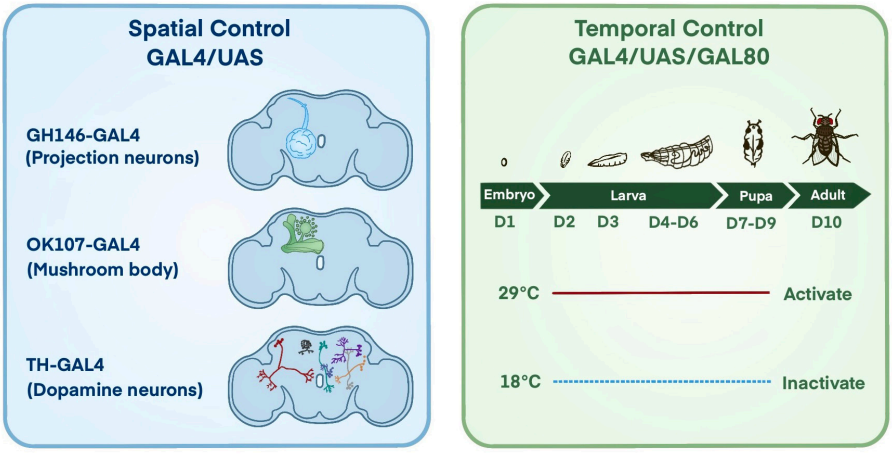


**Figure 1 | Spatiotemporal transgenic manipulation by the GAL4/UAS/GAL80 system.**
The GAL4/UAS (Gal4/upstream activating sequence) system provides spatial manipulation of genetic tools. GH146-GAL4, OK107-GAL4, and TH-GAL4 are specifically expressed in the projection neurons, mushroom bodies, and dopamine neurons of the fly brain. Combined with the UAS responder, the transgene is expressed only in those cells/tissues that express the GAL4 protein. GAL80 suppresses GAL4 activity at 18°C, but this inhibitory effect is relieved at 29°C. Thus, the expression of the transgene can be temporally controlled by modulating the temperature during specific developmental stages of the fly using the GAL4/UAS/GAL80 system. UAS: upstream activating sequence.

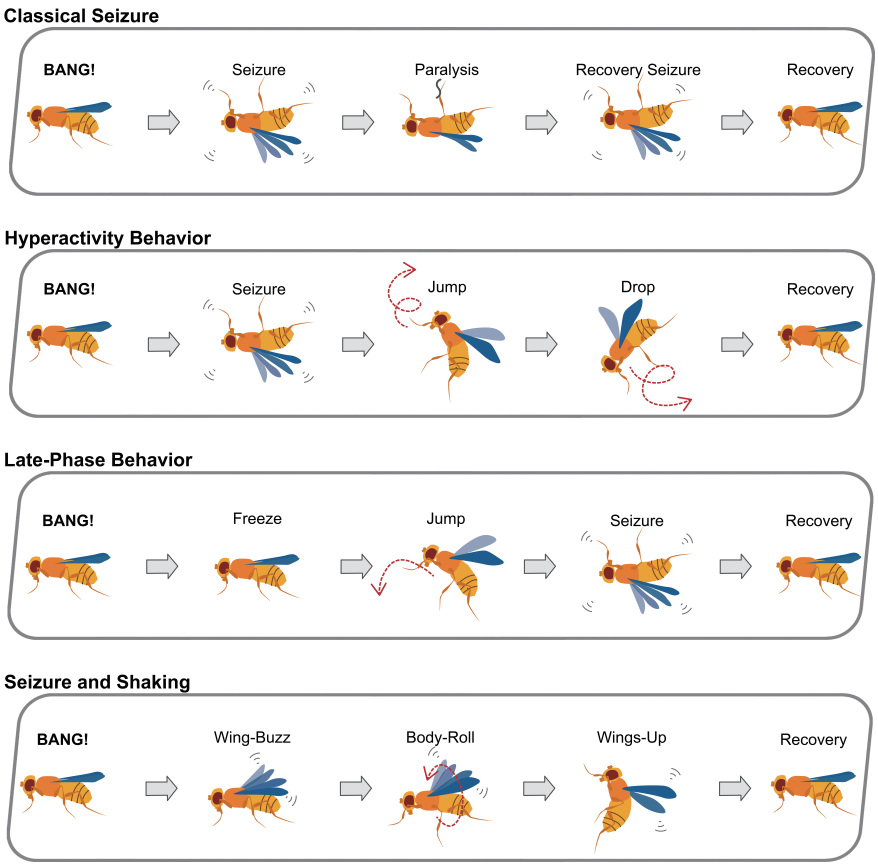


**Figure 2 | Classical and novel seizure behaviors of *Drosophila*.**
Classical seizure behavior contains the seizure, paralysis, and recovery stages. The other three novel seizure behaviors usually contain special behaviors at the seizure stage. For instance, hyperactivity behavior involves jumping and dropping, late-phase behavior involves freezing, and shaking behavior involves wing buzzing.

In addition to genetic mutation, advanced analytical techniques have revolutionized the behavioral analysis of seizures in *Drosophila*. High-throughput video tracking systems allow researchers to monitor large groups of flies simultaneously, capturing subtle changes in behavior associated with seizures. Electroconvulsive seizure assays provide a standardized method for inducing seizures, facilitating comparisons across different genetic backgrounds or experimental conditions. Seizure-induced locomotor deficits and optogenetic stimulation further enable the precise manipulation and observation of neuronal activity associated with seizures. Accurate quantification of seizure duration and frequency using advanced imaging and analysis techniques has emerged as a crucial aspect of *Drosophila* epilepsy research. By precisely measuring the timing and characteristics of epileptic-like behaviors, researchers can compare different genetic models and identify factors influencing the severity and recurrence of seizures in fruit flies.

