

De novo variants in *PLCG1* are associated with hearing impairment, ocular pathology, and cardiac defects

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Abstract

Phospholipase C isozymes (PLCs) hydrolyze phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate and diacylglycerol, important signaling molecules involved in many cellular processes. *PLCG1* encodes the PLC γ 1 isozyme that is broadly expressed. Hyperactive somatic mutations of *PLCG1* are observed in multiple cancers, but only one germline variant has been reported. Here we describe three unrelated individuals with *de novo* heterozygous missense variants in *PLCG1* (p.Asp1019Gly, p.His380Arg, and p.Asp1165Gly) who exhibit variable phenotypes including hearing loss, ocular pathology and cardiac septal defects. To model these variants *in vivo*, we generated the analogous variants in the *Drosophila* ortholog, *small wing* (*sl*). We created a null allele *sl*^{T2A} and assessed the expression pattern. *sl* is broadly expressed, including in wing discs, eye discs, and a subset of neurons and glia. Loss of *sl* causes wing size reductions, ectopic wing veins and supernumerary photoreceptors. We document that mutant flies exhibit a reduced lifespan and age-dependent locomotor defects. Expressing wild-type *sl* in *sl*^{T2A} mutant rescues the loss-of-function phenotypes whereas expressing the variants causes lethality. Ubiquitous overexpression of the variants also reduces viability, suggesting that the variants are toxic. Ectopic expression of an established hyperactive *PLCG1* variant (p.Asp1165His) in the wing pouch causes severe wing phenotypes, resembling those observed with overexpression of the p.Asp1019Gly or p.Asp1165Gly variants, further arguing that these two are gain-of-function variants. However, the wing phenotypes associated with p.His380Arg overexpression are

mild. Our data suggest that the *PLCG1* *de novo* heterozygous missense variants are pathogenic and contribute to the features observed in the probands.

eLife assessment

This **important** study reveals how *Drosophila* may be used to investigate the role of missense variants in the gene *PLCG1* related to human disease in case studies. The evidence that most of these variants have a gain-of-function effect in the fly is **convincing** and supportive of their pathogenic effect. With some additional control experiments to assess overexpression toxicity, this work would be of relevance to human and *Drosophila* geneticists alike.

Introduction

The inositol lipid-specific phospholipase C (PLC) isozymes are key signaling proteins that play critical roles in transducing signals from hormones, growth factors, neurotransmitters, and many extracellular stimuli ^{1,2,3}. The PLCs selectively hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) ^{4,5}. PIP2 functions as a membrane anchor for numerous proteins and affects membrane dynamics and ion transport ^{6,7,8}. The two products, IP3 and DAG, are important intracellular second messengers involved in Ca²⁺ signaling regulation and protein kinase C signaling activation, respectively ^{9,10}. Hence, PLC orchestrates diverse cellular processes and behaviors, including cell growth, differentiation, migration, and cell death ^{11,12,13,14}. There are at least thirteen PLC isozymes grouped in 6 classes (β, δ, ε, γ, η, ζ) in mammals with similar enzymatic function, but each PLC has its own spectrum of activators, expression pattern, and subcellular distribution ^{15,16,17}.

PLCG1 [MIM: 172420] encodes the PLCγ1 isozyme. PLCγ1 is directly activated by receptor tyrosine kinases (RTKs) as well as cytosolic receptors coupled to tyrosine kinases ¹⁸. Upon tyrosine phosphorylation, PLCγ1 undergoes conformational changes that release its autoinhibition upon which it associates with the plasma membrane to bind and hydrolyze its substrates ^{19,20,21}. There is a second PLCγ isozyme, PLCγ2, encoded by *PLCG2* [MIM: 600220]. Although these two isozymes have similar protein structure and activation mechanism, they are differentially expressed and regulated, and play non-redundant roles ^{22,23}. *PLCG2* is mostly expressed in cells of the hematopoietic system and mainly functions in immune response, causing human diseases associated with immune disorders ^{24,25,26,27}. However, *PLCG1* is ubiquitously expressed and is enriched in the central nervous system (CNS) ²⁸. *Plcg1* is essential in mice, and a null allele causes embryonic lethality with developmental defects in the vascular, neuronal, and immune system ^{29,30,31}. *PLCG1* has emerged as a possible driver for cell proliferation, and increased expression level of *PLCG1* has been observed in breast cancer, colon cancer, and squamous cell carcinoma ^{32,33,34}. Moreover, hyperactive somatic mutations of *PLCG1* have been observed in angiosarcomas and T cell leukemia/lymphomas ^{35,36,37}. However, the genotype-phenotype association of germline *PLCG1* variants has yet to be explored.

Results

Individuals with *de novo* heterozygous missense variants in *PLCG1* exhibit hearing impairment, ocular pathology, and cardiac defects

Here, we report three unrelated individuals with *de novo* heterozygous missense variants in *PLCG1* (GenBank: NM_002660.3). Proband 1 (c.3056A>G, p.Asp1019Gly) and proband 2 (c.1139A>G, p.His380Arg) were identified through the Undiagnosed Diseases Network (UDN) ⁴⁵ and proband 3 (c.3494A>G, p.Asp1165Gly) was identified via GeneMatcher ⁴⁶. The probands range in age from 5 years to 20 years. The phenotypes of the probands partially overlap but show a diverse spectrum. Pertinent shared features include deafness (probands 1 and 3), ophthalmologic abnormalities (probands 1 and 2), and cardiac septal defects (probands 1 and 3). Two of the three individuals show abnormal brain MRI (probands 1 and 2), and one has immune defects (proband 3). Additional variants identified in the probands are discussed in supplemental data. [As per medRxiv policy, the whole and detailed case history for the probands have been removed. To obtain more detailed information, please contact the authors]

The proband-associated missense *PLCG1* variants are located in conserved protein domains and are predicted to be deleterious

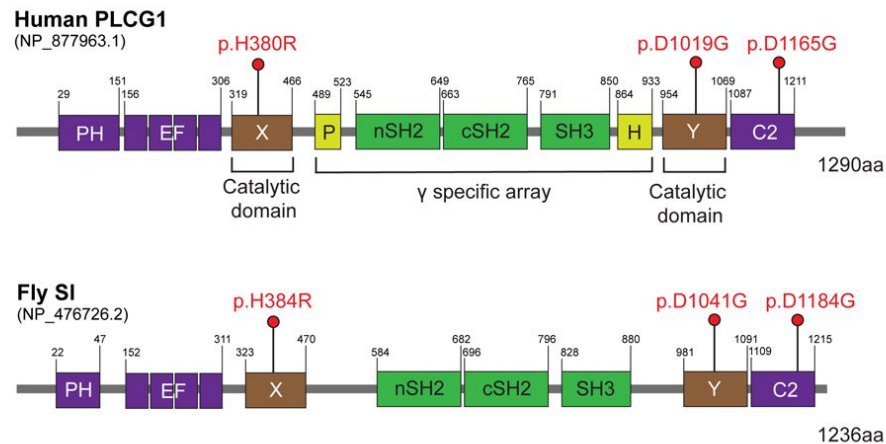
PLCG1 is predicted to be tolerant to loss-of-function alleles with a pLI score ⁴⁷ of 0.16, suggesting that loss of one copy of the gene is unlikely to cause haploinsufficiency in humans and consistent with the presence of many protein truncating variants in gnomAD ⁴⁸. However, the missense constraint Z score ⁴⁷ of *PLCG1* is 3.69, suggesting it is intolerant to missense variants. In addition, the prediction based on the DOMINO algorithm indicates that *PLCG1* variants are likely to be dominant ⁴⁹. The *in-silico* pathogenicity predictions suggest that these variants are likely to be pathogenic (Table S1) based on MARRVEL ⁵⁰.

The three variants identified from the probands map to different conserved domains of PLC γ 1, and each variant affects an amino acid residue that is conserved from flies to humans (Figure 1A and Figure 1B). The p.Asp1019Gly and p.His380Arg variants map to the catalytic core domains, and the p.Asp1165Gly is in the C-terminal C2 domain. PLC γ 1 contains other conserved domains including an N-terminal pleckstrin homology (PH) domain, four EF hand motifs, as well as a PLC γ -specific regulatory array that is composed of a split PH domain (sPH), two Src homology 2 (nSH2 and cSH2) domains and a Src homology 3 (SH3) domain.

small wing (sl) is the fly ortholog of human *PLCG1*

To obtain information about the nature of the proband-associated variants, we utilize *Drosophila* to model the variants *in vivo*. Flies have three genes encoding PLC isozymes (Table S2). Among them, *small wing (sl)* is predicted to be the ortholog of *PLCG1* with a DIOPT (DRSC Integrative Ortholog Prediction Tool) score of 17/18 (DIOPT version 9.0) ⁵¹. The encoded proteins share 39% identity and 57% similarity and are composed of similar conserved domains (Figure 1A). *sl* is also predicted to be the ortholog of *PLCG2* with a DIOPT score of 12/18. These data suggest that *sl* corresponds to two human genes encoding the PLC γ isozymes. We generated transgenic flies carrying the UAS-human *PLCG1* cDNAs for both the reference (UAS-*PLCG1*^{Reference}) and the variants (UAS-*PLCG1*^{D1019G}, UAS-*PLCG1*^{H380R}, and UAS-*PLCG1*^{D1165G}). Given the high level of protein sequence homology and the conservation of the affected amino acids (Figure 1B), we also generated transgenic flies for the reference and analogous variants in the fly *sl* cDNA, namely UAS-*sl*^{WT} and UAS-*sl*^{variants} (UAS-*sl*^{D1041G}, UAS-*sl*^{H384R}, and UAS-*sl*^{D1184G}). *sl* is on the X chromosome, and several alleles of *sl* have been isolated or generated previously, including *sl*²,

A The proband variants are in the conserved protein domains



B The proband variants affect conserved amino acids cross species



C *sl* reagents

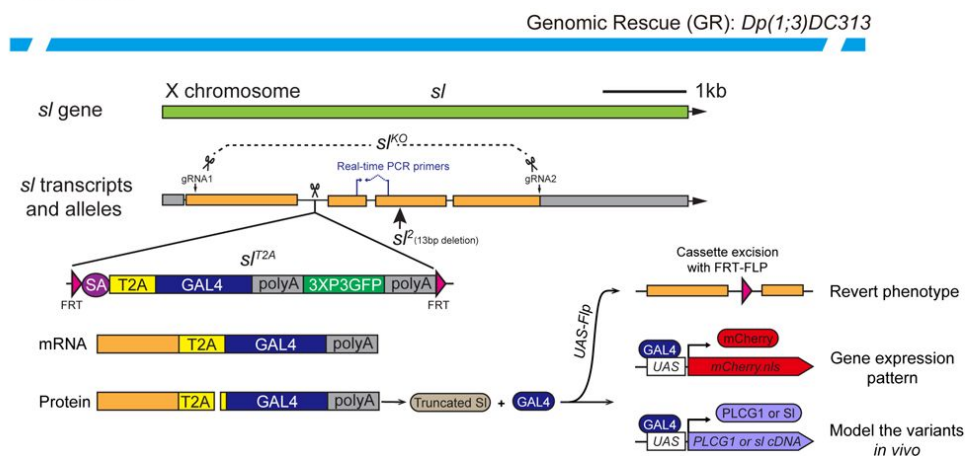


Figure 1.

The *PLCG1* homolog, *sl* is conserved in *Drosophila*

(A) Schematic of human *PLCG1* and fly *SI* protein domains and positions of the variants identified in the probands. Domain prediction is based on annotation from NCBI. Alignment of human *PLCG1* and the homologous proteins. The variants are marked with boxes.

(B) All the variants affect conserved amino acids (labeled in red). Isoforms for alignment: Human *PLCG1* NP_877963.1; Mouse *Plcg1* NP_067255.2; Zebrafish *plcg1* NP_919388.1; Fly *sl* NP_476726.2.

(C) Schematic of fly *sl* genomic span, transcript, alleles and the 92kb genomic rescue (GR) construct. Loss-of-function alleles of *sl* including *sl*² (13bp deletion ⁵²), *sl*^{KO} (CRISPR-mediated deletion of the gene span ⁵³), and *sl*^{T2A} (T2A cassette inserted in the first intron, ⁵⁴) are indicated. The T2A cassette in *sl*^{T2A} is flanked by FRT sites and can be excised by Flippase to revert loss-of-function phenotypes. GAL4 expression in *sl*^{T2A} under the control by *sl* endogenous promoter can be used to assess *sl* gene expression pattern by crossing with a *UAS-mCherry.nls* reporter line, or be used to model patient variants *in vivo* by crossing with human *PLCG1* cDNAs or corresponding fly *sl* cDNAs. The primer pair used for real-time PCR is indicated.

sl^{KO} and sl^{T2A} (Figure 1C). sl^2 carries a 13bp deletion in the third exon that leads to a frameshift and early stop gain⁵². sl^2 is a strong loss-of-function allele that causes small wing size, ectopic wing veins and extra R7 photoreceptors⁵². sl^{KO} was generated by CRISPR-mediated genomic editing that removes nearly the entire gene⁵³. sl^{T2A} allele was generated by inserting an FRT-SA-T2A-GAL4-polyA-FRT cassette as an artificial exon into the first coding intron of sl (Figure 1C)^{54,55}. The polyA arrests transcription, and sl^{T2A} is a strong loss-of-function allele (Figure S1A). The T2A viral sequence triggers ribosomal skipping and leads to the production of GAL4 proteins^{56,57} that are expressed in the proper spatial and temporal pattern of sl . This allows us to assess the expression pattern of sl by driving the expression of a *UAS-fluorescent protein*⁵⁴, or to assess the function of variants by expressing the human *UAS-reference/variant cDNAs*^{42,58–62}. In addition, the cassette is flanked by two FRT sites so that it can be excised from the cells that express the gene in the presence of *UAS-Flippase* and revert the mutant phenotypes (Figure 1C)⁵⁴.

