Exploiting fly models to investigate rare human neurological disorders

Tomomi Tanaka[1], Hyung-Lok Chung[1, 2, *]

https://doi.org/10.4103/NRR.NRR-D-23-01847

Date of submission: November 9, 2023

Date of decision: February 5, 2024

Date of acceptance: March 2, 2024

Date of web publication: April 3, 2024

From the Contents

Abstract

Rare neurological diseases, while individually are rare, collectively impact millions globally, leading to diverse and often severe neurological symptoms. Often attributed to genetic mutations that disrupt protein function or structure, understanding their genetic basis is crucial for accurate diagnosis and targeted therapies. To investigate the underlying pathogenesis of these conditions, researchers often use non-mammalian model organisms, such as *Drosophila* (fruit flies), which is valued for their genetic manipulability, cost-efficiency, and preservation of genes and biological functions across evolutionary time. Genetic tools available in *Drosophila*, including CRISPR-Cas9, offer a means to manipulate gene expression, allowing for a deep exploration of the genetic underpinnings of rare neurological diseases. *Drosophila* boasts a versatile genetic toolkit, rapid generation turnover, and ease of large-scale experimentation, making it an invaluable resource for identifying potential drug candidates. Researchers can expose flies carrying disease-associated mutations to various compounds, rapidly pinpointing promising therapeutic agents for further investigation in mammalian models and, ultimately, clinical trials. In this comprehensive review, we explore rare neurological diseases where fly research has significantly contributed to our understanding of their genetic basis, pathophysiology, and potential therapeutic implications. We discuss rare diseases associated with both neuron-expressed and glial-expressed genes. Specific cases include mutations in *CDK19* resulting in epilepsy and developmental delay, mutations in *TIAM1* leading to a neurodevelopmental disorder with seizures and language delay, and mutations in *IRF2BPL* causing seizures, a neurodevelopmental disorder with regression, loss of speech, and abnormal movements. And we explore mutations in *EMC1* related to cerebellar atrophy, visual impairment, psychomotor retardation, and gain-of-function mutations in *ACOX1* causing Mitchell syndrome. Loss-of-function mutations in *ACOX1* result in *ACOX1* deficiency, characterized by very-long-chain fatty acid accumulation and glial degeneration. Notably, this review highlights how modeling these diseases in *Drosophila* has provided valuable insights into their pathophysiology, offering a platform for the rapid identification of potential therapeutic interventions. Rare neurological diseases involve a wide range of expression systems, and sometimes common phenotypes can be found among different genes that cause abnormalities in neurons or glia. Furthermore, mutations within the same gene may result in varying functional outcomes, such as complete loss of function, partial loss of function, or gain-of-function mutations. The phenotypes observed in patients can differ significantly, underscoring the complexity of these conditions. In conclusion, *Drosophila* represents an indispensable and cost-effective tool for investigating rare neurological diseases. By facilitating the modeling of these conditions, *Drosophila* contributes to a deeper understanding of their genetic basis, pathophysiology, and potential therapies. This approach accelerates the discovery of promising drug candidates, ultimately benefiting patients affected by these complex and understudied diseases.

Key Words: ACOX1; *Drosophila melanogaster*; glia; lipid metabolism; model organisms; neuroinflammation; neurologic disorders; neuron; rare disease; VLCFA

Introduction

A group of rare neurological diseases with a spectrum of mild to severe neurological symptoms exists. Although rare individually, the collective impact of these diseases is substantial, affecting millions of people worldwide. These human diseases often have a genetic basis, caused by mutations in genes that alter protein function or structure (Ansar et al., 2019; Chung et al., 2020a, b; Lu et al., 2022; Marcogliese et al., 2022) and whose pathogenic function must be clarified for diagnosis and to develop effective therapies

[1]Department of Neurology, Houston Methodist Research Institute, Houston, TX, USA; [2]Department of Neurology, Weill Cornell Medical College, New York, NY, USA

***Correspondence to:** Hyung-Lok Chung, PhD, hchung2@houstonmethodist.org.
https://orcid.org/0000-0002-6848-0595 (Hyung-Lok Chung)

Funding: *This work was supported by Warren Alpert Foundation and Houston Methodist Academic Institute Laboratory Operating Fund (to HLC).*
How to cite this article: *Tanaka T, Chung HL (2025) Exploiting fly models to investigate rare human neurological disorders. Neural Regen Res 20(1):21-28.*

(Chow et al., 2017). When researching rare diseases, when a gene mutation is discovered for the first time, it is difficult to examine all candidate genes in mammals such as mice, both in terms of time and cost, when it is not sure whether it is the cause of the disease, it is unrealistic. To investigate the pathogenesis, rare neurological diseases are often modeled in non-mammalian model organisms like fruit flies (the "fly" model; *Drosophila melanogaster*), nematode worms (*Caenorhabditis elegans*), and zebrafish (*Danio rerio*), since they are a cost-effective and powerful initial platform for unraveling the roles of genes and variants. In particular, invertebrates such as *Caenorhabditis* and *Drosophila*, can easily solve this problem, as multiple human genes are aggregated into one corresponding gene. Maintaining fly stocks and conducting experiments with *Drosophila* is considerably less expensive than working with mammalian models or clinical trials in humans (Yamamoto et al., 2024). This cost-effectiveness allows researchers to explore a broader range of hypotheses and conduct larger-scale studies with high statistical power, ultimately accelerating the pace of discovery. On the other hand, although *Danio* is vertebrates, they are also widely used for genetic research. This is because *Danio* have advantages among vertebrates, such as being able to raise them at a relatively low cost, having transparent embryos that are easy to observe, and having a genome that is more similar to humans than *Drosophila* (Son et al., 2022). This is especially useful when invertebrates do not exhibit orthologs or appropriate disease phenotypes.

Specific genes can be studied in *Drosophila* due to evolutionary conservation of genes and their functions and, to better understand these diseases at the molecular level, analogous mutations can be introduced into the corresponding genes of *Drosophila*. These genetic manipulations in *Drosophila* can recreate aspects of disease phenotypes seen in humans, providing valuable insights into disease mechanisms and potential therapeutic targets (Ma et al., 2022).

Drosophila has a well-established and highly versatile genetic toolkit, with a wide range of techniques available to manipulate gene expression including knockdown (reducing gene activity), overexpression (increasing gene activity), humanization (Katanaev, 2023), and precise gene editing using more recent tools like CRISPR-Cas9 (Kanca et al., 2019). These genetic tools allow the functional assessment of individual genes to understand their role in various biological processes; for instance, even cognitive dysfunction can be modeled in *Drosophila*, which can help in identifying the mutations responsible for rare diseases (Marcogliese et al., 2022).