## Approaches for recording neural electrical activity

Excessive neuronal activity is one of the major features of epilepsy. Thus, neural activity recording is an indispensable procedure to validate epilepsy candidate genes. Nowadays, numerous neural activity recording techniques have been established to solve individual requirements in neurological studies. The characteristics of the neural activity recording methods used in the *Drosophila* model are summarized in **Table 5**.

### *Single-cell patch clamp recording*

The patch clamp, developed by Erwin Neher and Bert Sakmann in the late 1970s, is a powerful and widely used method for recording the electrical activity of individual cells, particularly neurons (Elamin et al., 2023).

The primary configurations, including whole-cell patch clamp and cell-attached patch clamp, allow researchers to acquire ion currents, voltage changes, and other electrophysiological parameters, which have since revolutionized our understanding of the functions of neurons, ion channels, and membrane conductance (Davie et al., 2006; Alcami et al., 2012; Petersen, 2017; Howard et al., 2022).

The patch clamp has a wide range of applications in neuroscience, cell biology, and pharmacology. Particularly, it is used to investigate the underlying mechanism of epilepsy. In cell culture, $K^+$ currents were recorded to identify disease-related variants of *KCNQ5* in genetic generalized epilepsy and elucidate the underlying mechanism (Krüger et al., 2022). In mouse models, miniature inhibitory postsynaptic currents of the $GABA_A$ receptor α1 subunit were recorded to investigate the underlying mechanism of juvenile myoclonic epilepsy (Arain et al., 2015). Recently, a cell-attached patch clamp was performed on *Drosophila* to validate the pathogenicity of *UNC13B* in epilepsy (Wang et al., 2021).

### *Synaptic signal recording*

Considering the gene mutations related to synaptic structure or function that have been observed in epileptic patients (Lammertse et al., 2020), alterations in synaptic connections and transmission should be considered when investigating the underlying mechanisms of epileptic candidate genes. Recently, evoked EPSP recording in *Drosophila*, using a stimulus electrode on the antennal lobe and a recording electrode on the mushroom body neuron, has been applied to assess synaptic activity from the antennal lobe to the mushroom body (Liu et al., 2023). Besides, this approach can also be applied to study timing-dependent synaptic plasticity (Qiao et al., 2022).

Additionally, the giant fiber system of *Drosophila* is a renowned neuronal circuit that facilitates the escape behavior response in flies. In flies, the two largest interneurons transmit signals from the brain to the mesothoracic neuromere, where each interneuron generates two distinct synapses. This characteristic makes the giant fiber system an appealing model for the study of neuronal circuitry (Augustin et al., 2011), which has been adopted to study the underlying mechanism of seizures and genetic alterations (Pavlidis and Tanouye, 1995; Lee and Wu, 2002).

**Table 5** | **Characteristics of neural activities recording methods for *Drosophilas***

| | Single-cell patch clamp | Synaptic signals | Calcium imaging |
|---|---|---|---|
| Advantage | Offers the advantage of directly recording current and voltage signals in living cells with submillisecond and subthreshold resolution (Peng et al., 2022b), making it one of the most effective methods for studying cellular activities. It enables precise control and measurement of membrane potentials, allowing for the determination of the activities of individual or specific types of ion channels. | This method accurately reflects the connections and interactions between neurons, which is crucial for studying the signal transduction (Liu et al., 2023). Moreover, it can also be adopted to study the timing-dependent synaptic plasticity (Qiao et al., 2022). | It enables visualization of neural activity over a wide range, from subcellular resolution to the entire organism with outstanding spatial resolution (Mann et al., 2017). Calcium imaging, with the use of specific calcium indicators, can reflect the activity state of neurons. |
| Limitation | The operation is challenging and requires specialized techniques and equipment. Furthermore, as it is a single-cell level measurement, it may not reflect more complex neuronal network activities such as interactions and population dynamics (Peng et al., 2022b). | Synaptic signal recording requires sophisticated equipment, involves challenging experimental techniques, and necessitates advanced instrument manipulation skills for dissecting and isolating specific regions at the cellular level. | Typically, the temporal resolution of calcium imaging is lower than that of electrophysiological techniques, requiring the use of changes in calcium ion concentration as an indirect measure of neuronal electrical activity. |

### *Field potential recording*

Local field potentials (LFPs) refer to the electrical activity recorded from a group of neurons in a localized area of the brain and are commonly used to study the collective behavior of neurons and their communication within a specific brain region. LFPs have provided insights into various brain processes, including sensory processing, motor control, and cognitive functions.

The study by Iyengar and Wu (2021) introduced an innovative approach for observing and analyzing LFPs in *Drosophila*, providing new insights into the intricate dynamics of neuronal activity during seizures. The researchers devised a pioneering method to monitor ensemble neural activity in tethered, behaving flies during seizures by recording LFPs from the *Drosophila* brain. Through the application of high-frequency stimulation across the brain, they effectively elicited a stereotypic sequence of electroconvulsive seizure spike discharges, accompanied by behavioral spasms. Importantly, the LFP signal during seizure episodes exhibited distinct characteristics, including large-amplitude oscillations and a temporal correlation with muscle spiking in the dorsal longitudinal muscle. These electroconvulsive seizure-related LFP events were clearly distinguished from LFP patterns observed during rest and flight.