We first assessed the expression pattern of sl by driving *UAS-mCherry.nls* (an mCherry that localizes to nuclei) with sl^{T2A} . sl is expressed in the 3rd larval wing discs and eye discs (Figure 2A), consistent with the loss-of-function phenotypes observed in the wings and eyes⁵². The expression pattern of sl in the wing discs is not homogenous. Higher expression levels are observed in the anterior compartment and along both the anterior/posterior and dorsal/ventral compartment boundaries (Figure 2A). The hemizygous sl^{T2A}/Y male flies and the trans-heterozygous sl^{T2A}/sl^2 or sl^{T2A}/sl^{KO} female flies show reduced wing size and ectopic wing veins (Figure 2B and Figure S1B), as well as additional photoreceptors in the eye (Figure 2C and Figure S1C). These phenotypes can be rescued by *UAS-Flippase* or by introducing a genomic rescue construct (*Dp(1;3)DC313*⁶³, Figure 1C) that covers the sl locus (Figure 2B and Figure 2C). These data show that all the observed phenotypes in sl^{T2A} mutants can be attributed to the loss of sl .

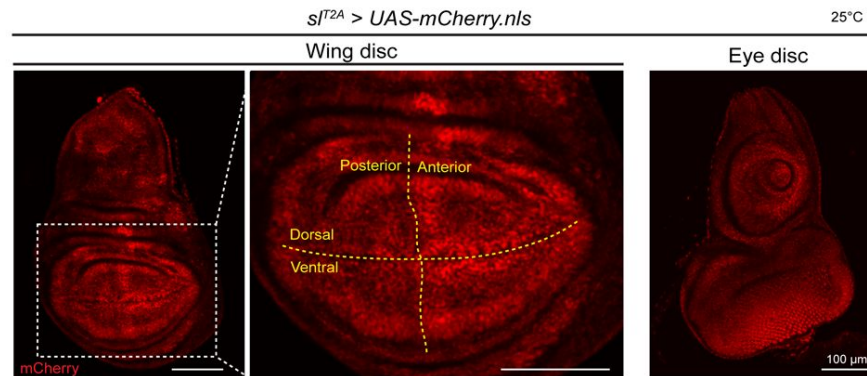
sl is expressed in the fly CNS and loss of sl causes longevity and locomotion defects

Given that human *PLCG1* is highly expressed in the central nervous system (CNS)²⁸ and that the probands present with neurologic phenotypes including hearing or vision deficits (Table 1), we investigated the expression pattern and the cell type specificity of sl in the CNS of flies. sl is expressed in the larval CNS as well as the adult brain, and co-staining with the pan-neuronal marker Elav⁶⁴ and glial marker Repo⁶⁵ show that sl is expressed in a subset of neurons and glia cells in the CNS (Figure 3A). We therefore assessed the longevity and climbing of sl^{T2A} flies. Compared to the wild-type w^{1118} flies, sl^{T2A}/Y hemizygous mutant flies show a shortened lifespan and a progressively reduced climbing ability. These phenotypes can be rescued by expression of the wild-type sl cDNA (sl^{T2A}/Y ; *UAS- sl^{WT}*) (Figure 3B).

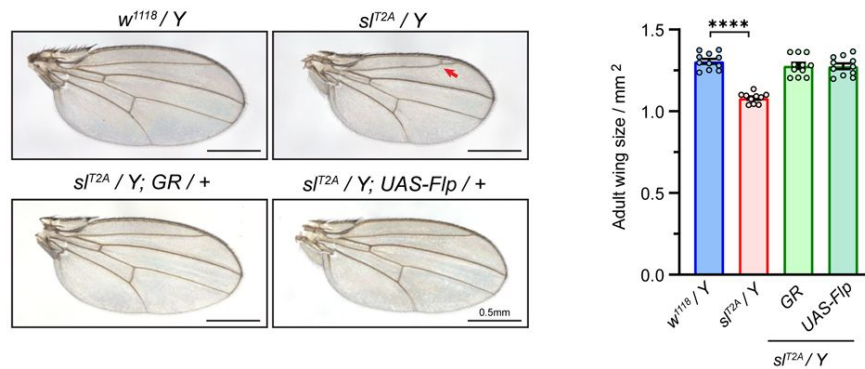
Functional assays in flies indicate that the *PLCG1* variants are toxic

To assess the impact of the variants, we expressed the sl variant cDNAs in the sl^{T2A}/Y hemizygous mutant males (sl^{T2A}/Y ; *UAS- $sl^{variants}$*) and compared their rescue ability with the wild-type sl (sl^{T2A}/Y ; *UAS- sl^{WT}*). As shown in Figure 4A, the sl^{T2A}/Y mutant flies (or the ones expressing a *UAS-Empty* control construct) have a slightly reduced eclosion rate but expression of the sl^{WT} cDNA fully rescues the percentage of eclosing progeny as measured by the Mendelian ratio. In contrast, expressing sl^{H384R} causes a severe reduction of the number of eclosing flies with very few escapers (sl^{T2A}/Y ; *UAS- sl^{H384R}*). Moreover, expression of the sl^{D1041G} or sl^{D1184G} leads to 100% lethality (Figure 4A). These data clearly indicate that the three variants are toxic.

A *sl* is expressed in the developing wing and eye discs



B *sl^{T2A}* causes ectopic veins and reduction in the size of the adult wings



C *sl^{T2A}* causes extra R7 photoreceptors in the adult eyes

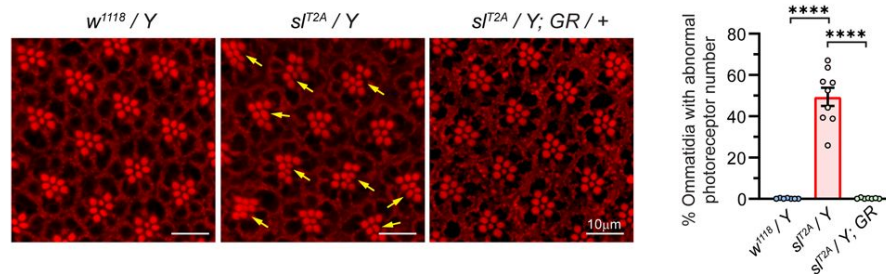


Figure 2.

***sl^{T2A}* is a loss-of-function allele causing phenotypes in wing and eye**

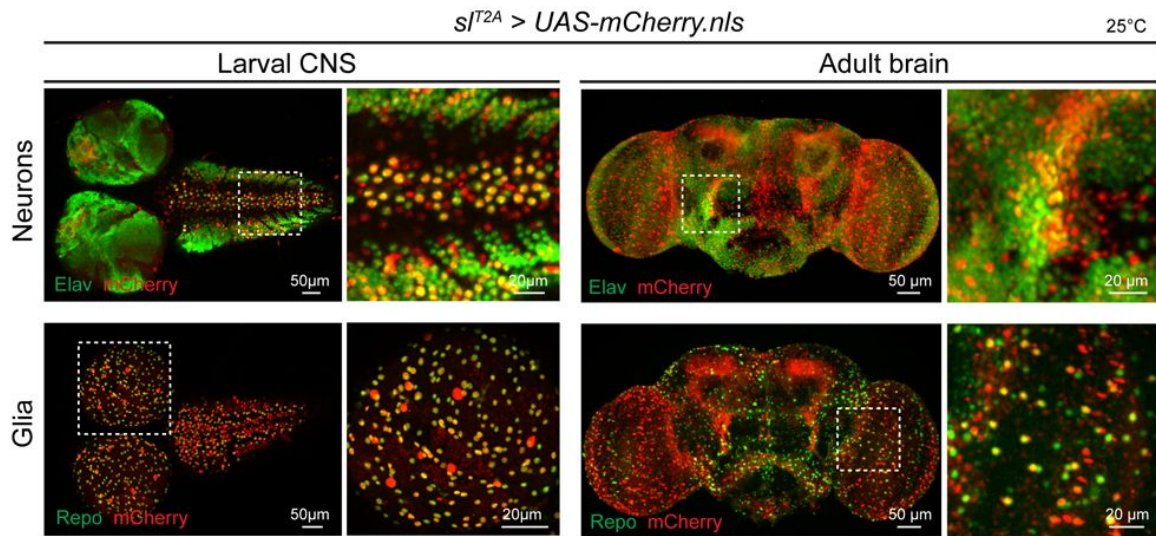
(A) *sl* expression in wing and eye. Expression of *UAS-mcherry.nls* (red) was driven by *sl^{T2A}* to label the nuclei of the cells that expressed *sl*. *sl* is expressed in the 3rd instar larval wing disc (left) and eye disc (right). Higher magnification image of the wing disc pouch region indicated by dashed rectangle is shown. The posterior/anterior and dorsal/ventral compartment boundaries are indicated by dashed lines in yellow. Scale bars, 100 μm

(B) *sl^{T2A}* causes wing size reduction and ectopic veins (arrowhead) in hemizygous mutant male flies.

The wing phenotypes can be rescued by introduction of a genomic rescue (GR) construct or the expression of Flippase. Scale bars, 0.5 mm. The quantification of adult wing size is shown in the right panel. Each dot represents the measurement of one adult wing sample. Unpaired t test, ****p < 0.0001, mean ± SEM.

(C) *sl^{T2A}* causes extra photoreceptors (arrows) in the hemizygous mutant flies. The eye phenotype can be rescued by introduction of a genomic rescue (GR) construct. The photoreceptor rhabdomeres stain positive for phalloidin labeling F-actin. Scale bars, 10 μm. The quantification is shown in the right panel. Each dot represents the measurement of one retina sample. Unpaired t test, ****p < 0.0001, mean ± SEM.

A *sl* is expressed in the CNS in a subset of neurons and glia



B Loss of *sl* causes behavioral defects including reduced lifespan and locomotor defect

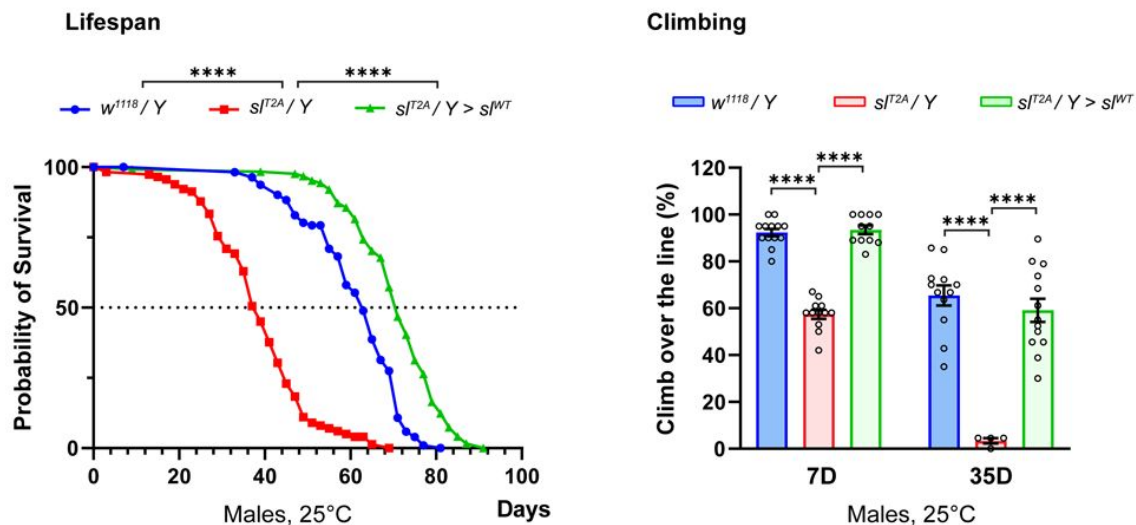


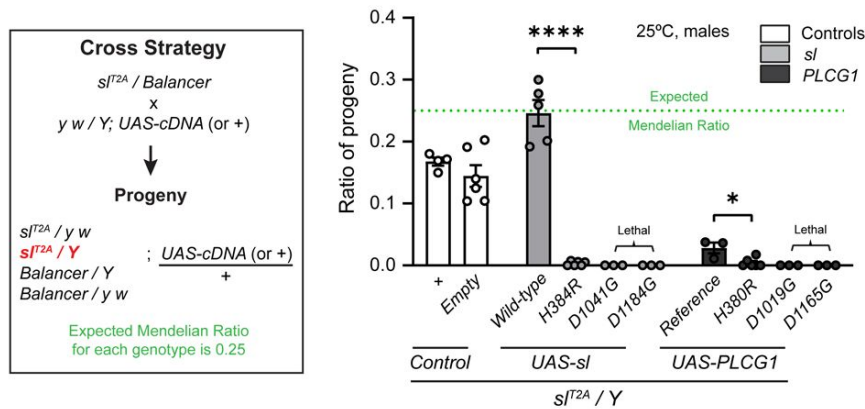
Figure 3.

sl is expressed in a subset of neurons and glia in the CNS, and loss of *sl* causes behavioral defects

(A) Expression pattern of *sl* in the central nervous system observed by sl^{T2A} -driven expression of $UAS-mCherry.nls$ reporter (red). In either larval or adult brain, *sl* is expressed in a subset of fly neurons and glia, which were labeled by pan-neuronal marker Elav (green, upper panel) and pan-glia marker Repo (green, lower panel). Higher magnification images of the regions indicated by dashed rectangles are shown. Scale bars, 20µm in the magnified images, 50µm in other images.