The fly central nervous system (CNS) contains around 100,000 neurons, a fraction of the billions of neurons found in the human brain. Despite its simplicity, key features of neuronal development, structure, and function are similar to those in more complex organisms, such as humans (Hirth et al., 1999; Pires-daSilva et al., 2003). *Drosophila* displays complex behaviors that can be easily studied and manipulated in a laboratory setting. Researchers can assess changes in behavior caused by genetic mutations associated with neurological diseases, providing insights into disease-related alterations in neural function. As a result, neural circuits and behaviors are easier to study in *Drosophila* at a level of detail currently unachievable in more complex organisms. The characteristics possessed by *Drosophila*, along with the ease of genetic manipulation in flies, indicate the utility of flies as a model organism in the study of neurological disorders. This, combined with the ease of genetic manipulation in flies, solidifies the position of flies as a model organism in rare neurological diseases.

The short generation time of *Drosophila* and the ease with which large numbers of *Drosophila* (Hales et al., 2015) can be bred and maintained make it possible to conduct large-scale genetic screens (Jay et al., 2021) and drug testing experiments (Deshpande et al., 2019; Lopez-Ortiz et al., 2023). In the context of rare neurological diseases, these characteristics of *Drosophila* make them a powerful tool for identifying potential drug candidates (Su, 2019). Researchers can expose thousands of flies carrying disease-associated mutations to various compounds and assess their impact on disease-related phenotypes (Chung et al., 2020b; Moore et al., 2023; Yin et al., 2023), allowing the rapid identification of promising therapeutic agents that can then be further investigated in mammalian models (Ansar et al., 2019; Chung et al., 2020b; Lin et al., 2023) and, ultimately, in clinical trials.

Given the complexity and scarcity of rare neurological diseases, *Drosophila* therefore represents a powerful and indispensable tool for their investigation. Here we provide a comprehensive overview of rare neurological diseases in which fly research has played a pivotal role in advancing our discovery and understanding of these conditions. We discuss the genetic basis of these diseases, explore the ways in which fly research has contributed to our knowledge, and discuss the potential therapeutic implications of these findings.

Search Strategy and Selection Criteria

Studies cited in this review were mainly published between 2010 and 2023 and searched from PubMed or Google Scholar databases. But also, we cited some studies to explain some mechanisms and to get original data even though it is older than 2010. The following keywords were used for this research: rare disease, gene, neurological disease, *drosophila melanogaster*, neuron, glia, variants, model organisms, zebrafish, and humanization.

Rare Genetic Diseases in Neurology

Nerves are present throughout the body and are the fundamental unit of the CNS, consisting of the brain and spinal cord; peripheral nerves originating from the CNS to spread throughout the body; and the autonomic nervous system, which regulates smooth muscle and other autonomic responses. Therefore, diseases of nerves can cause diverse abnormalities in function, resulting in a broad range of symptoms, including muscle diseases and cranial nerve diseases.

Patients with rare neurological diseases exhibit many different symptoms such as seizures, developmental delay, intellectual disability, sensory and motor disorders, and shortened survival, significantly affecting patients' quality of life and their families. Many of these rare diseases are

genetic, emphasizing the crucial role of unraveling the genetic foundation of rare neurological diseases in achieving precise diagnosis, prognostication, and the formulation of tailored therapeutic interventions. Significant progress has been made in identifying the genes associated with these conditions over recent decades, with advanced next-generation sequencing technology enabling the diagnosis of around 40%–50% of pediatric diseases by detecting variants of known disease-related genes through the use of whole-exome sequencing together with evaluation of copy number variations (Liu et al., 2019; Ngo et al., 2020; Investigators et al., 2021). Despite these advances, a substantial population of individuals still have undiagnosed diseases, and an estimated 6000 to 13,000 human genes are not yet linked to specific medical conditions (Bamshad et al., 2019).

Identifying the cause of a disease can be challenging, especially when patients have similar symptoms and where abnormalities in any element of the nervous system – neurons, glia, synapses, or myelin – can all be responsible. Nevertheless, we have successfully identified the causative genes of several rare diseases and exploited the fly model to elucidate their pathology (Ansar et al., 2018; Chung et al., 2020a, b, 2022; Lu et al., 2022), ultimately facilitating the discovery of new treatments in some cases (Chung et al., 2020b). Although technical discussions are reserved for comprehensive reviews (Yamamoto et al., 2014, 2024; Bellen et al., 2019; Harnish et al., 2019), below we provide concrete examples of how genetic manipulation in *Drosophila* (humanization, disease modeling, and gene overexpression) has been effectively used to explore the interplay between mutant genes in neurons and glia. We introduce five genes that are associated with human neurological rare diseases including cyclin-dependent kinase 19 (*CDK19*), TIAM Rac1-associated GEF 1 (*TIAM1*), Interferon regulatory factor 2 binding protein like (*IRF2BPL*), ER membrane protein complex subunit 1 (*EMC1*), and Acyl-CoA oxidase 1 (*ACOX1*), and we summarize them in **Additional Table 1**. We further demonstrated the cellular localization of the genes we covered, as shown in **Figure 1**. It is worth noting that a significant number of these genes are localized in the nucleus, which implies that alterations in transcription could lead to various neurological symptoms.

Rare diseases associated with neuron-expressed genes

Through the use of humanization and disease modeling techniques, we have contributed to our understanding of the disease mechanisms linked to mutations in *CDK19* (Chung et al., 2020a) and *TIAM1* (Lu et al., 2022), which ultimately result in neuronal abnormalities. Additionally, we used overexpression and disease modeling techniques in flies to study *IRF2BPL* (Marcogliese et al., 2018) causing several neuronal abnormalities.

Mutations in CDK19: developmental and epileptic encephalopathy 87 (DEE87)

Several genes have now been discovered that cause neurologic symptoms such as seizures, including infantile spasms, by studying *Drosophila* and *Danio*. We previously detected *de novo* missense mutations in the *CDK19* gene in three unrelated individuals displaying common symptoms of epilepsy, global developmental delay, and hypotonia, among others (Chung et al., 2020a). Zarate et al. (2021) also reported eleven individuals with *de novo* missense variants mapped to the kinase domain of *CDK19*, and summarized symptoms and phenotypes in fourteen individuals, including three individuals in our report, finding that 64% had seizures, including four with infantile spasms. Two recent papers (Sugawara et al., 2020; Yang et al., 2021) reported two more individuals, one with the same variant as an individual in our index report with a highly similar phenotype (Sugawara et al., 2020) and another with a different variant but also with a similar phenotype of early-onset refractory epilepsy (Yang et al., 2021).