### *Calcium imaging*

The concentration of calcium ions is critical as a cellular messenger, and fluctuations in intracellular calcium levels are crucial indicators of synaptic transmission and neuronal activity (Tian et al., 2009). Since its establishment in the 1970s, calcium imaging has proven to be an effective approach for monitoring neuronal activity (Moisescu et al., 1975; Blinks et al., 1976; Grienberger and Konnerth, 2012). In presynaptic terminals, the exocytosis of neurotransmitters is triggered by the influx of calcium (Neher and Sakaba, 2008). On the postsynaptic membrane, calcium transients in dendritic spines are crucial for inducing activity-dependent synaptic plasticity (Zucker, 1999). In the nucleus, calcium signals also play a vital role in regulating gene transcription (Lyons and West, 2011). Previously, calcium transients were observed in depolarizing neurons, indicating the alteration of intracellular calcium transients are strongly correlated with the electrical excitability of neurons (Neher and Sakaba, 2008; Russell, 2011). Due to the well-developed genetically encoded calcium indicators and confocal microscope techniques developed in recent decades, calcium imaging has been utilized to study the correlation between functional alterations and epilepsy-related genes in cell culture (Marsh, 1995), brain slices (Leweke et al., 1990), and *in vivo* (Hewapathirane et al., 2008; Yang and Yuste, 2017). Furthermore, calcium imaging has also been employed in flies to screen for anticonvulsive compounds (Streit et al., 2016).

A recently established *ex vivo* calcium imaging model was used to monitor epileptiform activities in GFP calmodulin protein 6 (GCaMP6)-expressing adult *Drosophila* (He et al., 2023a). This model preserves the integrity of the brain and protects neural networks from damage. The *ex vivo* approach offers a superior signal-to-noise

ratio, making it an efficient method for screening epilepsy-related genes and investigating the cellular mechanisms underlying epilepsy.

## Strategic Approaches Utilizing *Drosophila* for Studying Epilepsy-Related Genes

In the study of epilepsy in *Drosophila*, both forward and reverse genetics approaches can be valuable tools for understanding the genetic basis of the condition.

### Forward genetics approach

A particular strength of *Drosophila* is the possibility to perform unbiased screens for genes that regulate or mediate biological processes of interest, often referred to as forward genetics. Forward genetics involves inducing random mutations and then screening for specific phenotypes of interest (Sheardown et al., 2022). In the context of epilepsy research in *Drosophila*, forward genetics can be used to identify novel genes and pathways involved in seizure disorders.

In the forward genetics strategy, mutations are induced randomly, and animals are screened for a specific phenotype. These mutations can be induced chemically, for instance, using ethyl methane sulfonate or transposons. Alternatively, mutants can be isolated by screening an RNAi library or a collection of existing deficiencies. This unbiased approach aids in identifying uncharacterized mutations in known disease genes, leading to phenotypic expansion, as well as discovering genes not previously associated with disease. Forward genetics serves as a powerful method for uncovering previously unknown genes and revealing intricate biological phenomena.

### Reverse genetics approach

Virtually every gene of *Drosophila* is amenable to targeted manipulations through a wide range of available genetic strategies and tools, which are ideal for performing reverse genetics. Reverse genetics involves deliberately introducing mutations into specific genes of interest and then studying the resulting phenotypes (Adams and Sekelsky, 2002). In the context of epilepsy research in *Drosophila*, reverse genetics can be used to investigate the function of genes homologous to those implicated in human epilepsy.

In the reverse genetics approach, deliberate mutations are introduced into fly homologs of human genes, enabling researchers to meticulously examine their phenotypes *in vivo*. Three primary methods are employed to reduce or eliminate gene expression in flies: targeted gene disruption, as illustrated by technologies such as CRISPR/Cas9; transposon-mediated mutagenesis and excision of existing transposable elements; and gene silencing, achieved through RNAi or CRISPR (Losurdo et al., 2024). In addition to LOF studies, researchers can also overexpress a wild-type or mutant version of a human disease-causing gene (transgene) in flies to assess its effects in specific tissues.

By combining both forward and reverse genetics approaches in *Drosophila* research on epilepsy, researchers can gain insights into the underlying genetic mechanisms of the disorder and identify potential targets for therapeutic intervention. However, forward genetics suffers from low efficiency in identifying new genes and pathways, as mutations are randomly induced, making it challenging to study specific genes or pathways, and the understanding of the functional significance of identified genes is limited. Conversely, reverse genetics, while allowing targeted investigation of gene function, may lead to off-target effects and incomplete loss of gene function, as well as difficulties in reproducing complex genetic interactions and a limited understanding of noncoding regions.

### Diagnostic strategies

Building upon traditional genetic research strategies, a set of genetic methodologies, referred to as "diagnostic strategies," has recently been established to aid in the discovery of human disease-causing genes. This innovative approach involves systematic and targeted efforts to identify genes associated with specific human diseases, and it is currently frequently employed in the identification of candidate disease-causing genes for epilepsy. Unlike traditional forward and reverse genetics, which focus on one-way studies, the diagnostic strategy is characterized by a more proactive and disease-centric approach. Initiated by the patient's clinical phenotype, the diagnostic strategy employs a forward genetics-based screening approach to identify potential causative genes. Bioinformatics and computational analyses are then conducted to screen for candidate causative genes. The strategy subsequently transitions into a reverse genetics-based screening approach involving the processing and modification of target genes in *Drosophila*, thereby pinpointing epilepsy-causing genes and marking rare gene-associated variants.

This innovative diagnostic strategy can be divided into two main components: clinical screening and *in vivo* identification.

Part 1: Screening candidate epilepsy-causing genes using whole-exome sequencing technology. Diagnostic strategies in epilepsy typically commence with a targeted focus on patients, leveraging whole-exome sequencing to extract a multitude of candidate genetic variants associated with the disease phenotype. This sequencing extends to samples obtained from the patient, their parents, or siblings, yielding comprehensive genetic insights. The intricate analysis of extensive genomic data is made possible through advanced bioinformatics and computational tools, playing a pivotal role in variant identification, assessment of potential pathogenicity, and prioritization of candidate disease-causing genes. The reliance on collaborative efforts is a hallmark of diagnostic strategies, necessitating the formation of research networks that unite clinicians, geneticists, bioinformaticians, and other domain experts. This collaborative synergy serves multiple purposes: resource pooling, data sharing, and the establishment of large-scale studies.