(B) Loss of *sl* causes behavioral defects in longevity and locomotion. sl^{T2A} hemizygous flies have a shorter lifespan than w^{1118}/Y control flies. The median life span of sl^{T2A} and w^{1118}/Y flies is 40 days and 62 days respectively. The short lifespan in sl^{T2A} flies can be rescued by a UAS transgene that expresses wild-type *sl* cDNA (sl^{WT}). Fly locomotion ability was assessed by climbing assay (see methods). sl^{T2A} flies at the age of 7 days show reduced locomotion ability and become almost immobile at the age of 35 days. The reduced locomotion ability in sl^{T2A} flies can be fully rescued by sl^{WT} . For lifespan assay, Longrank test, **** $p < 0.0001$. For climbing assay, each dots represents one vial containing 18-22 flies for test. Unpaired t test, **** $p < 0.0001$.

A Expression of *UAS-sl* or *UAS-PLCG1* cDNAs in *sl^{T2A}* mutant flies



B Ubiquitous overexpression of *UAS-sl* or *UAS-PLCG1* using *Tub-GAL4*

<i>Tub-GAL4</i> > <i>UAS-sl</i> cDNA				29°C
Wild-type	H384R	D1041G	D1184G	
Viable	Semi-lethal (~36% lethal)	L1-L2 lethal	L1-L2 lethal	

<i>Tub-GAL4</i> > <i>UAS-PLCG1</i> cDNA				29°C
Reference	H380R	D1019G	D1165G	
Semi-lethal (~63% lethal)	Semi-lethal (~62% lethal)	L1-L2 lethal	L1-L2 lethal	

C The toxicity caused by overexpressing human *PLCG1* cDNA is dosage sensitive

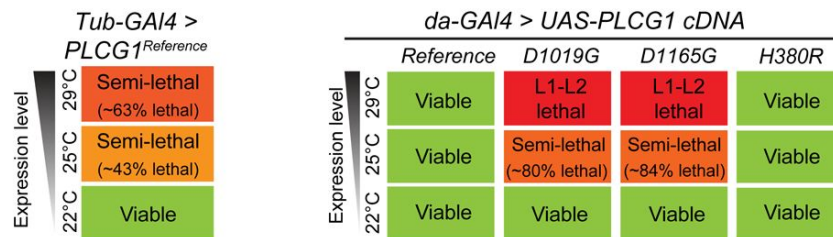


Figure 4.

The variant cDNAs are toxic

(A) Summary of the viability of expressing *sl* or *PLCG1* cDNAs in *sl^{T2A}* mutant flies. Cross strategy: heterozygous *sl^{T2A}* female flies were crossed to male flies carrying *UAS-cDNAs* or control (*UAS-Empty*) constructs, or crossed to the *y w* males as an extra control. The percentages of the hemizygous *sl^{T2A}* male progeny expressing different *UAS-cDNA* constructs were calculated. The expected Mendelian ratio is 0.25 (indicated by the green line in the graph). The dark gray columns represent human cDNAs, the light gray columns represent fly cDNAs, and the clear columns represent controls (*y w* or *UAS-Empty*). Each dot represents one independent replicate. Unpaired t test, **p* < 0.05, *****p* < 0.0001, mean ± SEM.

(B) Summary of the viability of ubiquitous overexpression of *sl* or *PLCG1* cDNAs driven by *Tub-GAL4*. Expression of wild-type fly *sl* cDNA shows no impact on viability whereas expression of the *sl* variants reduces viability. Expressing human *PLCG1* cDNAs at 29°C is toxic in flies. Expression of *PLCG1^{Reference}* or *PLCG1^{H380R}* causes semi-lethality, whereas expression of *PLCG1^{D1019G}* or *PLCG1^{D1165G}* is 100% lethal at early larval stage.

(C) Summary of the viability of ubiquitous overexpression *PLCG1* cDNAs using a strong driver *Tub-GAL4* (left panel) or a weaker driver *da-GAL4* (right panel) at different temperatures. The expression level rises with higher temperatures and decreases with lower temperatures, and the toxic impact on viability correlates with the expression level. Expression of *PLCG1^{D1019G}* or *PLCG1^{D1165G}* causes more severe toxicity than expression of *PLCG1^{Reference}* or *PLCG1^{H380R}*.

Given that the expression of these *sl* cDNAs is performed in a null mutant background, we opted to assess the phenotypes associated with ectopic expression assays by overexpressing the wild-type or variant *sl* cDNAs using a strong ubiquitous driver, *Tub-GAL4*. Overexpression of *sl*^{WT} shows no impact on viability. However, overexpression of *sl*^{H384R} leads to ~36% lethality, while overexpression of *sl*^{D1041G} or *sl*^{D1184G} results in 100% lethality at the L1-L2 larval stage (Figure 4B). Hence, ubiquitous overexpression assays cause similar but less severe lethal phenotypes than the expression assays driven by *sl*^{T2A}. This difference may arise from the different expression patterns and levels of *Tub-GAL4* and *sl*^{T2A}. Importantly, *sl*^{T2A} drives expression in the cells that normally express *sl*. In summary, the data argue that the *sl* variants are likely to be gain-of-function or neomorphic alleles and that *sl*^{D1041G} and *sl*^{D1184G} are very strong toxic alleles whereas *sl*^{H384R} is a milder allele.

To compare the *sl* and *PLCG1* associated phenotypes, we conducted similar assays using human *PLCG1* cDNAs. Expression of *PLCG1*^{Reference} in the *sl*^{T2A/Y} mutant flies (*sl*^{T2A/Y}; *UAS-PLCG1*^{Reference}) (Figure 4A) reduces viability by 90%, while the ubiquitous expression of *PLCG1*^{Reference} under the control of *Tub-GAL4* (*Tub-GAL4* > *UAS-PLCG1*^{Reference}) reduces viability by 63% (Figure 4B). These data argue that the reference human *PLCG1* cDNA is toxic in flies. Expression of *PLCG1*^{H380R} in the *sl*^{T2A/Y} mutant flies (*sl*^{T2A/Y}; *UAS-PLCG1*^{H380R}) leads to a modest increase in lethality when compared to *PLCG1*^{Reference} (Figure 4A), whereas *Tub-GAL4* > *UAS-PLCG1*^{H380R} is not worse than *PLCG1*^{Reference} (Figure 4B). In contrast, expression of *PLCG1*^{D1019G} or *PLCG1*^{D1165G} results in 100% lethality using either of the drivers (Figure 4A and 4B). In summary, expressing the human *PLCG1* in flies induces toxicity, and *PLCG1*^{H380R} has only slightly increased toxicity when compared to *PLCG1*^{Reference}. However, the *PLCG1*^{D1019G} or *PLCG1*^{D1165G} variants display a severe gain of toxicity.

Since overexpressing fly *sl*^{WT} does not cause viability issues (Figure 4B), the observed toxicity associated with *PLCG1*^{Reference} in flies may be due to the elevated expression of the human proteins. This hypothesis can be assessed by ectopic expression assays performed by raising the flies at different temperatures. The GAL4-UAS system is highly temperature-dependent since the promoter in the UAS construct contains an Hsp-70 promoter⁶⁶; at 29°C the expression level is much higher than at 25°C, and at 22°C the expression level is significantly lower than at 25°C⁶⁷. As shown in Figure 4C, the *Tub-GAL4* > *UAS-PLCG1*^{Reference} flies exhibit a high lethality ratio when raised at 29°C (~63% lethal). This lethality ratio decreased to ~43% when the flies are raised at 25°C whereas the flies are viable at 22°C. This shows that the toxicity is highly dependent on protein levels. Indeed, the expression levels can be further lowered by using a weak ubiquitous GAL4 driver, *da-GAL4*. The *da-GAL4* > *UAS-PLCG1*^{Reference} or *UAS-PLCG1*^{H380R} flies are viable at the three tested temperatures (29°C, 25°C, and 22°C). In contrast, *da-GAL4* > *UAS-PLCG1*^{D1019G} or *UAS-PLCG1*^{D1165G} flies are lethal at 29°C, semi-lethal (~80%-85% lethal) at 25°C, and viable at 22°C (Figure 4C, right panel). The animals that escape lethality at 25°C have smaller pupae (Figure S2) and a reduced adult body size, showing that growth is impeded. In summary, these data consistently confirmed that ubiquitous elevated expression levels of the human *PLCG1* is toxic in flies.

To assess other phenotypes, we used *nub-GAL4* to mostly drive the expression of *UAS-PLCG1* or *UAS-sl* cDNAs in the wing disc, a well-established model to study growth and differentiation in a tissue that is dispensable for viability⁶⁸. Overexpression of either *PLCG1*^{Reference} or *sl*^{WT} in the wing disc leads to slightly smaller wings with a ~10% reduction in size when compared to expressing the *UAS-Empty* control (Figure S3). Hence, both loss and overexpression of *sl* in the wing lead to a size reduction (Figure 2B and S3). This implies a potential dosage-dependent regulation on wing growth by the PLCy isozymes, although the underlying mechanism is unknown. Overexpression of the *PLCG1*^{H380R} or *sl*^{H384R} in the wing results in ~5% reduction in wing size when compared to the *PLCG1*^{Reference} or *sl*^{WT} (Figure S3). However, overexpression of *PLCG1*^{D1019G} or *PLCG1*^{D1165G} results in severe wing phenotypes characterized by notched wing margins, fused/thickened veins and reduced wing sizes (Figure 5A). Notably, overexpressing fly

sl^{D1041G} leads to very similar morphological defects as the corresponding human *PLCG1*^{D1019G} variant, indicating that the observed wing phenotypes are indeed due to alterations in *PLCG1/sl* functions. These data also argue that the human *PLCG1* functions in the same pathways as *sl*. Furthermore, overexpressing the *sl*^{D1184G} leads to pupal lethality (Figure 5A). This suggests that this variant has a more severe impact on development than the other variants and is not inconsistent with the observation that *nub-GAL4* drives some expression of *UAS-cDNA* in the nervous system as well.

We also assessed the effect of ectopic expression of human *PLCG1* on eye development using an eye-specific driver *eyeless-GAL4* (*ey-GAL4*). The expression of *PLCG1*^{Reference} or *PLCG1*^{H380R} in fly eyes leads to a mild reduction in eye size, while the expression of *PLCG1*^{D1019G} or *PLCG1*^{D1165G} leads to a severe reduction in eye size (Figure S4). In summary, the eye data are consistent with the wing data, showing that *PLCG1*^{D1019G} and *PLCG1*^{D1165G} are more toxic than *PLCG1*^{Reference}. On the other hand, the toxicity of *PLCG1*^{H380R} is milder and can be distinguished from that of the *PLCG1*^{Reference} only in certain contexts.

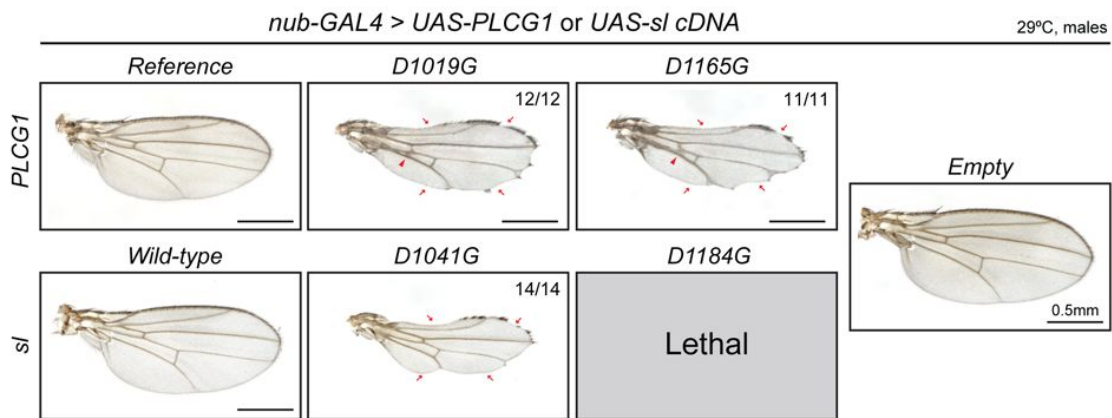
The p.Asp1019Gly and p.Asp1165Gly variants are gain-of-function variants

Previous studies have identified a very strong gain-of-function somatic *PLCG1* variant p.Asp1165His in adult T cell leukemia/lymphoma. This variant has been documented to cause a very dramatic increase in phospholipase activity *in vitro*. To characterize the impact of this hyperactive variant *in vivo*, we generated transgenic flies with this variant and tested *PLCG1*^{D1165H} in our tissue-specific expression assays. Overexpression of *PLCG1*^{D1165H} in the eye using *ey-GAL4* causes lethality at 29°C (Figure S4), arguing that it is highly toxic. As shown in Figure 5B, overexpressing *PLCG1*^{D1165H} in the wing using the *nub-GAL4* driver causes lethality at 29°C. However, these flies survive when they are raised at 25°C, yet the wings show severe morphological defects, including notched wing margins, thickened veins as well as reduced wing size. These phenotypes are similar to, but more severe than, the defects observed in the wings overexpressing *PLCG1*^{D1019G} or *PLCG1*^{D1165G} (Figure 5B). These data provide compelling evidence that these two variants are gain-of-function variants.