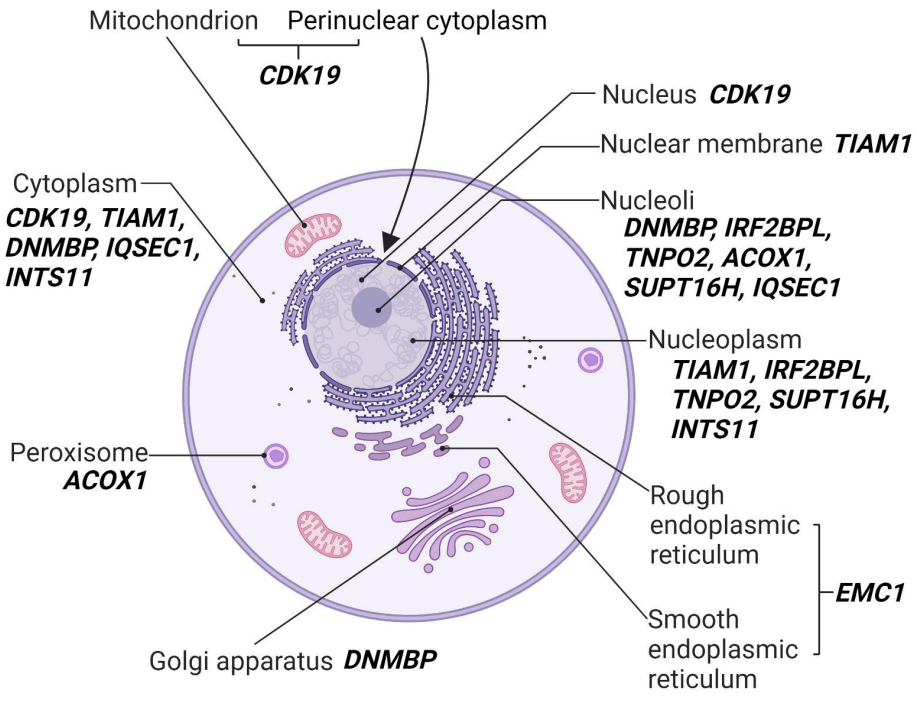

Figure 1 | Localization of the proteins that are associated with rare neurologic disorders in the cell (schematic drawing).
Adapted from "Structural Overview of an Animal Cell," by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates. ACOX1: Acyl-CoA oxidase 1; CDK19: cyclin-dependent kinase 19; DNMBP: dynamin binding protein; EMC1: ER membrane protein complex subunit 1; INTS11: integrator complex subunit 11; IQSEC1: IQ motif and Sec7 domain ArfGEF 1; IRF2BPL: interferon regulatory factor 2 binding protein like; SUPT16H: SPT16 homolog, facilitates chromatin remodeling subunit; TIAM1: TIAM Rac1-associated GEF 1; TNPO2: transportin 2.

CDK19 and its paralog *CDK8* are both members of the transcriptional CDKs. *CDK19*, as a constituent of the CDK module, collaborates with essential mediators to modulate the activity of RNA polymerase II and oversee transcriptional processes. In *Drosophila*, the sole equivalent of *CDK19* is *Cdk8*. To better understand the function of de novo missense mutations in *CDK19* in humans, we generated UAS-transgenic flies harboring human *CDK19* cDNA or *CDK19* variants found in individuals (Chung et al., 2020a). Lethality observed with loss of Cdk8 in flies was completely restored by co-expression of human *CDK19*, suggesting an evolutionarily conserved function in the nervous system. However, co-expression of variant *CDK19* failed to reverse lethality in flies, indicating that the *CDK19* variant is a loss-of-function (LoF) mutation. *CDK19* is highly expressed in the nervous system (Davie et al., 2018), and we observed *CDK19* expression in the perinuclear space and cytoplasm of neurons in flies for both the reference sequence and variant. When *CDK19* or *CDK19* variant was co-expressed in flies with neuron-specific *Cdk8* knockdown, co-expression of *CDK19* improved lethality and severe convulsive seizures but co-expression of variant *CDK19* failed to rescue. These results suggest that both *Cdk8* and *CDK19* share evolutionarily conserved functions. However, the knockdown of *Cdk8* via *repo-gal4* in glia did not lead to lethality or display any discernible behavioral defects such as impairments in climbing or longevity. This observation implies that *Cdk8*'s necessity is confined to neurons rather than glia. We showed that these variants exhibit strong LoF features, and it is evident that deleterious *CDK19* variants play a pathogenic role in a neurodevelopmental disorder with syndromic phenotypes. From this our findings and loss of Cdk8 causes an obvious loss of boutons and synapses at larval neuromuscular junctions, we argue that CDK19 plays a critical role in neurodevelopment and synapse formation and function, supporting the observation that it causes a neurodevelopmental syndrome with epilepsy in humans (Chung et al., 2020a). Zarate et al. (2021) further evaluated the toxicity of variant *CDK19* by co-expressing human *CDK19* or variant *CDK19* in zebrafish, and two representative amino acid substitutions (p. Y32H and p. G28R) were used to evaluate protein function using substrate phosphorylation and autophosphorylation assays. Using this method, they discovered that the two amino acid substitutions detected in individuals produced different *CDK19* LoF, it showed lower kinase activity than that of wild protein, and gain-of-function (GoF), it showed significantly higher kinase activity than that of wild protein, respectively.

Mutations in TIAM1: neurodevelopmental disorder with language delay and seizures (NEDLDS)

TIAM1 encodes the Ras-related C3 botulinum toxin substrate 1 (RAC1)-specific guanine nucleotide exchange factor. This protein is expressed in the cytoplasm (**Figure 1**) of almost all tissues, mostly found in the brain, and participates in brain development. In rodents, TIAM1 protein is expressed in dendrites and spines, being necessary for maintaining appropriate outgrowth during development. We identified five individuals from four families with bi-allelic *TIAM1* missense variants, all of whom experienced seizures, developmental delay, intellectual disability, and speech delay (Lu et al., 2022). The *Drosophila* homolog of *TIAM1* is still life (*sif*), a gene expressed mainly in neurons but not in glia. Based on its CNS expression pattern, we further examined whether *sif* plays a role both during development and in adult flies. Ubiquitous or neuron-specific knockdown of *sif* in flies resulted in climbing defects and seizure-like behavior, while glial-specific knockdown did not result in these abnormalities. Based on these results, *sif* LoF in neurons causes the observed phenotypes.