Part 2: Identification of candidate epilepsy-causing genes or rare epilepsy-associated variants in *Drosophila*. Once potential disease-associated genes are pinpointed through diagnostic strategies, the next crucial step involves conducting functional validation studies to unravel their role in disease pathogenesis. These studies employ a range of techniques, including model organisms, cell culture systems, and advanced *in vitro* and *in vivo* assays, to confirm how genetic variations impact cell function. In the context of *Drosophila* models used for identifying epilepsy candidate genes, the process typically begins with identifying the *Drosophila* homologs of the candidate causative genes associated with epilepsy. Subsequently, a *Drosophila* model is constructed for further investigation.

One approach involves knocking out the fly homolog or ortholog by integrating a GAL4 gene under the control of endogenous regulatory elements, followed by an assessment of the phenotype. If the phenotype can be rescued by expressing the wild-type UAS–human cDNA but not by the human variant, causality is established. Alternatively, *Drosophila* homozygous gene lines have been created by incorporating UAS and RNAi. These lines are then crossed with *Drosophila* lines expressing Gal4 specifically in cells, tissues, or organs, achieving conditional knockdown of the target gene. Temporal regulation of target genes can also be achieved using regulatory factors. For instance, Gal80, a negative regulator of Gal4, inhibits its transcriptional activation. The use of temperature-sensitive Gal80ts allows precise control over RNAi by altering the temperature. At lower temperatures, Gal80 maintains its activity, inhibiting Gal4, whereas at higher temperatures, Gal80 inactivation releases the inhibition, activating downstream DNA transcription (Tepe et al., 2023). The Gal4/UAS system facilitates targeted interference only when both Gal4 and UAS are present in the same *Drosophila*, simplifying strain conservation and the establishment of corresponding repositories.

In recent years, a growing number of reported epilepsy-causing genes have been identified. The use of *Drosophila* for screening epilepsy-related genes has proven to be an efficient diagnostic strategy. This approach not only aids in identifying epilepsy-causing genes or rare variants associated with epilepsy but also facilitates large-scale drug screening and the formulation of precise treatment plans (**Figure 3**).

#### *Utilizing Drosophila to identify partial epilepsy-associated UNC13B*

The *UNC13B* gene is responsible for encoding the presynaptic protein known as mammalian uncoordinated 13-2 (Munc13-2). This protein is prominently expressed in the brain, particularly in the cerebral cortex. It assumes a crucial role in synaptic vesicle priming and fusion, influencing neuronal excitability. Nevertheless, the functional implications of the *UNC13B* mutation in human diseases remained unclear until Wang et al. (2021) shed light on the subject. They identified eight unrelated Chinese patients with varying forms of partial epilepsy, all linked to heterozygous (seven patients) or compound heterozygous (one patient) variants in the *UNC13B* gene. This discovery emerged from a cohort of 446 partial epilepsy patients who underwent trio-based whole-exome sequencing. Encouragingly, all patients responded well to medication and exhibited normal development, suggesting a benign disease course.

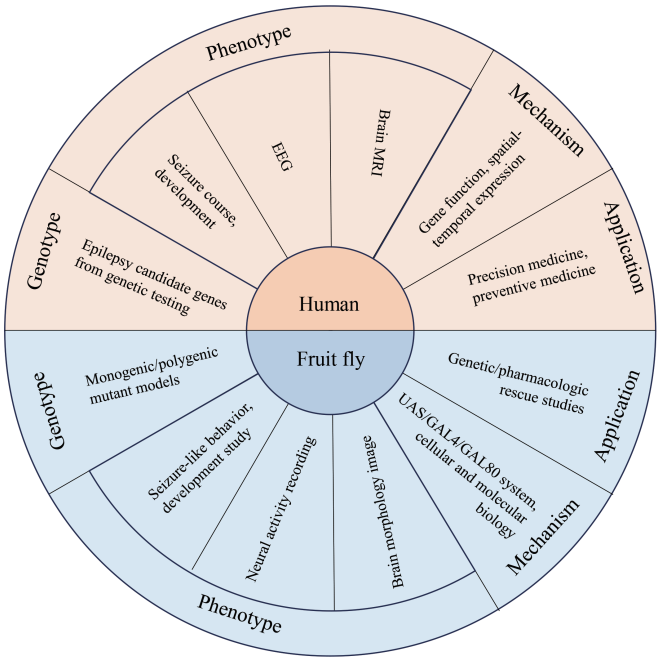


**Figure 3** | **Strategy of validation of epilepsy-related genes through a fly model.**
The pie chart illustrates the main mechanism or strategy of the validation of epilepsy-related genes through the fly model. EEG: Electroencephalography; MRI: magnetic resonance imaging; UAS: upstream activating sequence.

In their efforts to further identify *UNC13B* as a causative gene for epilepsy, Wang et al. (2021) utilized the UAS-GAL4 system to construct *tub-Gal4>Unc13b-RNAi Unc13b* knockdown flies. Intriguingly, the knockdown of *Unc13b* in *Drosophila* was lethal at the preadult stage, indicating a crucial role for the gene in early life. Through temporally controlled knockdown experiments, they achieved fly survival, and the resulting mutant flies exhibited an elevated seizure frequency and prolonged recovery time in the bang sensitivity test compared with those of the controls. Electrophysiological investigations of projection neurons revealed a significantly higher frequency of extracellular action currents in mutant flies compared to controls, although there was no discernible difference in action current amplitude. To assess potential alterations in brain morphology, UAS-mCD8::GFP was employed to generate *tub-Gal4>UAS-mCD8::GFP/UAS-Unc13b-RNAi* and *tub-Gal4>UAS-mCD8::GFP* strains, representing GFP-tagged *Unc13b* knockdown and control flies, respectively.