Discussion

A recent study reported an individual with a *de novo* heterozygous gain-of-function germline variant in *PLCG1*, p.Ser1021Phe. The proband exhibited an early-onset and severe immune dysregulation with autoimmune and autoinflammatory symptoms. Tao *et al.* performed *in vitro* and *ex vivo* experiments using cultured cell lines transfected with *PLCG1* constructs and peripheral blood mononuclear cells from the proband, respectively. They showed that the p.Ser1021Phe variant led to a 1.5-2 fold increase in intracellular IP3 production compared to controls. However, in our *in vivo* assays, overexpression of *PLCG1*^{S1021F} in wings or eyes does not cause obvious phenotypes when compared to *PLCG1*^{Reference} (Figure S5), which is clearly distinct from the other gain-of-function variants tested in our assays (p.Asp1019Gly, p.Asp1165Gly and p.Asp1165His) (Figure 5B). It is notable that the three probands reported here are discordant for immune-related phenotypes. Proband 1 with the p.Asp1019Gly variant has no immune dysregulation symptoms. Proband 2 with the p.His380Arg variant has a relapsing steroid responsive inflammatory encephalomyelitis, which is very different from the autoimmune symptoms reported by Tao *et al.* Finally, proband 3 with the p.Asp1165Gly variant presents with a T cell lymphocytopenia with recurrent infections suggesting that the individual is immune compromised. Hence, the immune phenotypes in the individuals reported here are very heterogeneous or absent, and inconsistent with an autoimmune disease. In summary, the immune-related phenotypes and their association with *PLCG1* variants will need to be explored in more depth.

A Wing-specific overexpression of *PLCG1*^{D1019G}, *PLCG1*^{D1165G} or their corresponding fly variants severely affect wing morphology



B Comparison between the established somatic hyperactive *PLCG1*^{D1165H} and the genetic *PLCG1*^{D1019G}, *PLCG1*^{D1165G} variants

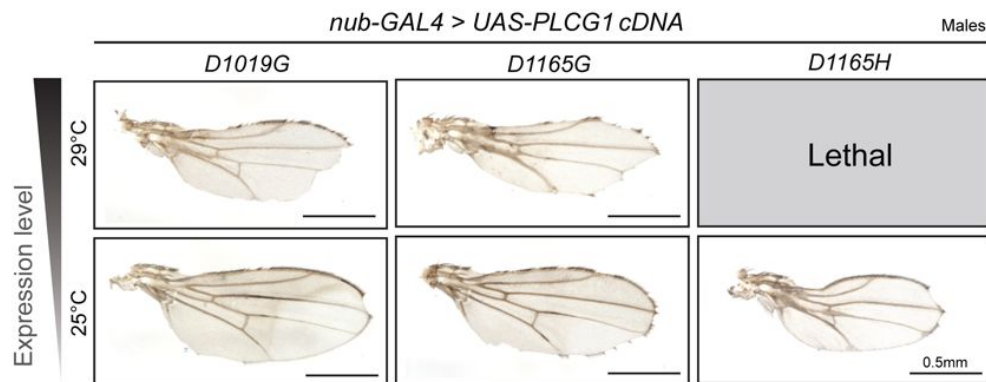


Figure 5.

The *PLCG1* p.Asp1019Gly and p.Asp1165Gly variants are likely to be gain-of-function alleles

(A) Wing-specific overexpression of *PLCG1*^{D1019G} or *PLCG1*^{D1165G} causes severe wing morphology defects including notched margin (arrows) and fused/thickened veins (arrowheads), fully penetrant (the penetration ratio is indicated). Note that overexpression of the fly *sl*^{D1041G} (corresponding to the human *PLCG1*^{D1019G}) shows similar phenotypes. Scale bars, 0.5mm.

(B) Wing-specific overexpression of an established hyperactive variant *PLCG1*^{D1165H} causes wing morphology phenotypes similar to, but more severe than that of *PLCG1*^{D1019G} or *PLCG1*^{D1165G}. Scale bars, 0.5mm.

Previously, studies based on biochemical assays and protein structures provided insights into how the variants studied here may affect the enzymatic activity of PLC γ 1 (the established protein structure of full-length rat Plcg1 is shown in **Figure 6A**). In its basal state, the PLC γ -specific regulatory array (sPH-nSH2-cSH2-SH3) forms autoinhibitory interfaces with the catalytic domains. Upon activation by the RTKs through binding to nSH2, PLC γ 1 is phosphorylated, which in turn induces the dissociation of the inhibitory cSH2 domain from the C2 domain. This triggers conformational rearrangements, allowing the enzyme to associate with the membrane and exposing the catalytic domains to allow hydrolysis of PIP2 ^{19,21,73,74}. As shown in **Figure 6A**, the location of the three variants modeled in this study (Asp1019Gly, His380Arg and Asp1165Gly) are either within the catalytic domains or at the intramolecular interfaces. The p.Asp1019Gly and p.Asp1165Gly variants impact crucial residues involved in autoinhibition. Specifically, the p.Asp1019Gly variant affects a residue located at the apex of the hydrophobic ridge within the Y box (**Figure 6B**), which is important for the interaction between the sPH domain and the Y box. This interaction is critical for the autoinhibition by blocking the membrane engagement of the catalytic core domain prior to enzymatic activation ^{20,75}. Notably, substitution of Asp1019 with Lys (D1019K) has been demonstrated to enhance basal phospholipase activity by approximately 15 fold *in vitro* ⁷⁶. Similarly, another hotspot somatic variant, p.Ser345Phe, located at the hydrophobic ridge within the X box, involved in the interaction between the sPH domain and X box, has also been verified to be hyperactive ^{37,77}. In contrast, the p.Ser1021Phe variant described by Tao *et al.* ⁷² lies outside the hydrophobic ridge of the interface between the sPH domain and Y box (**Figure 6B**). On the other hand, the p.Asp1165Gly variant affects a residue situated within a loop of the C2 domain (**Figure 6C**). The Asp1165 residue plays a key role in stabilizing the interaction between the cSH2 domain and the C2 domain to maintain the autoinhibited state ⁷⁸. As mentioned above, the somatic variant p.Asp1165His leads to significantly elevated phospholipase activity *in vitro* ^{71,73}, and results in severe phenotypes *in vivo* (**Figure 5B**). In contrast, the p.His380Arg variant impacts the His380 residue within the X box, situated near the bound Ca²⁺ cofactor in the catalytic core (**Figure 6D**). His380 plays a role in coordination of phosphate 1 of IP3 ⁷⁴. While this residue may not be key to the autoinhibition, it is important to the phospholipase activity. Substitution of His380 with Phe or Ala (H380F, H380A) has been reported to suppress PIP2 hydrolysis and IP3 production ^{79,80}. Substitution of the His380 with Arg in p.His380Arg variant may create a more basic environment, impacting the lipase activity in a distinct way. Our *in vivo* data are consistent with the published *in vitro* data, strengthening our conclusion that the variants are pathogenic and impact the protein function.

In summary, we report three individuals with *de novo* heterozygous missense variants in *PLCG1* presenting with disease features that encompass ophthalmologic, hearing, and cardiac defects with variable expressivity. Our functional assays provide compelling *in vivo* evidence that the *PLCG1* variants alter normal protein function. However, additional genetic variants (see Table 1) and environmental factors may contribute to some of the diverse phenotypes observed in these three individuals. Moreover, the PLC γ 1 isozyme is an integral component of multiple signaling pathways, and the outcomes of its dysregulation are expected to be context dependent. Hence, different variants of *PLCG1* may impact diverse cellular processes in different tissues and cells, leading to a range of pathological changes. Even when the affected residues are in close proximity to each other, their impact on protein function can be different ⁷³. Moreover, given that the p.Asp1019Gly and p.Asp1165Gly variants are hyperactive, potential therapeutic targets include specific inhibitors of *PLCG1*, such as antisense approaches that target the altered nucleotides ^{81,82}.

Data and code availability

This study did not generate datasets. All reagents developed in this study are available upon request.

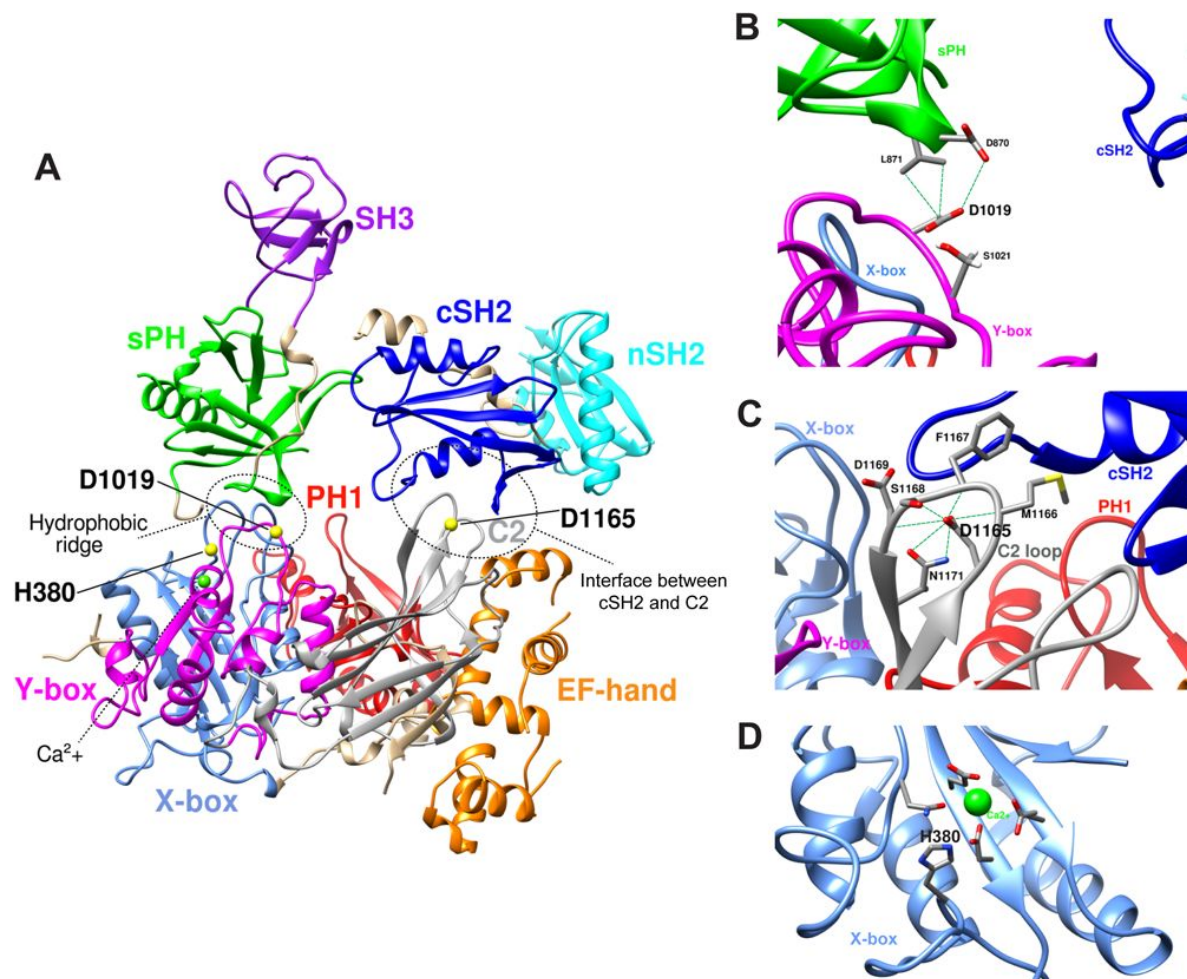


Figure 6.

***PLCG1* variants affect important residues**

(A) 3D structure of full-length rat *Plcg1* (PDB code: 6PBC; rat *Plcg1* shares 96.9% amino acid identity with human *PLCG1*). The conserved protein domains are labeled with different colors. Two major intracellular interfaces are circled by dashed lines: 1- The hydrophobic ridge between the sPH domain and the catalytic core (X-box and Y-box); and 2- The interface between the cSH2 domain and the C2 domain. The three residues affected by the variants are indicated by yellow balls.

(B) Enlarged views of the Asp1019 residue within the autoinhibition interface between sPH domain and the Y box. The potential interactions with nearby residues are indicated. Notably, the Ser1021 residue is outside of the hydrophobic ridge and shows no predicted interaction with the residues in the sPH domain.

(C) Enlarged views of the Asp1165 residue within the autoinhibition interface between the cSH2 domain and the C2 domain. The potential interactions with nearby residues are indicated.

(D) Enlarged view of the His380 residue within the X-box catalytic domain, in proximity to the Ca^{2+} cofactor. Structural analysis was performed via UCSF Chimera ⁸³.

Conflict of Interest Statement

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing completed at Baylor Genetics Laboratories.

Data Availability

All data produced in the present study are available upon reasonable request to the authors.

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Author contributions

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Supplemental Data

Additional variants identified in the probands

Proband 1 carries an intragenic duplication in *PSD3*. *PSD3* has not been associated with a Mendelian disorder but is potentially associated with an autosomal dominant arthrogryposis [10](#). Hence, it may underlie the joint defects observed in proband 1.

Proband 2 has compound heterozygous missense variants in *ERAP2* and *SEMA3G*. *ERAP2* [MIM: 609497] has not been associated with a Mendelian disorder. It encodes an ER-residential metalloaminopeptidase that functions in the major histocompatibility class I antigen presentation pathway. Some variants in *ERAP2* are associated with a susceptibility to autoimmune diseases

such as ankylosing spondylitis and Crohn's disease [2](#)[3](#)[4](#)[5](#). Given that proband 2 exhibits neuroinflammation and encephalitis, these phenotypes may be associated with the *ERAP2* variants. *SEMA3G* (Semaphorin 3G) has not been associated with a Mendelian disorder. However, a homozygous missense variant in *SEMA3G* was observed in two affected siblings from a consanguineous family. The siblings exhibited dysmorphic features as well as developmental delay [5](#)[6](#).