We assessed the function associated with TIAM1 variants and compared them to the TIAM1 Ref cDNA function in *Drosophila* model. We generated UAS-transgenic flies harboring human TIAM1 cDNA or TIAM1 variants found in individuals. Lethality observed with loss of *sif* in flies was partially rescued by co-expression of human TIAM1, but one of the three variants had reduced rescue ability. This finding suggests that this variant is a partial LoF. In ectopic expression studies, both wild type *sif* and TIAM1 Ref are toxic, whereas the three variants show reduced toxicity, suggesting that they are partial LoF variants.

Previously, Cheng et al. (2021) reported Tiam1 knockout mice have decreased spine density, simplified dendritic arbors, and decreased miniature excitatory postsynaptic currents in the hippocampus, our findings also provide evidence that sif is important for appropriate neural function and that TIAM1 variants observed in the individuals are disruptive, thus implicating loss of TIAM1 cause neuron abnormalities and phenotypes in humans.

Of note, we previously studied *sif* in flies after discovering that twelve individuals with infantile cataracts had a homozygous LoF variant in *DNMBP*, which is also a *sif* homolog participating in fly eye development and enriched in lens-secreting cells, specifically corneal lens-forming cells (Ansar et al., 2018). Assigning two human homologs to a single *Drosophila* gene is not surprising, since *Drosophila* genes are often close homologs of multiple human genes because *Drosophila* has 13,600 genes (Adams et al., 2000), and humans have an estimated about 20,000 genes (Amaral et al., 2023).

Mutations in IRF2BPL: neurodevelopmental disorder with regression, abnormal movements, loss of speech, and seizures (NEDAMSS)

Marcogliese et al. (2018) reported neurological symptoms in seven individuals caused by heterozygous variants of *IRF2BPL*. Five of these individuals harbored nonsense variants and experienced similar clinical symptoms such as seizures, severe neurodevelopmental regression, hypotonia, progressive ataxia, and a lack of coordination. The other two individuals had missense variants and showed similar symptoms, albeit milder than those with nonsense alleles.

The fly homolog of *IRF2BPL* is *pits* (protein interacting with Ttk69 and Sin3A), which is located on the X chromosome and shares a similar protein structure to human *IRF2BPL*, albeit with low similarity. *IRF2BPL* is localized to the nucleoplasm (**Figure 1**), and Pits is expressed in the cell bodies and nuclei of many neurons as well as their axons. They are particularly present in the mushroom bodies, the learning and memory centers required for balance, hearing, and motor coordination in insects. Using the fly model, the authors found that all the

nonsense variants observed in individuals were LoF and one of the missense variants was partial LoF. Furthermore, individuals harboring nonsense variants in *IRF2BPL* exhibited a significant progressive motor dysfunction. Similarly, in flies, reduced *Pits* expression in neurons was associated with a climbing impairment that worsened with age, suggesting that *Pits* plays fundamental roles in CNS development and maintenance. Additionally our results provide further support for the involvement of IRF2BPL variants in neurological symptoms, expanding the phenotype to include neurodevelopmental regression Moreover, experiments conducted with *Drosophila* as model organisms reinforce the significance of IRF2BPL in both embryonic development and the maintenance of neurons. Given the high conservation of IRF2BPL, the abundance of its fly counterpart, *pits*, in the nervous system during both development and adulthood adds weight to its crucial role in these processes (Marcogliese et al., 2018).

Rare diseases associated with glial-expressed genes

Neurological human disease can also be caused by variants in genes expressed in non-neuronal cells, such as glia. We discovered that variants in *EMC1* and *ACOX1* led to glial abnormalities. Variants in *EMC1* have been linked to various clinical conditions, including neurodevelopmental disorders (Harel et al., 2016). Several potentially pathogenic *EMC1* variants have been identified, which are associated with a range of clinical phenotypes in humans. These predominantly function as LoF alleles when using Xenopus as a modeling system (Marquez et al., 2020).

Mutations in EMC1: cerebellar atrophy, visual impairment, and psychomotor retardation (CAVIPMR)

In nine individuals with *EMC1* variants, the main features were global developmental delay and hypotonia. We reported three unrelated children with severe to profound developmental delay, truncal hypotonia, seizures, and cortical visual impairments (Chung et al., 2022). The study found that one of the localized protein domains of the three reported *EMC1* variants was not specifically conserved in fly *EMC1*, despite the sole homolog of *EMC1* in flies being *EMC1*. To further investigate this, the study analyzed this domain and predicted functional homologous mutations in the fly based on the structural characteristics of the amino acids using Clustal Omega A (Sievers et al., 2011). We also verified the pathogenic nature of the three *EMC1* variants in human by employing transgenic flies carrying the homologous fly *EMC1* mutations. This approach enabled us to investigate the functional distinctions between the reference gene and variants. *EMC1* is known to be expressed in the cytoplasm of almost all cells (**Figure 1**). Our findings also showed that *EMC1* was expressed in both neurons and glia, and knockdown and overexpression of fly *EMC1* in glia caused lethality and climbing impairment, respectively, while knockdown and overexpression of fly *EMC1* in neurons did not produce any phenotype. Therefore, both loss and overexpression of *EMC1* are toxic, but only when expressed in glia. The study suggested that the three affected individuals had partially lost *EMC1* function, contributing to a more detailed understanding of the phenotype spectrum associated with EMC1 variants. This study provides evidence of pathogenicity for EMC1 variants within a defined domain and indicates that the primary defect in EMC1-associated disease lies in glia rather than neurons. However, it remains to be determined which glia are susceptible to loss or gain of *EMC1* function in humans.

Previously, Richard et al.(2013) reported that the EMC complex maintains ER homeostasis, and a deficiency of the EMC complex induced ER stress and an unfolded protein response. Especially, ER stress and the unfolded protein response are closely associated with many myelin and myelinating glia disorders (Clayton et al., 2016). We infer that EMC1 variants identified in individuals cause ER stress in glia, leading to neurologic phenotypes, but this needs to be further explored.