***Exploring the epileptogenic mechanism of the familial focal epilepsy-related gene NPRL3 in Drosophila melanogaster***

The mTORC1 pathway plays a pivotal role in modulating responses to environmental stimuli, including nutrients and growth factors. The GATOR1 protein complex, comprising NPRL3, DEPDC5 (DEP domain-containing protein 5), and NPRL2 (nitrogen permease regulator-like 2), exerts an inhibitory influence on the mTORC1 pathway. Since 2013, an increasing number of reported mutations in GATOR1 have been associated with diverse phenotypes in individuals with epilepsy, with varying degrees of disease severity. Most NPRL3 mutations result in nonsense-mediated mRNA decay or loss of function. However, the precise mechanisms through which these mutations activate the mTOR pathway and downstream signaling events triggered by mTOR activation remain unknown.

Du et al. (2023) reported three families with focal epilepsy and identified three novel heterozygous germline mutations in the NPRL3 gene through linkage analysis, whole-exome sequencing, and Sanger sequencing. To elucidate the detailed pathogenic mechanism, this study is the first to utilize a *Drosophila* model, revealing that *nprl3* RNAi in *Drosophila* induces epileptic-like behavior in a bang sensitivity test. Furthermore, knockdown of any component of the *Drosophila* GATOR1 complex (*nprl3*, *nprl2*, and *iml1*) induced epileptic-like behavior in *Drosophila*. These findings further support the involvement of defects in GATOR1 signaling in epileptogenesis. Additionally, global knockdown of *nprl3* led to significant overgrowth of the synaptic terminal at the NMJ. Synapse turnover, a highly dynamic and regulated process, was affected, with increased bouton numbers in *nprl3* RNAi flies, suggesting that *nprl3* inhibits bouton formation and/or promotes bouton elimination at the NMJ. Elevated glutamate receptor levels in these flies indicated an excitation–inhibition imbalance, a major contributor to seizures, as type I boutons primarily contain the excitatory neurotransmitter glutamate. These morphological changes in larval NMJs demonstrate developmental defects in these flies, potentially contributing to epilepsy-like behaviors in adults. To validate this, attempts to specifically knock down *nprl3* during development via the tub-GAL80$^{ts}$ system were unsuccessful, with flies not surviving to adulthood, possibly because of a combination of *nprl3* deficiency and high developmental temperatures (29°C). Knocking down *nprl3* specifically in adults did not enhance epilepsy-like behavior, strongly suggesting that the lack of nprl3 during development is necessary for the epilepsy-like phenotype.

***Unraveling the role of MTSS2 and its point mutation (R671W) in epilepsy-related phenotypes through Drosophila modeling***

MTSS2, also known as MTSS1L, binds to plasma membranes and influences their curvature. It is prominently expressed in the central nervous system and is implicated in activity-dependent synaptic plasticity. Despite its known functions, the gene did not exhibit a clear association with any human disease, and its biological role remained elusive until Huang et al. (2022) conducted Trio exome sequencing on five patients from unrelated families with intellectual developmental disorders with ocular anomalies and distinctive facial features. In their study, they identified a *de novo* heterozygous missense mutation (R671W) in the *MTSS2* gene.

To investigate the impact of the R671W variant, the researchers modeled it in *Drosophila*. They created a *mimT2A-GAL4* allele by integrating a CRISPR-Mediated Integration Cassette into a shared intron of all *mim* transcripts. The splice acceptor facilitated the incorporation of T2A-GAL4 into the mRNA, leading to transcription termination and truncation of the mim mRNA due to the poly(A) tail. The viral T2A sequence arrested translation but allowed the production of GAL4 under the control of the endogenous regulatory elements of *mim*. Consequently, the *mimT2A-GAL4* allele expressed UAS-cDNA in a pattern analogous to *mim*.

In *Drosophila*, the fly ortholog gene mim displayed widespread expression in most neurons and some glia of the developing larval central nervous system and adult brain. Notably, it was enriched in neurons associated with learning and memory. Loss of the *mim* gene resulted in a reduced life span, impaired locomotor behavior, and diminished synaptic transmission in adult flies. The expression of the human reference cDNA for MTSS2 restored the phenotype, whereas the c.2011C-T variant exhibited reduced rescue ability, suggesting an LOF nature. Elevated expression of the reference cDNA had no impact on life span or locomotor activity, but elevated expression of the variant induced defects akin to *mim* loss of function, indicating a potential dominant-negative role of the variant in flies.

Besides the above examples, recent works have collectively exploited the rapid genetic manipulability and high-throughput capabilities of *Drosophila* to elucidate the complex genetic relationships and mechanisms underlying epilepsy in the context of various neurological disorders. For example, Salpietro et al. (2024) identified distinct neurological syndromes associated with biallelic variants in GTPBP1 and GTPBP2, whereas Li et al. (2024a) explored how malfunctioning spliceosome components like U2AF2 affect neurodevelopment, using *Drosophila* to model these complex interactions. Roshandel et al. (2023) investigated *SLCO5A1* and synaptic assembly genes related to impulsivity in juvenile myoclonic epilepsy using *Drosophila* for functional characterization, and Accogli et al. (2023) demonstrated the critical role of neuron navigator 2 in brain development. Yap et al. (2021) linked *OGDHL* biallelic variants to a

spectrum of neurodevelopmental issues, including epilepsy; Manivannan et al. (2022) validated the role of *FZR1* variants in developmental and epileptic encephalopathies; and Ghosh et al. (2018) modeled the disease mechanisms using *Drosophila* to investigate the effects of mutations in *ADPRHL2* on pediatric stress-induced epileptic ataxia syndrome.