Proband 3 carries a *de novo* missense variant in *PKP2* [MIM: 602861]. *PKP2* encodes Pakophilin-2 and has been associated with dominant arrhythmogenic right ventricular dysplasia 9 [MIM: 609040] [6](#)[7](#)[8](#)[9](#). However, this proband was born with septal defects.

Expression of human *PLCG1* reduces the viability of *sl*^{T2A} hemizygotes, and *PLCG1*^{Reference} shows no obvious rescue of the phenotypes in the wings and eyes caused by loss of *sl*

We assessed if human *PLCG1* could effectively serve as a functional substitute for fly *sl* and rescue the loss-of-function phenotypes observed in *sl* mutant flies. However, only a small fraction of the *sl*^{T2A}/Y mutant hemizygotes expressing *PLCG1*^{Reference} can survive to adults (**Figure 4A** [10](#)). In addition to the obvious toxicity, *PLCG1*^{Reference} fails to rescue the phenotypes observed in the wings or eyes of the *sl* mutant flies (**Figure S6** [11](#)), which is fully rescued by fly *sl*^{WT} (**Figure 2** [12](#)). This suggests that despite their high DIOPT score, human *PLCG1* cannot fully substitute for fly *sl*. It is possible that during the course of evolution, *PLCG1* has acquired more specialized functions. For example, an essential step for enzymatic activation of mammalian PLC γ is the binding of its nSH2 domain to specific phosphotyrosines on the RTKs through a specific binding motif [9](#)[13](#). However, Thackeray *et al.* found that the consensus motif is absent in the intracellular domain of the *Drosophila* EGF receptor homolog DER [10](#)[14](#), one of the three RTKs in *Drosophila* which is required for wing vein differentiation and photoreceptor formation [11](#)[15](#)[16](#).

Supplemental Figures and Legends

Supplemental Tables

Table S1. Pathogenicity prediction of the proband variants

Table S2. Mammalian PLC coding genes and their fly orthologs

Table S3. Fly strains used in the experiments

Table S4. Primers used in the experiments

Material and methods

Recruitment of the probands

Formal consents for genetic testing and publication were obtained from all probands or their family members. Probands 1 and 2 were recruited through the Undiagnosed Diseases Network (UDN) and were evaluated through the clinical research protocol of the National Institutes of Health Undiagnosed Diseases (15-HG-0130), which was approved by the National Human Genome Research Institute (NHGRI). Proband 3 was recruited through GeneMatcher.

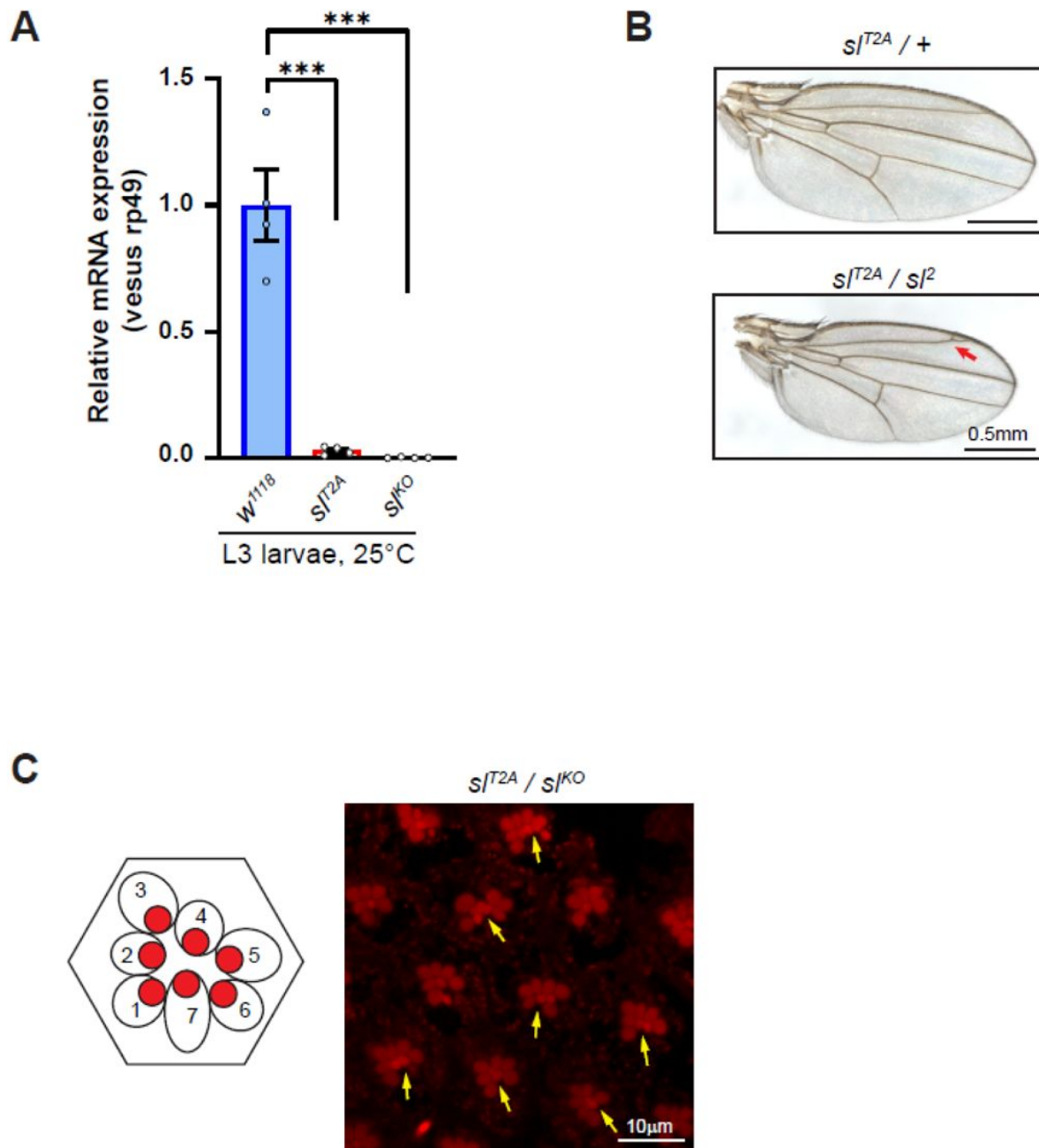


Figure S1.

***sl*^{T2A} is a loss-of-function allele causing wing and eye phenotypes**

(A) Relative *sl* mRNA expression are <5% and <1% in *sl*^{T2A} and *sl*^{KO} mutant larvae when compared to controls (*w*¹¹¹⁸). The primers used for real-time PCR are shown in **Figure 2A**. Each dot represents a replicate per genotype. Unpaired t test, ***p<0.001.

(B) Representative images showing that *sl*^{T2A}/*sl*² trans-heterozygous mutant flies have smaller wing and ectopic veins (indicated by arrow). Scale bars, 0.5mm.

(C) Representative images showing that *sl*^{T2A}/*sl*^{KO} trans-heterozygous mutant flies have extra photoreceptors (indicated by arrows). The schematic of the section of an ommatidia presenting seven photoreceptors (the R8 photoreceptor is not visible in such section) is shown. The photoreceptor rhabdomeres stain positive for phalloidin labeling F-actin. Scale bar, 10μm.

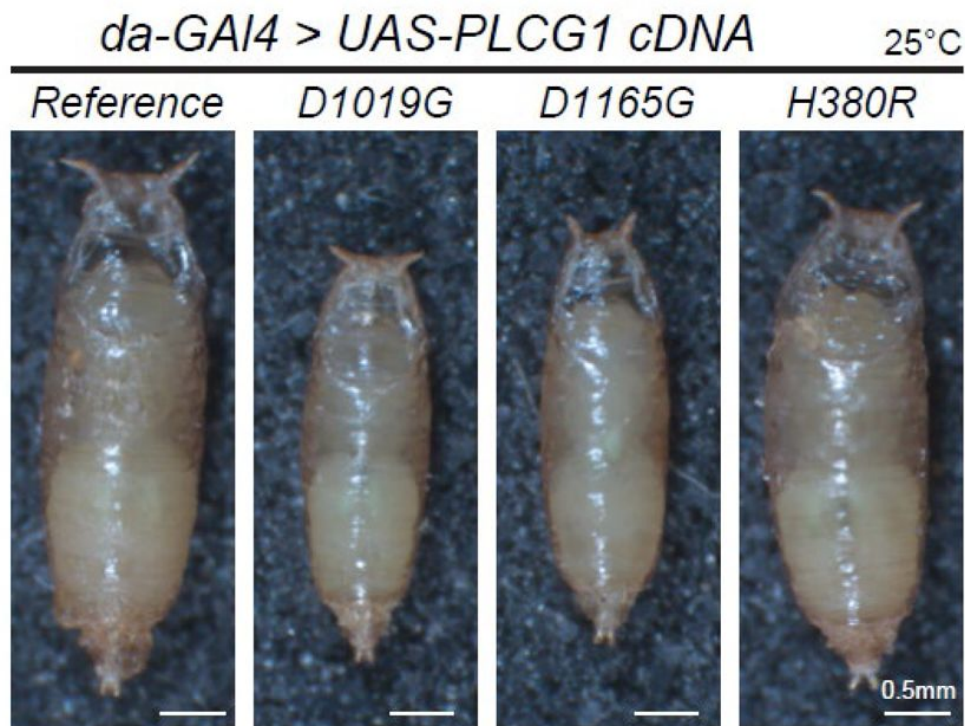


Figure S2.

Ubiquitous overexpressing of *PLCG1*^{D1019G} or *PLCG1*^{D1165G} driven by *da-GAL4* causes pupal size reduction

Representative images showing the pupal size of animals overexpressing *PLCG1* cDNAs by *da-GAL4* at 25°C. Overexpression of *PLCG1*^{D1019G} or *PLCG1*^{D1165G} causes pupae size reductions when compared to *PLCG1*^{Reference}. Overexpression of *PLCG1*^{H380R} is indistinguishable from *PLCG1*^{Reference}. Scale bars, 0.5mm.

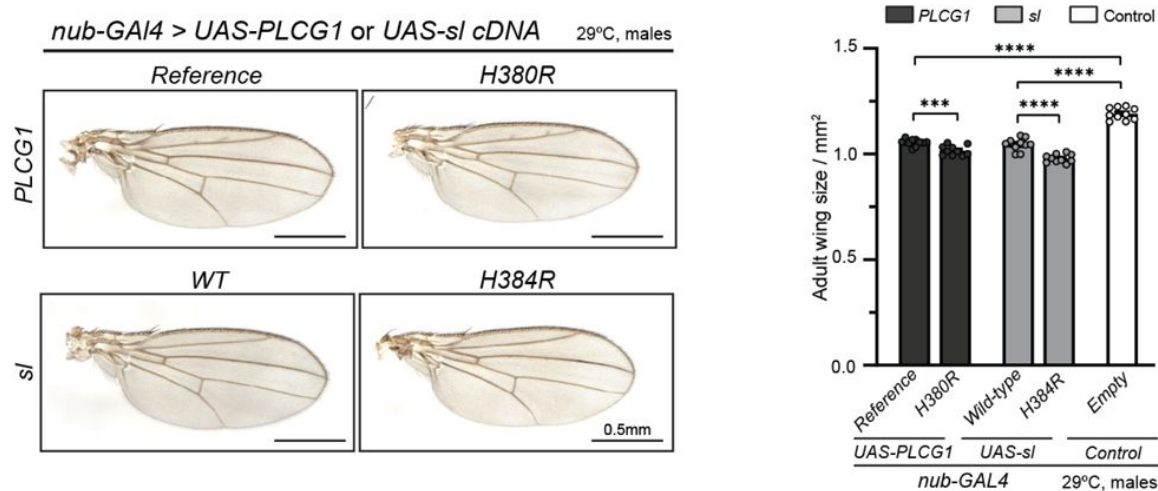


Figure S3.

Wing-specific overexpression of *PLCG1*^{Reference} or *sl*^{WT} causes wing size reduction, while overexpressing the *PLCG1*^{H380R} or *sl*^{H384R} further reduces wing size

Representative images showing that wing-specific overexpression of *PLCG1*^{Reference} or *sl*^{WT} causes ~15% wing size reduction compared to the *UAS-Empty* control construct. Overexpression of *PLCG1*^{H380R} or *sl*^{H384R} causes a further ~5% size reduction. Each dot represents one measured adult wing. Unpaired t test, ***p < 0.001, ****p < 0.0001, mean ± SEM. Scale bars, 0.5mm

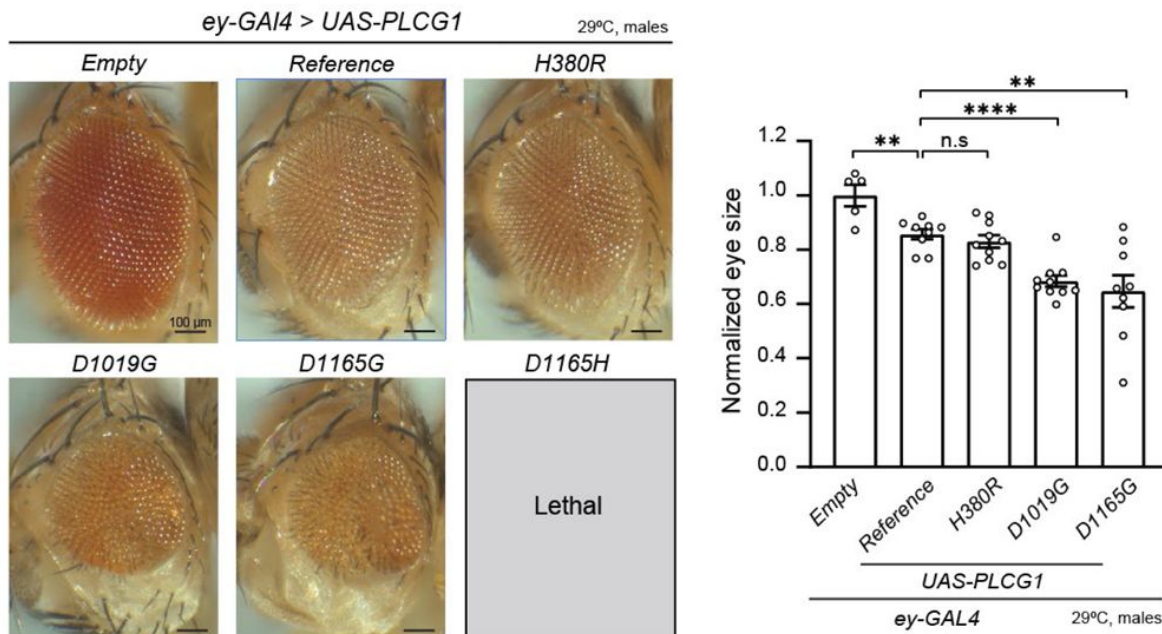


Figure S4.