GoF mutations in ACOX1: Mitchell syndrome

We recently reported three pediatric individuals from different families harboring a novel heterozygous mutation in *ACOX1*. These individuals developed progressive myeloneuropathy and sensorineural hearing loss, with a nerve biopsy of one individual showing degenerating Schwann cells (Chung et al., 2020b). We therefore investigated the impact of *ACOX1* deficiency in flies, creating two *ACOX1*-deficient *Drosophila* alleles using CRISPR. One allele reduced the expression of *ACOX1* and a second knockout allele completely abrogated *ACOX1* expression. Using this model, *ACOX1* was found to be essential for fly survival, and LoF either partially or completely resulted in lethality. Adult flies with reduced *ACOX1* expression exhibited decreased locomotor performance, retinal degeneration, and reduced lifespan. The loss of *ACOX1* function in humans causes early onset and severe diseases like white matter demyelination, cognition decrease, loss of learning and speaking skills, loss of vision and hearing, hypotonia, and shortness of lifespan (Chung et al., 2020b).

Given that *ACOX1* encodes the initial and rate-determining enzyme within the peroxisomal b oxidation pathway responsible for the degradation of very-long-chain fatty acid (VLCFA), we initially hypothesized that the observed toxicity was due to the accumulation of VLCFAs. However, we found that individuals with the novel genetic abnormality did not exhibit VLCFA accumulation. Instead, the *ACOX1* variants in these individuals are stable to degradation, indicating a GoF variant. On further investigation, this variant was shown to promote oxidative activity, leading to oxidative damage and glial degeneration as the core pathology responsible for the disease phenotype. To investigate the consequences of increased oxidative activity, we overexpressed an equivalent fly mutation, *dACOX1*, using 20 different driver lines across various cell types, determining that only overexpression in wrapping glia resulted in a severe loss of viability, emphasizing the heightened sensitivity of wrapping glia to overactive *dACOX1* (Chung et al., 2020b).

Our findings therefore suggested that the GoF variants in *ACOX1* heightened the generation of reactive oxygen species, resulting in oxidative damage and glial degeneration. Supporting this, a potent antioxidant, N-acetyl cysteine amide, significantly enhanced motor function and survival rates in flies expressing mutant *dACOX1*. In human patients,

administration of NAC led to remarkable improvements in peripheral nerve function. Unfortunately, NAC does not cross the blood-brain barrier, so these patients still succumb to rapidly progressive CNS disease. We named this novel *ACOX1*-mediated disease Mitchell's disease in honor of the first patient who received NAC supplementation.

Loss of function mutations in ACOX1: ACOX1 deficiency

VLCFAs are the primary fatty acid components of myelin. When elevated, VLCFAs stiffen the plasma membrane, which is essential for optimal myelin function. However, when VLCFAs accumulate in *ACOX1* deficiency, this results in rapid and severe deterioration of nervous system function due to glial degeneration.

In a recent study, we reported that an increase in VLCFA levels in glial cells was associated with climbing impairments and a shortened lifespan in flies (Chung et al., 2023). This increase was also associated with an elevation in sphingosine-1-phosphate (S1P), which was produced by the glial cells in response to increased VLCFA levels. S1P was secreted by glial cells and transported to neurons, leading to neuroinflammation through cellular immune responses (seen as melanization in flies) and activation of the IMD nuclear factor-κB immune signaling pathway, a surrogate of neuroinflammation. This process also triggered the activation of CNS macrophages via the nuclear factor-κB pathway, ultimately resulting in neuronal toxicity.

Fortunately, bezafibrate (a medication that reduces VLCFA synthesis) and fingolimod (inhibits S1P signaling) mitigated the toxicity caused by increased VLCFA levels and excessive S1P signaling, respectively.

The susceptibility of these variants in CNS is described in **Figure 2**.

Conclusion and Perspective

Patients with rare neurological diseases can exhibit a wide range of clinical features, but sometimes similarities can be identified. While variants in the same gene usually result in similar human phenotypes (Lu et al., 2022; Tepe et al., 2023), even variants in different genes that cause abnormalities in neurons or glia can sometimes share common clinical findings (Ansar et al., 2019; Chung et al., 2022). However, variants in the same gene can also lead to different outcomes, such as complete loss of function, partial loss of function, or gain of function (Marcogliese et al., 2018; Chung et al., 2020b; Goodman et al., 2021), depending on the specific variant. Moreover, even variants in the same gene can cause significantly different patient phenotypes, with underlying mechanisms that exhibit substantial variation. For example, loss of function and gain of function variants of ACOX1 have distinct effects, where the gain of function leads to elevated levels of reactive oxygen species and glial degeneration, while loss of function causes VLCFA accumulation and glial degeneration (Chung et al., 2020b, 2023).

Model organisms such as flies are extremely useful for determining whether a human mutation is disease-causing. Immunostaining tissue from genetically-modified flies can be useful for determining the localization of gene expression. Techniques such as knockdown, overexpression, humanization, and disease modeling can also be applied to model organisms to help identify disease-causing genes. By reproducing the same phenotypic system as in humans, it may be possible to identify the gene responsible for a given disease. It is important to note that a significant number of the proteins that are encoded by the pathogenic genes we discovered are nucleoproteins (**Figure 1**). This means that if there are any mutations in the genes that encode these crucial nucleoproteins, they may exhibit the unique disease phenotypes discussed in this review.