## *Drosophila Melanogaster* Drive Precision Medicine Development for Epilepsy

**Drug therapy: screening and repurposing**

Drug repurposing, or repositioning, is an innovative approach to identifying new uses for existing drugs. This strategy is particularly advantageous in epilepsy research, as it allows for the rapid evaluation of already approved drugs, significantly reducing the time and cost associated with traditional drug development. This approach leverages the existing safety and pharmacokinetic data of these drugs, expediting the path to clinical application. By employing fruit fly models of epilepsy, researchers can efficiently test a wide range of compounds for their ability to reduce seizure activity, which can also aid in the identification of potential side effects and toxicities.

One of the key advantages of using fruit flies in drug repurposing is the ability to perform high-throughput screens. Thousands of compounds can be tested rapidly, identifying those that exhibit potential antiepileptic effects (Song and Tanouye, 2008). For example, researchers can use electrophysiological recordings to monitor seizure-like activity in the brains of fruit flies exposed to various drugs. Compounds that reduce seizure frequency or severity should be prioritized for further investigation. However, the most convenient method is to feed seizure-sensitive *Drosophila* mutants a panel of drugs to be screened and then select for reversion of seizure-like behaviors or paralytic behavior (Reynolds et al., 2004). By observing changes in the locomotor activity of fruit flies, researchers can infer the efficacy of drug candidates. Drugs that restore normal movement patterns in epileptic flies are considered promising candidates for repurposing. Antiepileptic drug (AED) efficacy in feeding experiments has been determined by measuring the reduction in paralytic recovery time for bss or eas mutants. The AEDs phenytoin, gabapentin, potassium bromide, and carbenoxolone have all been deemed effective in *Drosophila* based on reduction of BS mutant recovery time (Tan et al., 2004; Song and Tanouye, 2006; Stilwell et al., 2006).

In addition to identifying potential new uses for existing drugs, fruit fly models can help elucidate the mechanisms by which these drugs exert their effects (Tickoo and Russell, 2002). By studying the interaction between repurposed drugs and specific genetic pathways in *Drosophila*, researchers can gain insights into the underlying biology of epilepsy. This knowledge can inform the design of more targeted therapies and guide clinical trials. Epilepsy treatment outcomes often vary among patients due to genetic differences, the heterogeneous nature of epilepsy syndromes, and variations in drug metabolism and brain biochemistry (Heavin et al., 2019; Wolking et al., 2020a, b, 2021). This variability poses a challenge in identifying universally effective therapies. The use of *Drosophila* mutants provides a powerful model to study this complexity. By using genetically diverse strains of *Drosophila*, researchers can mimic the genetic and biochemical variability observed in human patients. This allows the identification of drugs that may act in specific genetic contexts or against specific molecular pathways. For example, studies using *Drosophila* models with mutations in genes such as *TBC1D24* and *KCNA1* have shown how different genetic backgrounds can alter the efficacy of drugs such as antioxidants or channel modulators. This approach may shed light on why certain AEDs are effective in some individuals but not others, supporting more personalized and targeted therapies for epilepsy patients.

A recent study involving *TBC1D24* mutations highlights the role of *Drosophila* in drug repurposing. *TBC1D24* mutations are linked to epilepsy and related phenotypes (Balestrini et al., 2016). The compound heterozygous mutations R360H and G501R in Tre2/Bub2/Cdc16, the lysin motif, and the domain catalytic (TLDc) domain of TBC1D24 were identified in patients with Rolandic epilepsy and exercise-induced dystonia. Functional studies in *Drosophila* showed that the TBC1D24G501R mutation led to defects in locomotion and synaptic vesicle trafficking, which were alleviated by antioxidant treatments like N-acetylcysteine amide or α-tocopherol (Lüthy et al., 2019). This study adds another tile to a possible therapeutic strategy for *TBC1D24*-associated diseases, namely, combining agents that increase synaptic phosphatidyl-D-myo-inositol-4,5-bisphosphate (PI(4,5)P2) levels, which target TBC dysfunction, together with antioxidants, which target TLDc mutant hypersensitivity to ROS.

*KCNA1* mutations, which lead to episodic ataxia type 1, disrupt cerebellar function and cause epilepsy (Browne et al., 1994; Adelman et al., 1995). Niflumic acid, an analgesic and anti-inflammatory drug, has been found to enhance Kv1.1 channel activity. Niflumic acid increases Kv1.1 current amplitudes by boosting the channel's open probability, shifting the voltage dependence, and slowing OFF-gating current decay (Servettini et al., 2023). This mitigates the functional defects associated with episodic ataxia type 1 and restores normal cerebellar function. Niflumic acid's effectiveness has been demonstrated in mouse models of episodic ataxia type 1 and Shaker (Kv1.1) mutant fruit flies, indicating its potential as a therapeutic agent (Papazian et al., 1987; Tempel et al., 1987).

Similarly, *Drosophila* models expressing human *KCNT1* mutations (G288S, R398Q, and R928C) replicate severe, treatment-resistant epilepsy (Hussain et al., 2024). Screening with various AEDs revealed that cannabidiol significantly reduced seizures in these models, whereas other drugs had mixed or negative effects. These findings indicate the utility of *Drosophila* for identifying promising new treatments for *KCNT1*-related epilepsy.

Overall, the use of fruit flies in drug repurposing efforts for epilepsy offers a cost-effective and efficient method to identify new therapeutic options. This approach accelerates the transition from bench to bedside, facilitates high-throughput screening and provides insights into drug mechanisms, supporting the development of personalized treatments by leveraging existing medications and providing hope for improved treatments for epilepsy patients.