Eye-specific overexpression of human *PLCG1* leads to size reduction in the eyes, and expression of the *PLCG1*^{D1019G} or *PLCG1*^{D1165G} causes more severe phenotype than expression of *PLCG1*^{Reference} or *PLCG1*^{H380R}

Representative images showing that eye-specific overexpression of *PLCG1*^{Reference} or *PLCG1*^{H380R} causes ~15% eye size reduction compared to the *UAS-Empty* control construct, whereas overexpression of *PLCG1*^{D1019G} or *PLCG1*^{D1165G} causes a ~30% size reduction. Overexpression of *PLCG1*^{D1165H} causes lethality. Each dot represents one measured adult eye. Unpaired t test, **p<0.01, ****p < 0.0001, ns: not significant, mean ± SEM. Scale bars, 100µm.

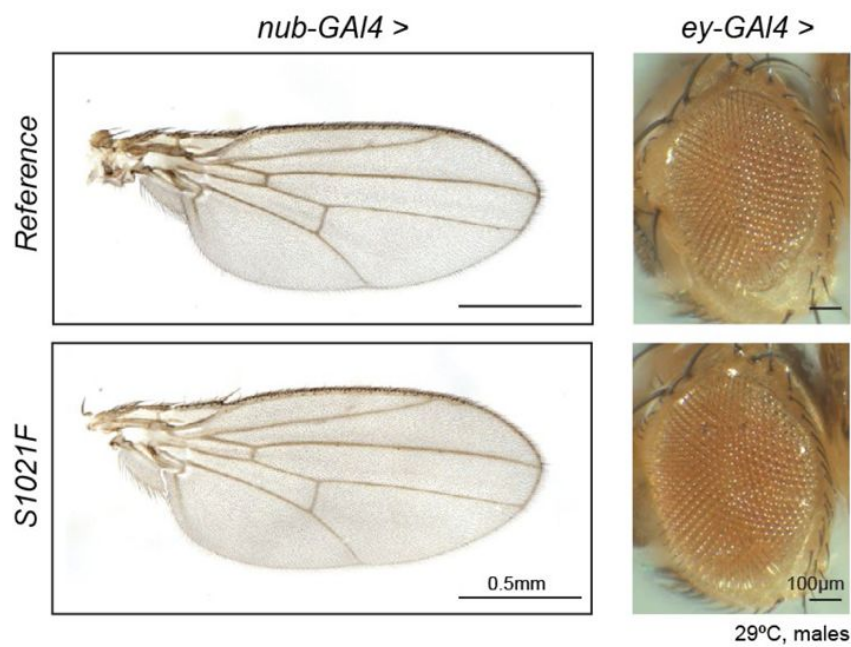


Figure S5.

Ectopic expression of *PLCG1*^{*S1021F*} causes indistinguishable phenotypes in the wings or eyes compared to *PLCG1*^{Reference}

Representative images showing the adult wings and eyes with *PLCG1*^{*S1021F*} overexpression using *nub-GAL4* or *ey-GAL4*, respectively. There is no obvious morphological difference compared to *PLCG1*^{Reference}. Scale bars, 0.5mm in the wing images, 100µm in the eye images.

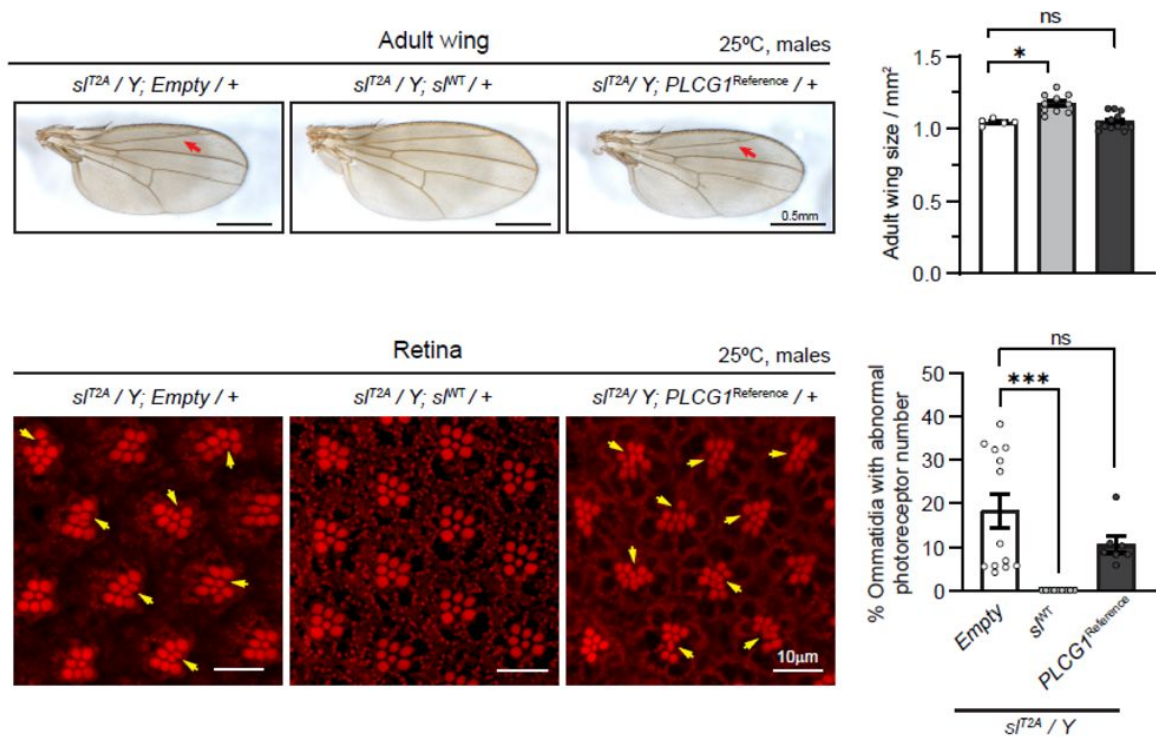


Figure S6.

Expressing human *PLCG1* does not rescue wing and eye phenotypes associated with *sI^{T2A}*

Representative images showing the adult wings (upper panel) and the photoreceptors (lower panel) expressing *PLCG1^{Reference}* or *sI^{WT}*. Expression of *sI^{WT}* rescues the loss-of-function phenotypes including wing size reduction, ectopic veins (indicated by red arrows) and extra photoreceptors (indicated by yellow arrows), whereas expression of the *PLCG1^{Reference}* or *UAS-Empty* shows no rescue. Scale bars, 0.5mm for the wing images and 10μm for the photoreceptor images. The photoreceptor rhabdomeres stain positive for phalloidin labeling F-actin. Quantification of the wing size (upper right panel) and the photoreceptors (lower right panel) are shown. Each dot represents one wing or one retina sample, respectively. Unpaired t test, ***p<0.001, *p<0.05. ns: not significant.

***Drosophila* husbandry and generation of transgenic flies**

The fly strains used in this study (listed in Table S3) were generated in house or obtained from the Bloomington *Drosophila* Stock Center (BDSC). All the flies were raised and maintained on standard fly food at room temperature unless specified. The *sl*^{T2A} allele was outcrossed with *w*¹¹¹⁸ to clean up the genetic background. The strains used in this study were listed in Supplemental Table S3.

To generate the *UAS-cDNA* transgenic lines, human *PLCG1* cDNA was obtained from Horizon Discovery (MHS6278-213246131, clone ID 9052656), and fly *sl* cDNA was obtained from *Drosophila* Genomics Resource Center (DGRC, RE62235). The coding sequence (CDS) of *PLCG1*^{Reference} and *sl*^{WT} were amplified using iProof™ High-Fidelity DNA Polymerase Kit (BioRad, #1725301), purified using QIAEX II Gel Extraction Kit (QIAGEN, #20021), sub-cloned into the Gateway compatible entry vector pDONR223 by BP cloning (BP clonase II, Thermo Fisher Scientific, #11789020) and sequentially cloned into the destination vector pGW-attB-HA by LR cloning (LR clonase II, Thermo Fisher Scientific, #11791100) ¹⁴. The variants were generated by site-directed mutagenesis strategy using Q5 Hot Start High-Fidelity 2x Master Mix (NEB, #M0494S) and *DpnI* restriction enzyme (NEB, # R0176L). All the constructs were sanger verified and injected, and inserted into the VK33 (*PBac{y[+]-attP}VK00033*) docking site using ϕ C31 mediated transgenesis ^{15,16}. Primers are listed in Supplemental Table S4.

***Drosophila* behavioral assays**

The climbing assay to examine the negative geotaxis and locomotion ability of the flies was performed as previously described ^{17,18} with some modifications. 18-22 flies per vial were transferred to an empty plastic vial and given 20min to rest prior to being tested. The flies were tapped to the bottom of the vial and were allowed to climb for 15s. The percentage of flies per vial that climbed over 5cm were calculated. The maximum distance from the bottom to the top is 18.5cm.

For the lifespan assay, newly eclosed male flies were collected and maintained at 25 °C (10 flies per vial). The flies were transferred to a new vial and the number of dead flies was counted every two days.

Immunostaining

Fly tissues were dissected in 1x PBS, fixed in 4% paraformaldehyde for 20min at room temperature, and washed in PBS (3 x 10min). For antibody staining, samples were treated with PBST (Triton X-100 in PBS, 0.1% for larval tissues, 2% for adult brain), 5% normal goat serum, and incubated in primary antibody overnight at 4°C. Samples were washed with 0.1% PBST (3 x 10min) and incubated with secondary antibody for 2h at room temperature (in darkness) and washed in 0.1% PBST (3 x 10min). Primary antibodies: rat anti-*Drosophila* Elav (1:250, DSHB, #7E8A10); mouse anti-*Drosophila* Repo (1:50, DSHB, #8D12). Secondary antibodies: goat anti-rat-647 (1:250, Jackson ImmunoResearch, #112-605-003), goat anti-mouse-Cy5 (1:250, Invitrogen, #A10524). Larval discs were mounted in Vectashield (Vector Labs #H1200 and #H1000). Larval CNS and adult brain were mounted in Rapiclear (Cedarlane, #RC147001). For adult retinas, flies are reared at 25 °C under 12-h light/dark conditions. Retinas were isolated from 5-7 day old flies. Heads were dissected in PBS and fixed in 3.7% formaldehyde overnight at 4°C. The samples were rinsed with 0.1% PBST and the retinas were subsequently dissected and incubated with PBST-diluted phalloidin 647 (1:100, Invitrogen, #A22287) for 1h. Retinas were washed in 0.1% PBST and mounted in Vectashield. The images were obtained with a confocal microscope (Leica SP8X or Zeiss Airyscan LSM 880) and processed using the ImageJ-FIJI software ¹⁹.

Imaging of adult fly wings and eyes

To prepare the samples of adult fly wings, the wing blades were dissected and mounted in a glycerol/ethanol 1/1 mixture. Only wings from the same gender were compared to each other since females have larger wings than males when raised in the same conditions. To prepare the samples of adult fly eyes, the flies were frozen and placed onto a double-side stick tape with one eye facing up. The samples were imaged using bright field Stereomicroscope (Leica MZ16 or Leica Z16 APO). Image Pro Plus 7.0 software was used to for extended depth-of-field images. The image processing and the measurement of the total areas of the wing blades or eyes were conducted using the ImageJ-FIJI software¹⁹.

Real-time PCR

Real-time PCR was performed as previously described²⁰ with modifications. All-In-One 5X RT MasterMix (abm, #G592), iTaq Universal SYBR Green Master Mix (BioRad#1725120) and BioRad C1000 Touch Cyclor were used. Primers are listed in Supplemental Table S4.