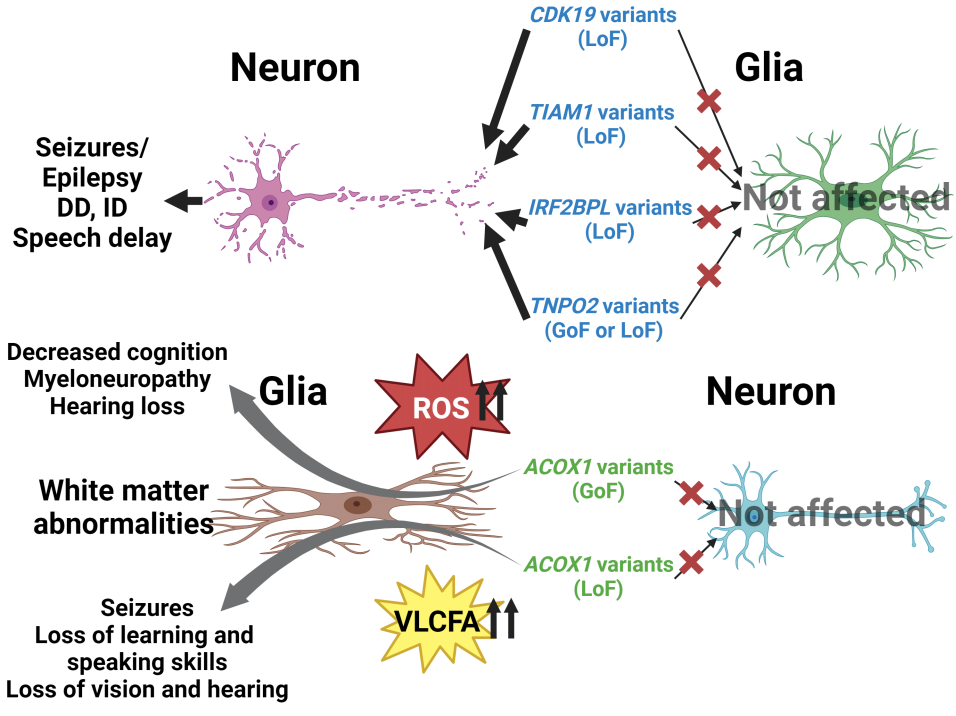

Figure 2 | Pathogenicity of variants in neurons and glia. Each gene is expressed in different tissues and cells, and variants can be pathogenic in neurons or in glia. Created with BioRender.com. ACOX1: Acyl-CoA oxidase 1; CDK19: cyclin-dependent kinase 19; DD: Developmental delay; GoF: gain of function; ID: intellectual disability; IRF2BPL: interferon regulatory factor 2 binding protein like; LoF: loss of function; ROS: reactive oxygen species; TIAM1: TIAM Rac1-associated GEF 1; TNPO2: transportin 2; VLCFA: very-long-chain fatty acid.

Author contributions: *Manuscript conception and design, analysis and interpretation, manuscript writing, and approval of the final version of the manuscript: TT, HLC.*

Conflicts of interest: *The authors declare no conflicts of interest.*

Data availability statement: *All relevant data are included in the paper and its Additional file.*

Additional file:

Additional Table 1: *Examples of rare neurological diseases studied in Drosophila.*

References

Adams MD, et al. (2000) The genome sequence of Drosophila melanogaster. Science 287:2185-2195.

Amaral P, et al. (2023) The status of the human gene catalogue. Nature 622:41-47.

Ansar M, et al. (2018) Bi-allelic loss-of-function variants in DNMBP cause infantile cataracts. Am J Hum Genet 103:568-578.

Ansar M, et al. (2019) Bi-allelic variants in IQSEC1 cause intellectual disability, developmental delay, and short stature. Am J Hum Genet 105:907-920.

Bamshad MJ, Nickerson DA, Chong JX (2019) Mendelian gene discovery: fast and furious with no end in sight. Am J Hum Genet 105:448-455.

Bellen HJ, Wangler MF, Yamamoto S (2019) The fruit fly at the interface of diagnosis and pathogenic mechanisms of rare and common human diseases. Hum Mol Genet 28:R207-214.

Cabet S, Lesca G, Labalme A, Des Portes V, Guibaud L, Sanlaville D, Pons L (2020) Novel truncating and missense variants extending the spectrum of EMC1-related phenotypes, causing autism spectrum disorder, severe global development delay and visual impairment. Eur J Med Genet 63:103897.

Cheng J, Scala F, Blanco FA, Niu S, Firozi K, Keehan L, Mulherkar S, Froudarakis E, Li L, Duman JG, Jiang X, Tolias KF (2021) The Rac-GEF Tiam1 promotes dendrite and synapse stabilization of dentate granule cells and restricts hippocampal-dependent memory functions. J Neurosci 41:1191-1206.

Chiu CY, Leng S, Martin KA, Kim E, Gorman S, Duhl DM (1999) Cloning and characterization of T-cell lymphoma invasion and metastasis 2 (TIAM2), a novel guanine nucleotide exchange factor related to TIAM1. Genomics 61:66-73.

Chow CY, Reiter LT (2017) Etiology of human genetic disease on the fly. Trends Genet 33:391-398.

Chung HL, Mao X, Wang H, Park YJ, Marcogliese PC, Rosenfeld JA, Burrage LC, Liu P, Murdock DR, Yamamoto S, Wangler MF, Undiagnosed Diseases N, Chao HT, Long H, Feng L, Bacino CA, Bellen HJ, Xiao B (2020a) De novo variants in CDK19 are associated with a syndrome involving intellectual disability and epileptic encephalopathy. Am J Hum Genet 106:717-725.

Chung HL, et al. (2020b) Loss- or gain-of-function mutations in ACOX1 cause axonal loss via different mechanisms. Neuron 106:589-606.

Chung HL, Rump P, Lu D, Glassford MR, Mok JW, Fatih J, Basal A, Marcogliese PC, Kanca O, Rapp M, Fock JM, Kamsteeg EJ, Lupski JR, Larson A, Haninbal MC, Bellen H, Harel T (2022) De novo variants in EMC1 lead to neurodevelopmental delay and cerebellar degeneration and affect glial function in Drosophila. Hum Mol Genet 31:3231-3244.

Chung HL, Ye Q, Park YJ, Zuo Z, Mok JW, Kanca O, Tattikota SG, Lu S, Perrimon N, Lee HK, Bellen HJ (2023) Very-long-chain fatty acids induce glial-derived sphingosine-1-phosphate synthesis, secretion, and neuroinflammation. Cell Metab 35:855-874.

Clayton BLL, Popko B (2016) Endoplasmic reticulum stress and the unfolded protein response in disorders of myelinating glia. Brain Res 1648:594-602.

Cook DR, Rossman KL, Der CJ (2014) Rho guanine nucleotide exchange factors: regulators of Rho GTPase activity in development and disease. Oncogene 33:4021-4035.

Davie K, et al. (2018) A single-cell transcriptome atlas of the aging drosophila brain. Cell 174:982-998.

Deshpande P, Gogia N, Singh A (2019) Exploring the efficacy of natural products in alleviating Alzheimer's disease. Neural Regen Res 14:1321-1329.

Ferdinandusse S, Denis S, Hogenhout EM, Koster J, van Roermund CW, IJlst L, Moser AB, Wanders RJ, Waterham HR (2007) Clinical, biochemical, and mutational spectrum of peroxisomal acyl-coenzyme A oxidase deficiency. Hum Mutat 28:904-912.

Gardiner J, Marc J (2010) Disruption of normal cytoskeletal dynamics may play a key role in the pathogenesis of epilepsy. Neuroscientist 16:28-39.

Gavrilovici C, Jiang Y, Kiroski I, Teskey GC, Rho JM, Nguyen MD (2020) Postnatal role of the cytoskeleton in adult epileptogenesis. Cereb Cortex Commun 1:tgaa024.