**Gene therapy: targeting genes for precision medicine**

Gene therapy aims to alleviate disease by introducing genetic material into target cells to restore proper physiological function (Turner et al., 2021). For epilepsy, gene therapies target neurons in the central nervous system. This necessitates either intrathecal delivery to bypass the blood–brain barrier or systemic administration of therapies capable of crossing the blood–brain barrier to reach the entire brain. Most gene therapies are packaged in adeno-associated viruses (AAVs), with AAV9 being commonly used due to its blood–brain barrier-permeable capsid (Cheah et al., 2021). Beyond delivery methods, there are various approaches to gene therapy, each tailored to address different aspects of the genetic disorder.

***Gene replacement therapy***

Gene replacement therapy involves introducing a normal copy of a gene to compensate for defective versions. In *Drosophila*, this approach is used to study the effects of replacing mutated genes with functional ones. For example, researchers can use transgenic flies with AAV vectors to express a wild-type version of ion channel genes affected by LOF mutations. This allows for the assessment of restored neuronal function and seizure reduction. *Drosophila* models enable researchers to analyze how successful gene replacement impacts seizure phenotypes and neuronal health.

***Gene editing***

CRISPR/Cas9 technology allows precise modifications of the genome and is particularly useful for correcting specific mutations (Doudna, 2020). In *Drosophila*, CRISPR/Cas9 can be applied to repair dominant mutations in epilepsy-related genes like SCN1A and SCN8A (Goldberg, 2020; Turner et al., 2021). By directly editing these genes in *Drosophila* models, researchers can evaluate how correcting mutations affects neuronal excitability and seizure activity. This approach provides critical insights into the feasibility of gene editing for treating genetic epilepsy and helps refine strategies for human applications.

***Gene silencing***

Gene silencing techniques, including RNAi and antisense oligonucleotides, are used to reduce the expression of toxic or overactive genes (Rinaldi and Wood, 2018). In *Drosophila*, RNAi can be employed to target and knock down specific genes associated with epilepsy, particularly those with GOF mutations. For instance, RNAi constructs can be designed to silence overactive ion channels or other deleterious proteins, allowing researchers to study the effects on seizure severity and neuronal function (Rodriguez-Lebron and Paulson, 2006). Similarly, antisense oligonucleotides can be used to decrease the expression of problematic genes,

offering a means to modulate gene activity and evaluate therapeutic potential.

***Gene expression modification***

Techniques such as CRISPR activation and CRISPR interference are used to modulate gene expression (La Russa and Qi, 2015). In *Drosophila*, CRISPR activation can upregulate genes with LOF mutations, while CRISPR interference can inhibit overactive genes. These techniques allow researchers to explore how altering gene expression impacts epilepsy-related phenotypes. By employing these methods, researchers can gain insights into how modifying gene activity affects seizure control and neuronal function, providing valuable information for developing targeted therapies.

Despite their advantages, *Drosophila* models present challenges. Efficient delivery of gene therapies and the relevance of findings to human systems are ongoing concerns. Additionally, ensuring safety and efficacy in *Drosophila* models is crucial for translating these approaches to clinical use. Future research will need to address these challenges, optimize delivery methods, and validate findings in more complex systems to advance gene therapy for epilepsy.

## Challenges and Future Directions

**Genetic consultation and preventive medicine**

Using fruit flies to identify potential epilepsy-causing genes or mutations can be a powerful tool in genetic counseling and preventive medicine (Nabbout and Kuchenbuch, 2020; Knowles et al., 2022; Smith et al., 2023).

Genetic consultation begins with a thorough review of personal and family medical histories, helping to identify potential genetic causes of epilepsy. Genetic testing follows, including diagnostic tests to pinpoint specific mutations, predictive tests to assess the risk for asymptomatic individuals, and carrier testing for family planning. Genetic counseling then provides insights into inheritance patterns, implications of genetic findings, and guidance on treatment options and lifestyle changes (Pal et al., 2010). In this context, *Drosophila* can be used to identify and characterize genes that may be involved in seizure disorders. Researchers can introduce specific gene mutations into *Drosophila* or manipulate gene expression to observe the resulting phenotypes. This approach helps identify candidate genes that might contribute to epileptic conditions in humans.

Preventive medicine focuses on reducing the impact of genetic epilepsy through early intervention and personalized care. Primary prevention involves genetic counseling and testing to guide reproductive choices and early intervention programs for at-risk newborns. Secondary prevention emphasizes regular monitoring and targeted interventions to manage seizures early and prevent complications. Tertiary prevention aims to optimize treatment by using genetic information to select and adjust AEDs and develop individualized care plans. By integrating genetic information into treatment plans, healthcare providers can tailor AEDs based on genetic variations affecting drug metabolism and recommend lifestyle adjustments to avoid specific seizure triggers. Early detection and management are crucial, involving regular check-ups and intervention strategies to prevent the progression of epilepsy. For families with epilepsy history planning to have children, options such as preimplantation genetic diagnosis and prenatal testing can reduce the risk of passing on genetic conditions. Third-generation *in vitro* fertilization technology can also be used for screening embryos without potential pathogenic variants.

Overall, genetic consultation and preventive medicine are crucial in managing genetic epilepsy, offering personalized care and proactive strategies that enhance patient outcomes and support affected families.