References

1. Berridge MJ, Irvine RF (1984) **Inositol trisphosphate, a novel second messenger in cellular signal transduction** *Nature* **312**
2. Exton JH (1996) **Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins** *Annu Rev Pharmacol Toxicol* **36**:481–509
3. Balla T (2013) **Phosphoinositides: tiny lipids with giant impact on cell regulation** *Physiol Rev* **93**:1019–1137
4. Majerus PW, Connolly TM, Deckmyn H, et al. (1986) **The metabolism of phosphoinositide-derived messenger molecules** *Science* **234**
5. Nishizuka Y (1984) **Turnover of inositol phospholipids and signal transduction** *Science* **225**
6. Hilgemann DW (2007) **Local PIP(2) signals: when, where, and how?** *Pflugers Arch* **455**:55–67
7. Hilgemann DW, Feng S, Nasuhoglu C (2001) **The complex and intriguing lives of PIP2 with ion channels and transporters** *Sci STKE* **111**
8. Suh BC, Hille B (2008) **PIP2 is a necessary cofactor for ion channel function: how and why?** *Annu Rev Biophys* **37**:175–195
9. Nishizuka Y (1992) **Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C** *Science* **258**
10. Berridge MJ (1993) **Inositol trisphosphate and calcium signalling** *Nature* **361**
11. Yang YR, Choi JH, Chang JS, et al. (2012) **Diverse cellular and physiological roles of phospholipase C-gamma1** *Adv Biol Regul* **52**:138–151
12. Cocco L, Follo MY, Manzoli L, Suh PG (2015) **Phosphoinositide-specific phospholipase C in health and disease** *J Lipid Res* **56**:1853–1860

13. Gomes DA, de Miranda MC, Faria J, Rodrigues MA (2021) **The basis of nuclear phospholipase C in cell proliferation** *Adv Biol Regul* **82**
14. Asano S, Maetani Y, Ago Y, Kanematsu T (2022) **Phospholipase C-related catalytically inactive protein enhances cisplatin-induced apoptotic cell death** *Eur J Pharmacol* **933**
15. Suh PG, Park JI, Manzoli L, et al. (2008) **Multiple roles of phosphoinositide-specific phospholipase C isozymes** *BMB Rep* **41**:415–434
16. Kadamur G, Ross EM (2013) **Mammalian phospholipase C** *Annu Rev Physiol* **75**:127–154
17. Katan M, Cockcroft S (2020) **Phospholipase C families: Common themes and versatility in physiology and pathology** *Prog Lipid Res* **80**
18. Gresset A, Sondek J, Harden TK (2012) **The phospholipase C isozymes and their regulation** *Subcell Biochem* **58**:61–94
19. Gresset A, Hicks SN, Harden TK, Sondek J (2010) **Mechanism of phosphorylation-induced activation of phospholipase C-gamma isozymes** *J Biol Chem* **285**:35836–35847
20. Hajicek N, Keith NC, Siraliev-Perez E, et al. (2019) **Structural basis for the activation of PLC-gamma isozymes by phosphorylation and cancer-associated mutations** *Elife* **8**
21. Nosbisch JL, Bear JE, Haugh JM (2022) **A kinetic model of phospholipase C-gamma1 linking structure-based insights to dynamics of enzyme autoinhibition and activation** *J Biol Chem* **298**
22. Homma Y, Takenawa T, Emori Y, Sorimachi H, Suzuki K (1989) **Tissue- and cell type-specific expression of mRNAs for four types of inositol phospholipid-specific phospholipase C** *Biochem Biophys Res Commun* **164**:406–412
23. Regunathan J, Chen Y, Kutlesa S, et al. (2006) **Differential and nonredundant roles of phospholipase Cgamma2 and phospholipase Cgamma1 in the terminal maturation of NK cells** *J Immunol* **177**:5365–5376
24. Yu P, Constien R, Dear N, et al. (2005) **Autoimmunity and inflammation due to a gain-of-function mutation in phospholipase C gamma 2 that specifically increases external Ca²⁺ entry** *Immunity* **22**:451–465
25. Ombrello MJ, Remmers EF, Sun G, et al. (2012) **Cold urticaria, immunodeficiency, and autoimmunity related to PLCG2 deletions** *N Engl J Med* **366**:330–338
26. Zhou Q, Lee GS, Brady J, et al. (2012) **A hypermorphic missense mutation in PLCG2, encoding phospholipase Cgamma2, causes a dominantly inherited autoinflammatory disease with immunodeficiency** *Am J Hum Genet* **91**:713–720
27. Neves JF, Doffinger R, Barcena-Morales G, et al. (2018) **Novel PLCG2 Mutation in a Patient With APLAID and Cutis Laxa** *Front Immunol* **9**
28. Consortium G. Human genomics (2015) **The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans** *Science* **348**

29. Ji QS, Winnier GE, Niswender KD, et al. (1997) **Essential role of the tyrosine kinase substrate phospholipase C-gamma1 in mammalian growth and development** *Proc Natl Acad Sci U S A* **94**:2999–3003
30. Liao HJ, Kume T, McKay C, Xu MJ, Ihle JN, Carpenter G (2002) **Absence of erythropoiesis and vasculogenesis in Plcg1-deficient mice** *J Biol Chem* **277**:9335–9341
31. Arteaga CL, Johnson MD, Todderud G, Coffey RJ, Carpenter G, Page DL (1991) **Elevated content of the tyrosine kinase substrate phospholipase C-gamma 1 in primary human breast carcinomas** *Proc Natl Acad Sci U S A* **88**:10435–10439
32. Noh DY, Lee YH, Kim SS, et al. (1994) **Elevated content of phospholipase C-gamma 1 in colorectal cancer tissues** *Cancer* **73**:36–41
33. Xie Z, Chen Y, Liao EY, Jiang Y, Liu FY, Pennypacker SD (2010) **Phospholipase C-gamma1 is required for the epidermal growth factor receptor-induced squamous cell carcinoma cell mitogenesis** *Biochem Biophys Res Commun* **397**:296–300
34. Park JG, Lee YH, Kim SS, et al. (1994) **Overexpression of phospholipase C-gamma 1 in familial adenomatous polyposis** *Cancer Res* **54**:2240–2244
35. Kunze K, Spieker T, Gamerding U, et al. (2014) **A recurrent activating PLCG1 mutation in cardiac angiosarcomas increases apoptosis resistance and invasiveness of endothelial cells** *Cancer Res* **74**:6173–6183
36. Behjati S, Tarpey PS, Sheldon H, et al. (2014) **Recurrent PTPRB and PLCG1 mutations in angiosarcoma** *Nat Genet* **46**:376–379
37. Vaque JP, Gomez-Lopez G, Monsalvez V, et al. (2014) **PLCG1 mutations in cutaneous T-cell lymphomas** *Blood* **123**:2034–2043
38. Bischof J, Bjorklund M, Furger E, Schertel C, Taipale J, Basler K (2013) **A versatile platform for creating a comprehensive UAS-ORFeome library in Drosophila** *Development* **140**:2434–2442
39. Bischof J, Maeda RK, Hediger M, Karch F, Basler K (2007) **An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases** *Proc Natl Acad Sci U S A* **104**:3312–3317
40. Venken KJ, He Y, Hoskins RA, Bellen HJ (2006) **P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in D. melanogaster** *Science* **314**
41. Madabattula ST, Strautman JC, Bysice AM, et al. (2015) **Quantitative Analysis of Climbing Defects in a Drosophila Model of Neurodegenerative Disorders** *J Vis Exp* **100**
42. Lu S, Hernan R, Marcogliese PC, et al. (2022) **Loss-of-function variants in TIAM1 are associated with developmental delay, intellectual disability, and seizures** *Am J Hum Genet* **109**:571–586
43. Ravenscroft TA, Janssens J, Lee PT, et al. (2020) **Drosophila Voltage-Gated Sodium Channels Are Only Expressed in Active Neurons and Are Localized to Distal Axonal Initial Segment-like Domains** *J Neurosci* **40**:7999–8024
44. Schneider CA, Rasband WS, Eliceiri KW (2012) **NIH Image to ImageJ: 25 years of image analysis** *Nat Methods* **9**:671–675

45. Splinter K, Adams DR, Bacino CA, et al. (2018) **Effect of Genetic Diagnosis on Patients with Previously Undiagnosed Disease** *N Engl J Med* **379**:2131–2139
46. Sobreira N, Schiettecatte F, Valle D, Hamosh A (2015) **GeneMatcher: a matching tool for connecting investigators with an interest in the same gene** *Hum Mutat* **36**:928–930
47. Lek M, Karczewski KJ, Minikel EV, et al. (2016) **Analysis of protein-coding genetic variation in 60,706 humans** *Nature* **536**
48. Karczewski KJ, Francioli LC, Tiao G, et al. (2020) **The mutational constraint spectrum quantified from variation in 141,456 humans** *Nature* **581**
49. Quinodoz M, Royer-Bertrand B, Cisarova K, Di Gioia SA, Superti-Furga A, Rivolta C (2017) **DOMINO: Using Machine Learning to Predict Genes Associated with Dominant Disorders** *Am J Hum Genet* **101**:623–629
50. Wang J, Al-Ouran R, Hu Y, et al. (2017) **MARRVEL: Integration of Human and Model Organism Genetic Resources to Facilitate Functional Annotation of the Human Genome** *Am J Hum Genet* **100**:843–853
51. Hu Y, Comjean A, Rodiger J, et al. (2021) **org-the database of the Drosophila RNAi screening center and transgenic RNAi project: 2021 update** *Nucleic Acids Res* **49**:D908–D915
52. Thackeray JR, Gaines PC, Ebert P (1998) **Carlson JR. small wing encodes a phospholipase C-(gamma) that acts as a negative regulator of R7 development in Drosophila** *Development* **125**:5033–5042
53. Trivedi D, Cm V, Bisht K, et al. (2020) **A genome engineering resource to uncover principles of cellular organization and tissue architecture by lipid signaling** *Elife* **9**
54. Lee PT, Zirin J, Kanca O, et al. (2018) **A gene-specific T2A-GAL4 library for Drosophila** *eLife* **7**
55. Diao F, Ironfield H, Luan H, et al. (2015) **Plug-and-play genetic access to drosophila cell types using exchangeable exon cassettes** *Cell Rep* **10**:1410–1421
56. Diao F, White BH (2012) **A novel approach for directing transgene expression in Drosophila: T2A-Gal4 in-frame fusion** *Genetics* **190**:1139–1144
57. Donnelly MLL, Luke G, Mehrotra A, et al. (2001) **Analysis of the aphthovirus 2A/2B polyprotein ‘cleavage’ mechanism indicates not a proteolytic reaction, but a novel translational effect: a putative ribosomal ‘skip’** *J Gen Virol* **82**:1013–1025
58. Pan X, Alvarez AN, Ma M, et al. (2023) **Allelic strengths of encephalopathy-associated UBA5 variants correlate between in vivo and in vitro assays** *medRxiv*
59. Huang Y, Lemire G, Briere LC, et al. (2022) **The recurrent de novo c. 2011C>T missense variant in MTSS2 causes syndromic intellectual disability** *Am J Hum Genet*
60. Lu S, Ma M, Mao X, et al. (2022) **De novo variants in FRMD5 are associated with developmental delay, intellectual disability, ataxia, and abnormalities of eye movement** *Am J Hum Genet* **109**:1932–1943

61. Ma M, Zhang X, Zheng Y, et al. (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
62. Huang Y, Ma M, Mao X, et al. (2022) **Novel dominant and recessive variants in human ROBO1 cause distinct neurodevelopmental defects through different mechanisms** *Hum Mol Genet* **31**:2751–2765
63. Venken KJ, Popodi E, Holtzman SL, et al. (2010) **A molecularly defined duplication set for the X chromosome of *Drosophila melanogaster*** *Genetics* **186**:1111–1125
64. Robinow S, White K (1991) **Characterization and spatial distribution of the ELAV protein during *Drosophila melanogaster* development** *J Neurobiol* **22**:443–461
65. Sepp KJ, Schulte J, Auld VJ (2001) **Peripheral glia direct axon guidance across the CNS/PNS transition zone** *Dev Biol* **238**:47–63
66. Fischer JA, Giniger E, Maniatis T, Ptashne M (1988) **GAL4 activates transcription in *Drosophila*** *Nature* **332**
67. Nagarkar-Jaiswal S, Lee PT, Campbell ME, et al. (2015) **A library of MiMICs allows tagging of genes and reversible, spatial and temporal knockdown of proteins in *Drosophila*** *Elife* **4**
68. Blair SS (2007) **Wing vein patterning in *Drosophila* and the analysis of intercellular signaling** *Annu Rev Cell Dev Biol* **23**:293–319
69. Ross J, Kuzin A, Brody T (2015) **Odenwald WF. cis-regulatory analysis of the *Drosophila* pdm locus reveals a diversity of neural enhancers** *BMC Genomics* **16**
70. Kataoka K, Nagata Y, Kitanaka A, et al. (2015) **Integrated molecular analysis of adult T cell leukemia/lymphoma** *Nat Genet* **47**:1304–1315
71. Siraliev-Perez E, Stariha JTB, Hoffmann RM, et al. (2022) **Dynamics of allosteric regulation of the phospholipase C-gamma isozymes upon recruitment to membranes** *Elife* **11**
72. Tao P, Han X, Wang Q, et al. (2023) **A gain-of-function variation in PLCG1 causes a new immune dysregulation disease** *J Allergy Clin Immunol* **152**:1292–1302
73. Liu Y, Bunney TD, Khosa S, et al. (2020) **Structural insights and activating mutations in diverse pathologies define mechanisms of deregulation for phospholipase C gamma enzymes** *EBioMedicine* **51**
74. Le Huray KIP, Bunney TD, Pinotsis N, Kalli AC, Katan M (2022) **Characterization of the membrane interactions of phospholipase Cgamma reveals key features of the active enzyme** *Sci Adv* **8**
75. Ellis MV, James SR, Perisic O, Downes CP, Williams RL, Katan M (1998) **Catalytic domain of phosphoinositide-specific phospholipase C (PLC) Mutational analysis of residues within the active site and hydrophobic ridge of plcdelta1.** *J Biol Chem*
76. Hajicek N, Charpentier TH, Rush JR, Harden TK, Sondek J (2013) **Autoinhibition and phosphorylation-induced activation of phospholipase C-gamma isozymes** *Biochemistry* **52**:4810–4819