Geetha TS, Lingappa L, Jain AR, Govindan H, Mandloi N, Murugan S, Gupta R, Vedam R (2018) A novel splice variant in EMC1 is associated with cerebellar atrophy, visual impairment, psychomotor retardation with epilepsy. Mol Genet Genomic Med 6:282-287.

Goodman LD, et al. (2021) TNPO2 variants associate with human developmental delays, neurologic deficits, and dysmorphic features and alter TNPO2 activity in Drosophila. Am J Hum Genet 108:1669-1691.

Goto A, Hoshino M, Matsuda M, Nakamura T (2011) Phosphorylation of STEF/Tiam2 by protein kinase A is critical for Rac1 activation and neurite outgrowth in dibutyryl cAMP-treated PC12D cells. Mol Biol Cell 22:1780-1790.

Hales KG, Korey CA, Larracuente AM, Roberts DM (2015) Genetics on the fly: a primer on the drosophila model system. Genetics 201:815-842.

Harel T, et al. (2016) Monoallelic and biallelic variants in EMC1 identified in individuals with global developmental delay, hypotonia, scoliosis, and cerebellar atrophy. Am J Hum Genet 98:562-570.

Harnish JM, Deal SL, Chao HT, Wangler MF, Yamamoto S (2019) In vivo functional study of disease-associated rare human variants using drosophila. J Vis Exp doi: 10.3791/59658.

Hirth F, Reichert H (1999) Conserved genetic programs in insect and mammalian brain development. Bioessays 21:677-684.

Hoshino M, Sone M, Fukata M, Kuroda S, Kaibuchi K, Nabeshima Y, Hama C (1999) Identification of the stef gene that encodes a novel guanine nucleotide exchange factor specific for Rac1. J Biol Chem 274:17837-17844.

Investigators GPP, et al. (2021) 100,000 genomes pilot on rare-disease diagnosis in health care- preliminary report. N Engl J Med 385:1868-1880.

Jay TR, Kang Y, Jefferson A, Freeman MR (2021) An ELISA-based method for rapid genetic screens in Drosophila. Proc Natl Acad Sci U S A 118:e2107427118.

Kanca O, Zirin J, Garcia-Marques J, Knight SM, Yang-Zhou D, Amador G, Chung H, Zuo Z, Ma L, He Y, Lin WW, Fang Y, Ge M, Yamamoto S, Schulze KL, Hu Y, Spradling AC, Mohr SE, Perrimon N, Bellen HJ (2019) An efficient CRISPR-based strategy to insert small and large fragments of DNA using short homology arms. Elife 8:e51539.

Katanaev VL (2023) Humanization for neurological disease modeling: a roadmap to increase the potential of Drosophila model systems. Animal Model Exp Med 6:230-236.

Lin G, Tepe B, McGrane G, Tipon RC, Croft G, Panwala L, Hope A, Liang AJH, Zuo Z, Byeon SK, Wang L, Pandey A, Bellen HJ (2023) Exploring therapeutic strategies for infantile neuronal axonal dystrophy (INAD/PARK14). Elife 12:e82555.

Liu P, et al. (2019) Reanalysis of clinical exome sequencing data. N Engl J Med 380:2478-2480.

Lopez-Ortiz C, Gracia-Rodriguez C, Belcher S, Flores-Iga G, Das A, Nimmakayala P, Balagurusamy N, Reddy UK (2023) Drosophila melanogaster as a translational model system to explore the impact of phytochemicals on human health. Int J Mol Sci 24:13365.

Lu S, Hernan R, Marcogliese PC, Huang Y, Gertler TS, Akcaboy M, Liu S, Chung HL, Pan X, Sun X, Oguz MM, Oztoprak U, de Baaij JHF, Ivanisevic J, McGinnis E, Guillen Sacoto MJ, Chung WK, Bellen HJ (2022) Loss-of-function variants in TIAM1 are associated with developmental delay, intellectual disability, and seizures. Am J Hum Genet 109:571-586.

Ma M, Moulton MJ, Lu S, Bellen HJ (2022) 'Fly-ing' from rare to common neurodegenerative disease mechanisms. Trends Genet 38:972-984.

Ma M, Zhang X, Zheng Y, Lu S, Pan X, Mao X, Pan H, Chung HL, Wang H, Guo H, Bellen HJ (2023) The fly homolog of SUPT16H, a gene associated with neurodevelopmental disorders, is required in a cell-autonomous fashion for cell survival. Hum Mol Genet 32:984-997.

Marcogliese PC, et al. (2018) IRF2BPL is associated with neurological phenotypes. Am J Hum Genet 103:245-260.

Marcogliese PC, et al. (2022) Drosophila functional screening of de novo variants in autism uncovers damaging variants and facilitates discovery of rare neurodevelopmental diseases. Cell Rep 38:110517.

Marquez J, Criscione J, Charney RM, Prasad MS, Hwang WY, Mis EK, Garcia-Castro MI, Khokha MK (2020) Disrupted ER membrane protein complex-mediated topogenesis drives congenital neural crest defects. J Clin Invest 130:813-826.

Moore MC, Taylor DT (2023) Effects of valproate on seizure-like activity in Drosophila melanogaster with a knockdown of Ube3a in different neuronal populations as a model of Angelman Syndrome. Epilepsy Behav Rep 24:100622.

Ngo KJ, et al. (2020) A diagnostic ceiling for exome sequencing in cerebellar ataxia and related neurological disorders. Hum Mutat 41:487-501.

Pires-daSilva A, Sommer RJ (2003) The evolution of signalling pathways in animal development. Nat Rev Genet 4:39-49.

Richard M, Boulin T, Robert VJ, Richmond JE, Bessereau JL (2013) Biosynthesis of ionotropic acetylcholine receptors requires the evolutionarily conserved ER membrane complex. Proc Natl Acad Sci U S A 110:E1055-1063.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539.

Son M, Kim DY, Kim CH (2022) Disease modeling of rare neurological disorders in zebrafish. Int J Mol Sci 23:3946.

Spence EF, Soderling SH (2015) Actin out: regulation of the synaptic cytoskeleton. J Biol Chem 290:28613-28622.

Su TT (2019) Drug screening in Drosophila; why, when, and when not? Wiley Interdiscip Rev Dev Biol 8:e346.

Suchowerska AK, Fath T (2014) Cytoskeletal changes in diseases of the nervous system. Front Biol 9:5-17.

Sugawara Y, Mizuno T, Moriyama K, Ishiwata H, Kato M, Nakashima M, Mizuguchi T, Matsumoto N (2020) Cerebrospinal fluid abnormalities in developmental and epileptic encephalopathy with a de novo CDK19 variant. Neurol Genet 6:e527.