**Investigation of polygenic effects in epilepsy using *Drosophila***

In a prior study, we identified two pathogenic variants concurrently in YWHAZ and ARHGAP4 through whole-exome sequencing of a patient with intellectual disability and global developmental delay as well as febrile seizures (Wan et al., 2023). This finding strongly supports the notion that neurogenetic disorders can arise due to diverse genetic factors along with other specific factors (Williams and Battaglia, 2013; Weber et al., 2017; Koeleman, 2018; Ellis et al., 2020; Perucca et al., 2020). Additionally, genome-wide association studies targeting common forms of epilepsy have pinpointed common genetic risk variants associated with various epilepsy types, exhibiting small effect sizes (median odds ratios of 1.33) unexpectedly (Hindorff et al., 2009). Contrastingly, polygenic risk scores, amalgamating the effect sizes of numerous variants into a single score, have shown promise in stratifying individuals with and without epilepsy, demonstrating the potential reliability of PRS-based predictions for clinical applications (Khera et al., 2018). In the GE-Epi25-EUR cohort, genome-wide polygenic risk scores for generalized epilepsy were notably elevated in patients with GE compared with those in control groups (Leu et al., 2019). Consequently, in future research, it will be crucial to explore the interplay among common and rare genetic variations, along with other modifications, that may predispose individuals to epilepsy (Gramm et al., 2020).

Nevertheless, the absence of robust guidelines and the considerable workload have imposed limitations on exhaustive animal studies aimed at elucidating the mechanisms underlying epilepsy development by delving into the complex relationships between variants. *Drosophila* has emerged as a robust model for epilepsy research owing to its ability to map genetic interactions, underpinned by potent resources and methodologies, including genome-wide RNAi transgenic stocks (Dietzl et al., 2007; Ni et al., 2011), mapped transposon insertion alleles, the Gal4/UAS system, and the recently pioneered CRISPR/Cas9 system. Of particular note is the integration of RNAi with the Gal4/UAS system, affording the capacity for achieving double- or even triple-knockdown effects. This not only obviates the need for arduous and costly experiments inherent in other animal models but also provides a compelling rationale for investigating interactions between genetic knockdown, knockout and knock-in *Drosophila*. Such inquiries serve as a foundational exploration for comprehending behavioral and other neurobiological phenotypes, offering reliable methodologies for discerning critical gene–gene interactions likely pertinent to human polygenic disorders and traits.

**Limitations and challenges**

In summary, the use of fruit flies to study epilepsy has become a valuable approach in neuroscience research. However, there are several limitations to the use of fruit flies as model organisms for epilepsy research that must be carefully considered. One of the main limitations is the genetic and physiological differences between fruit flies and humans. Although fruit flies share a large portion of their genome with humans, including many genes involved in epilepsy, the complexity of the human brain and its associated neural networks cannot be fully reproduced in fruit flies. Human epilepsy often involves complex interactions between various brain regions and neurotransmitter systems that are absent or greatly simplified in fruit flies. Second, the human brain is composed of approximately 86 billion neurons, each of which forms countless synaptic connections, forming a highly complex network. In contrast, the fruit fly brain contains approximately 100,000 neurons, which, while sufficient to study basic neuronal functions and behaviors, lack the complexity required to model the full spectrum of human epilepsy. This difference in neural circuits limits the extent to which fruit fly findings can be directly generalized to humans. In addition, the manifestation of epileptic seizures in fruit flies is very different from that in humans. While researchers can induce epilepsy-like activity in fruit flies through genetic manipulation or chemical exposure, the behavioral and electrophysiological features of these seizures do not fully mimic the various types of seizures observed in human epilepsy patients. This discrepancy presents a challenge for studying the underlying mechanisms and potential treatments for different forms of epilepsy. The ultimate goal of epilepsy research is to develop effective treatments for human patients. While the fruit fly model has played an important role in identifying genes and pathways involved in seizure susceptibility, the translational relevance of these findings is often limited. Compounds that show promise in alleviating seizures in fruit flies may not produce the same effects in mammalian systems due to differences in drug metabolism, blood–brain barrier permeability, and overall physiology. Despite these limitations, fruit flies remain a valuable model organism for ethical and practical considerations. Fruit flies allow for high-throughput genetic screens and rapid generation of transgenic lines, which can facilitate the discovery of novel genetic factors involved in epilepsy. However, to extrapolate data from fruit fly to human conditions, researchers can complement fruit fly studies with mammalian models, such as mice or rats, to validate the findings and ensure their relevance to human epilepsy.

## Conclusion

**The potential of *Drosophila* as a valuable tool for validating epilepsy-related genes**

*Drosophila*, or fruit flies, have significant potential as a valuable tool for validating epilepsy-related genes. They share conserved genetic pathways with humans, allowing researchers to mimic human genetic variations efficiently. Fruit flies exhibit relevant behaviors and offer electrophysiological capabilities for studying neuronal function. High-throughput screening is feasible, and genetic complementation studies can be conducted. *Drosophila* serves as a cost-effective and rapid model for generating insights into the role of epilepsy-related genes, facilitating cross-species validation in mammalian models and human studies.

**The role of such validation in advancing precision medicine approaches for epilepsy**

Validating epilepsy-related genes, particularly using models like *Drosophila*, is instrumental in advancing precision medicine for epilepsy. These findings identify specific genes as potential therapeutic targets, paving the way for personalized treatments based on individual genetic profiles. This validation aids in drug discovery, patient stratification, predictive biomarker development, early intervention, research prioritization, and improved diagnostics. Overall, understanding the genetic basis of epilepsy enhances the effectiveness of treatments and care, improving the lives of individuals with this condition.

**The broader implications of this research for understanding and treating other complex neurological disorders**

Research on epilepsy-related genes and the use of model organisms like *Drosophila* has broad implications for understanding and treating various complex neurological disorders. These implications include shared genetic pathways, the application of precision medicine principles, drug development opportunities, cross-disease biomarkers, genetic testing advancements, comprehensive neurobiology insights, cross-species models, collaborative research efforts, and ethical considerations. This research not only enhances our understanding of epilepsy but also has the potential to benefit a wide range of neurological conditions by uncovering common genetic factors and guiding innovative therapeutic strategies.

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