77. Manso R, Rodriguez-Pinilla SM, Gonzalez-Rincon J, et al. (2015) **Recurrent presence of the PLCG1 S345F mutation in nodal peripheral T-cell lymphomas** *Haematologica* **100**:e25–27
78. DeBell K, Graham L, Reischl I, Serrano C, Bonvini E, Rellahan B (2007) **Intramolecular regulation of phospholipase C-gamma1 by its C-terminal Src homology 2 domain** *Mol Cell Biol* **27**:854–863
79. Smith MR, Liu YL, Matthews NT, Rhee SG, Sung WK, Kung HF (1994) **Phospholipase C-gamma 1 can induce DNA synthesis by a mechanism independent of its lipase activity** *Proc Natl Acad Sci U S A* **91**:6554–6558
80. Wada J, Rathnayake U, Jenkins LM, et al. (2022) **In vitro reconstitution reveals cooperative mechanisms of adapter protein-mediated activation of phospholipase C-gamma1 in T cells** *J Biol Chem* **298**
81. Thakur S, Sinhari A, Jain P, Jadhav HR (2022) **A perspective on oligonucleotide therapy: Approaches to patient customization** *Front Pharmacol* **13**
82. Khvorova A, Watts JK (2017) **The chemical evolution of oligonucleotide therapies of clinical utility** *Nat Biotechnol* **35**:238–248
83. Pettersen EF, Goddard TD, Huang CC, et al. (2004) **UCSF Chimera--a visualization system for exploratory research and analysis** *J Comput Chem* **25**:1605–1612
84. Bayram Y, Karaca E, Coban Akdemir Z, et al. (2016) **Molecular etiology of arthrogryposis in multiple families of mostly Turkish origin** *J Clin Invest* **126**:762–778
85. Ebrazeh M, Ezzatifar F, Torkamandi S, et al. (2021) **Association of the genetic variants in the endoplasmic reticulum aminopeptidase 2 gene with ankylosing spondylitis susceptibility** *Int J Rheum Dis* **24**:567–581
86. Franke A, McGovern DP, Barrett JC, et al. (2010) **Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci** *Nat Genet* **42**:1118–1125
87. International Genetics of Ankylosing Spondylitis C, Cortes A, Hadler J, et al. (2013) **Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci** *Nat Genet*
88. Oleari R, Andre V, Lettieri A, et al. (2021) **A Novel SEMA3G Mutation in Two Siblings Affected by Syndromic GnRH Deficiency** *Neuroendocrinology* **111**:421–441
89. Dalal D, Molin LH, Piccini J, et al. (2006) **Clinical features of arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in plakophilin-2** *Circulation* **113**:1641–1649
90. Gerull B, Heuser A, Wichter T, et al. (2004) **Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy** *Nat Genet* **36**:1162–1164
91. Hakui H, Kioka H, Sera F, et al. (2022) **Refractory Ventricular Arrhythmias in a Patient With Dilated Cardiomyopathy Caused by a Nonsense Mutation in BAG5** *Circ J* **86**
1. Bayram Y, Karaca E, Coban Akdemir Z, et al. (2016) **Molecular etiology of arthrogryposis in multiple families of mostly Turkish origin** *J Clin Invest* **126**:762–778

2. Ebraze M, Ezzatifar F, Torkamandi S, et al. (2021) **Association of the genetic variants in the endoplasmic reticulum aminopeptidase 2 gene with ankylosing spondylitis susceptibility** *Int J Rheum Dis* **24**:567–581
3. Franke A, McGovern DP, Barrett JC, et al. (2010) **Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci** *Nat Genet* **42**:1118–1125
4. International Genetics of Ankylosing Spondylitis C, Cortes A, Hadler J, et al. (2013) **Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci** *Nat Genet*
5. Oleari R, Andre V, Lettieri A, et al. (2021) **A Novel SEMA3G Mutation in Two Siblings Affected by Syndromic GnRH Deficiency** *Neuroendocrinology* **111**:421–441
6. Dalal D, Molin LH, Piccini J, et al. (2006) **Clinical features of arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in plakophilin-2** *Circulation* **113**:1641–1649
7. Gerull B, Heuser A, Wichter T, et al. (2004) **Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy** *Nat Genet* **36**:1162–1164
8. Hakui H, Kioka H, Sera F, et al. (2022) **Refractory Ventricular Arrhythmias in a Patient With Dilated Cardiomyopathy Caused by a Nonsense Mutation in BAG5** *Circ J* **86**
9. Songyang Z, Shoelson SE, Chaudhuri M, et al. (1993) **SH2 domains recognize specific phosphopeptide sequences** *Cell* **72**:767–778
10. Thackeray JR, Gaines PC, Ebert P (1998) **Carlson JR. small wing encodes a phospholipase C-(gamma) that acts as a negative regulator of R7 development in Drosophila** *Development* **125**:5033–5042
11. Dickson B, Hafen E (1994) **Genetics of signal transduction in invertebrates** *Curr Opin Genet Dev* **4**:64–70
12. Freeman M (1996) **Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye** *Cell* **87**:651–660
13. Schweitzer R, Shilo BZ (1997) **A thousand and one roles for the Drosophila EGF receptor** *Trends Genet* **13**:191–196
14. Bischof J, Bjorklund M, Furger E, Schertel C, Taipale J, Basler K (2013) **A versatile platform for creating a comprehensive UAS-ORFeome library in Drosophila** *Development* **140**:2434–2442
15. Bischof J, Maeda RK, Hediger M, Karch F, Basler K (2007) **An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases** *Proc Natl Acad Sci U S A* **104**:3312–3317
16. Venken KJ, He Y, Hoskins RA, Bellen HJ (2006) **P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in D. melanogaster** *Science* **314**
17. Madabattula ST, Strautman JC, Bysice AM, et al. (2015) **Quantitative Analysis of Climbing Defects in a Drosophila Model of Neurodegenerative Disorders** *J Vis Exp* **100**

18. Lu S, Hernan R, Marcogliese PC, et al. (2022) **Loss-of-function variants in TIAM1 are associated with developmental delay, intellectual disability, and seizures** *Am J Hum Genet* **109**:571–586
19. Schneider CA, Rasband WS, Eliceiri KW (2012) **NIH Image to ImageJ: 25 years of image analysis** *Nat Methods* **9**:671–675
20. Ravenscroft TA, Janssens J, Lee PT, et al. (2020) **Drosophila Voltage-Gated Sodium Channels Are Only Expressed in Active Neurons and Are Localized to Distal Axonal Initial Segment-like Domains** *J Neurosci* **40**:7999–8024

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Reviewer #1 (Public Review):

Summary:

This manuscript provides an initial characterization of three new missense variants of the PLCG1 gene associated with diverse disease phenotypes, utilizing a *Drosophila* model to investigate their molecular effects *in vivo*. Through the meticulous creation of genetic tools, the study assesses the small wing (sl) phenotype - the fly's ortholog of PLCG1 - across an array of phenotypes from longevity to behavior in both sl null mutants and variants. The findings indicate that the *Drosophila* PLCG1 ortholog displays aberrant functions. Notably, it is demonstrated that overexpression of both human and *Drosophila* PLCG1 variants in fly tissue leads to toxicity, underscoring their pathogenic potential *in vivo*.

Strengths:

The research effectively highlights the physiological significance of sl in *Drosophila*. In addition, the study establishes the *in vivo* toxicity of disease-associated variants of both human PLCG1 and *Drosophila* sl.

Weaknesses:

The study's limitations include the human PLCG1 transgene's inability to compensate for the *Drosophila* sl null mutant phenotype, suggesting potential functional divergence between the species. This discrepancy signals the need for additional exploration into the mechanistic nuances of PLCG1 variant pathogenesis, especially regarding their gain-of-function effects *in vivo*.

Overall:

The study offers compelling evidence for the pathogenicity of newly discovered disease-related PLCG1 variants, manifesting as toxicity in a *Drosophila* *in vivo* model, which substantiates the main claim by the authors. Nevertheless, a deeper inquiry into the specific *in vivo* mechanisms driving the toxicity caused by these variants in *Drosophila* could significantly enhance the study's impact.

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Reviewer #2 (Public Review):

The manuscript by Ma et al. reports the identification of three unrelated people who are heterozygous for de novo missense variants in PLCG1, which encodes phospholipase C-gamma 1, a key signaling protein. These individuals present with partially overlapping phenotypes including hearing loss, ocular pathology, cardiac defects, abnormal brain imaging results, and immune defects. None of the patients present with all of the above phenotypes. PLCG1 has also been implicated as a possible driver for cell proliferation in cancer.

The three missense variants found in the patients result in the following amino acid substitutions: His380Arg, Asp1019Gly, and Asp1165Gly. PLCG1 (and the closely related PLCG2) have a single *Drosophila* ortholog called small wing (sl). sl-null flies are viable but have small wings with ectopic wing veins and supernumerary photoreceptors in the eye. As all three amino acids affected in the patients are conserved in the fly protein, in this work Ma et al. tested whether they are pathogenic by expressing either reference or patient variant fly or human genes in *Drosophila* and determining the phenotypes produced by doing so.

Expression in *Drosophila* of the variant forms of PLCG1 found in these three patients is toxic; highly so for Asp1019Gly and Asp1165Gly, much more modestly for His380Arg. Another variant, Asp1165His which was identified in lymphoma samples and shown by others to be hyperactive, was also found to be toxic in the *Drosophila* assays. However, a final variant,

Ser1021Phe, identified by others in an individual with severe immune dysregulation, produced no phenotype upon expression in flies.

Based on these results, the authors conclude that the PLCG1 variants found in patients are pathogenic, producing gain-of-function phenotypes through hyperactivity. In my view, the data supporting this conclusion are robust, despite the lack of a detectable phenotype with Ser1021Phe, and I have no concerns about the core experiments that comprise the paper.

Figure 6, the last in the paper, provides information about PLCG1 structure and how the different variants would affect it. It shows that the His380, Asp1019, and Asp1165 all lie within catalytic domains or intramolecular interfaces and that variants in the latter two affect residues essential for autoinhibition. It also shows that Ser1021 falls outside the key interface occupied by Asp1019, but more could have been said about the potential effects of Ser1021Phe.

Overall, I believe the authors fully achieved the aims of their study. The work will have a substantial impact because it reports the identification of novel disease-linked genes, and because it further demonstrates the high value of the *Drosophila* model for finding and understanding gene-disease linkages.

<https://doi.org/10.7554/eLife.95887.1.sa1>

Reviewer #3 (Public Review):

Summary:

The paper attempts to model the functional significance of variants of PLCG2 in a set of patients with variable clinical manifestations.

Strengths:

A study attempting to use the *Drosophila* system to test the function of variants reported from human patients.

Weaknesses:

Additional experiments are needed to shore up the claims in the paper. These are listed below.

Major Comments:

(1) Does the pLI/ missense constraint Z score prediction algorithm take into consideration whether the gene exhibits monoallelic or biallelic expression?

(2) Figure 1B: Include human PLCG2 in the alignment that displays the species-wide conserved variant residues.

(3) Figure 4A:

Given that

(i) sl is predicted to be the fly ortholog for both mammalian PLCγ isozymes: PLCG1 and PLCG2 [Line 62]

(ii) they are shown to have non-redundant roles in mammals [Line 71] and

(iii) reconstituting PLCG1 is highly toxic in flies, leading to increased lethality.

This raises questions about whether sl mutant phenotypes are specifically caused by the absence of PLG1 or PLCG2 functions in flies. Can hPLCG2 reconstitution in sl mutants be used as a negative control to rule out the possibility of the same?

- (4) Do *slT2A/Y; UAS-PLCG1* Reference flies survive when grown at 22°C? Since transgenic fly expressing *PLCG1* cDNA when driven under ubiquitous *gal4s*, *Tubulin* and *Da*, can result in viable progeny at 22°C, the survival of *slT2A/Y; UAS-PLCG1* Reference should be possible.
and similarly
Does *slT2A* flies exhibit the phenotypes of (i) reduced eclosion rate (ii) reduced wing size and ectopic wing veins and (iii) extra R7 photoreceptor in the fly eye at 22°C?
If so, will it be possible to get a complete rescue of the *slT2A* mutant phenotypes with the *hPLCG1* cDNA at 22°C? This dataset is essential to establish *Drosophila* as an ideal model to study the *PLCG1* de novo variants.
- (5) Localisation and western blot assays to check if the introduction of the de novo mutations can have an impact on the sub-cellular targeting of the protein or protein stability respectively.
- (6) Analysing the nature of the reported gain of function (experimental proof for the same is missing in the manuscript) variants:
Instead of directly showing the effect of introducing the de novo variant transgenes in the *Drosophila* model especially when the full-length *PLCG1* is not able to completely rescue the *slT2A* phenotype;
(i) Show that the gain-of-function variants can have an impact on the protein function or signalling via one of the three signalling outputs in the mammalian cell culture system: (i) inositol-1,4,5-trisphosphate production, (ii) intracellular Ca^{2+} release or (iii) increased phosphorylation of extracellular signal-related kinase, p65, and p38.
OR
(ii) Run a molecular simulation to demonstrate how the protein's auto-inhibited state can be disrupted and basal lipase activity increased by introducing D1019G and D1165G, which destabilise the association between the C2 and cSH2 domains. The H380R variant may also exhibit characteristics similar to the previously documented H335A mutation which leaves the protein catalytically inactive as the residue is important to coordinate the incoming water molecule required for PIP2 hydrolysis.
- (7) Clarify the reason for carrying out the wing-specific and eye-specific experiments using *nub-gal4* and *eyless-gal4* at 29°C despite the high *gal4* toxicity at this temperature.
- (8) For the sake of completeness the authors should also report other variants identified in the genomes of these patients that could also contribute to the clinical features.

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