Tepe B, et al. (2023) Bi-allelic variants in INTS11 are associated with a complex neurological disorder. Am J Hum Genet 110:774-789.

Yamamoto S, et al. (2014) A drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. Cell 159:200-214.

Yamamoto S, Kanca O, Wangler MF, Bellen HJ (2024) Integrating non-mammalian model organisms in the diagnosis of rare genetic diseases in humans. Nat Rev Genet 25:46-60.

Yang S, Yu W, Chen Q, Wang X (2021) A novel variant of CDK19 causes a severe neurodevelopmental disorder with infantile spasms. Cold Spring Harb Mol Case Stud 7:a006082.

Yin P, Cai Y, Cui T, Berg AJ, Wang T, Morency DT, Paganelli PM, Lok C, Xue Y, Vicini S, Wang T (2023) Glial sphingosine-mediated epigenetic regulation stabilizes synaptic function in Drosophila models of Alzheimer's disease. J Neurosci 43:6954-6971.

Yoshizawa M, Hoshino M, Sone M, Nabeshima Y (2002) Expression of stef, an activator of Rac1, correlates with the stages of neuronal morphological development in the mouse brain. Mech Dev 113:65-68.

Zarate YA, et al. (2021) CDK19-related disorder results from both loss-of-function and gain-of-function de novo missense variants. Genet Med 23:1050-1057.

C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y

Additional Table 1 Examples of rare neurological diseases studied in *Drosophila*

Human gene	Human phenotype	Variant function	Fly homolog	Strategy in flies	Fly phenotype with variants	Fly gene expression	Human protein localization in cell	Reference
CDK19	Infant spasm(epilepsy) DD Speech delay Hypotonia Dysmorphic features (OMIM #618916)	LoF ·Missense	*Cdk8*	Humanization Disease modeling	Semi-lethal Bang sensitivity	Neuron in adult and larval CNS	·Nucleus ·Perinuclear cytoplasm ·Cytoplasm ·Mitochondrion (Not published)	Chung et al., 2020a; Zarate et al., 2021; Yang et al., 2021; Sugawara et al., 2020
TIAM1	Seizures DD ID Speech delay (OMIM #619908)	LoF ·Bi-allelic missense	*sif*	Humanization Disease modeling	Semi-lethal Severe climbing defect Bang sensitivity	Neuron The neuropil of the CNS	·Nuclear membrane ·Nucleoplasm ·Cytoplasm	Lu et al., 2022
DNMBP	Infantile cataracts (OMIM #618415)	LoF ·Nonsense ·Frameshift deletion ·Frameshift	*sif*	Disease modeling	Morphological and functional defects in developing eyes	Lens-secreting cells	·Nucleoli ·Golgi apparatus ·Cytoplasm	Ansar et al., 2018
IRF2BPL	Seizures Neurodevelopmental disorder with regression Loss of speech Abnormal movements (OMIM #618088)	LoF ·Nonsense ·Missense	*Pits*	Overexpression Disease modeling	Progressive abnormalities in climbing and bang sensitive with aging	Neuron Third-instar larvae: whole CNS, enriched in MB Adult: cell bodies of MB	·Nucleoli ·Nucleoplasm ·Axons	Marcogliese et al., 2018
TNPO2	Seizures DD ID Speech impaired Dysmorphic features (OMIM #619556)	GoF and LoF ·Missense ·Deletion	*dTnpo*	Overexpression Humanization Disease modeling	Homozygous lethal *dTnpo* loss in eyes and wings causes dysmorphisms	Neuron Larva: cell bodies of MB neurons, and ventral nerve cord. Adult: optic lobe, MB cell bodies, and the	·Nucleoli ·Nucleoplasm	Goodman et al., 2021

						central complex		
EMC1[*]	Seizures DD Speech delay Hypotonia Cortical visual impairment (OMIM # 616875)	LoF ·Nonsense ·Missense ·Bi-allelic missense	*EMC1*	Humanization Overexpression (Knockdown)	Semi-lethal or Pupal lethal A motor deficit at Day 7 and are very short-lived	Neuron and Glia Pathogenic in glia.	·Endoplasmic reticulum	Harel et al., 2016; Geetha et al., 2018; Cabet et al., 2020; Chung et al., 2022
ACOX1	Seizures Decreased cognition Loss of learning and speaking skills Loss of vision and hearing Hypotonia (OMIM #264470)	LoF ·Missense ·In-frame deletions	*dACOX1*	Knock down Overexpression	Developmental delay Pupal death Reduced lifespan Impaired synaptic transmission, Glial and axonal loss	Glia Especially perineural glia and wrapping glia	·Nucleoli ·Peroxisomes	Ferdinandusse et al., 2007; Chung et al., 2020b
	Mitchell Syndrome; Decreased cognition Progressive myeloneuropathy Sensorineural hearing loss Ataxia (OMIM #618960)	GoF ·Missense		Overexpression Disease modeling	Semi lethal Unable to fly, walk, or feed, and they die in less than 2 days			Chung et al., 2020b; Chung et al., 2023
SUPT16H	Epilepsy DD ID/Cognitive delay Dysmorphism and brain structural abnormalities (OMIM #619480)	LoF ·Missense	*dre4*	Overexpression Humanization	Pupal lethal, reduced lifespan.	Glia Especially perineural glia and sub perineurial glia are pathogenic.	·Nucleoli fibrillar center ·Nucleoplasm ·Chromosome	Ma et al., 2023
IQSEC1	Epilepsy DD ID Speech delay Hypotonia Motor developmental delay (OMIM #618687)	LoF ·Missense	*schizo*	Humanization	Embryonic lethal. The vision of these flies is either very severely impaired or lost.	Neuron and glia Larva: broadly localized in the nervous system in embryos. Adult: neuropil and cell bodies	·Nucleoli ·Nucleoli rim ·Cytoplasm	Ansar et al., 2019
INTS11	DD ID	LoF ·Missense	*dIntS11*	Overexpression Humanization	Semi-lethal, shortened lifespan,	Neurons and glia.	·Nucleoplasm ·Cytoplasm	Tepe et al., 2023

	Language delay Impaired motor development (OMIM #620428)	·Duplication ·Deletion		Disease modeling	bang sensitivity, and affect locomotor activity.	Pathogenic in glia		

*: Phenotypic expansion. DD: Developmental delay; GoF: gain of function; ID: intellectual disability; LoF: loss of function; MB: mushroom body; Pits: protein interacting with Ttk69 and Sin3A; Sif: stil life.