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Mouse models of PI(3,5)P₂ deficiency with impaired lysosome function

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Abstract

The endo-lysosomal system and autophagy are essential components of macromolecular turnover in eukaryotic cells. The low-abundance signaling lipid PI(3,5)P₂ is a key regulator of this pathway. Analysis of mouse models with defects in PI(3,5)P₂ biosynthesis have revealed the unique dependence of the mammalian nervous system on this signaling pathway. This insight led to the discovery of the molecular basis for several human neurological disorders, including Charcot-Marie-Tooth Disease and Yunis-Varon Syndrome. Spontaneous mutants, conditional knockouts, transgenic lines and gene-trap alleles of *Fig4*, *Vac14* and *Pikfyve* (*Fab1*) in the mouse have provided novel information regarding the role of PI(3,5)P₂ *in vivo*. This review summarizes what has been learned from mouse models and highlights the utility of manipulating complex signaling pathways *in vivo*.

Keywords

FIG4; VAC14; FAB1; PIKFYVE; lysosome; autophagy; transgenic; conditional; neurological mutant

1. Introduction

Low molecular weight effectors regulate many aspects of cell biology through specific interactions with protein recognition domains. Phosphatidyl inositols are amphipathic lipids that are associated with cell membranes and interact with cytoplasmic proteins through their polar domains. PI(3,5)P₂ (phosphatidyl-inositol (3,5) bisphosphate) is a low-abundance member of this family that regulates vesicle maturation and trafficking in the endosomal / lysosomal pathway in eukaryotic cells (Volpicelli-Daley and De Camilli 2007; McCartney et al, 2013). PI(3,5)P₂ was first identified in 1997 as a component of the yeast vacuole and mammalian cells (Dove et al, 1997; Whiteford et al, 1997). Reduction of PI(3,5)P₂ in yeast

results in an enlarged vacuole due to defects in vacuole fission and retrograde traffic to the Golgi (Mitchell et al, 2006; Bonangelino et al, 2002; Gary et al, 2002). Hyperosmotic shock in yeast results in elevation of PI(3,5)P₂ within ten minutes, suggesting a role in environmental adaptation (Deux et al, 2006). The signals regulating PI(3,5)P₂ in mammalian cells have not been identified.

In yeast, PI(3,5)P₂ is generated by a protein complex that includes the lipid 5-kinase *Pikfyve*, the 5-phosphatase *Fig4*, and the scaffolding protein *Vac14*, and these three proteins are highly conserved in mammalian genomes (Jin et al, 2008; Botelho et al 2008; Sbrissa et al 2007). Initial studies in mammalian cells utilized reduction of PI(3,5)P₂ by transfection of COS7 cells with a dominant negative mutant of the PIKfyve kinase (Ikonomov et al, 2002) and transfection of HEK cells with an siRNA to VAC14 (Sbrissa et al, 2004). Both treatments resulted in cell vacuolization reminiscent of the enlarged vacuole in yeast mutants, indicating that the role of PI(3,5)P₂ is conserved in eukaryotic cells.

The discovery of spontaneous mouse mutations affecting *Fig4* and *Vac14*, and the generation of targeted mutations of all three proteins in the mouse germ line, has produced insight into the role of this pathway in tissues of the intact animal. Although these genes are expressed in all mammalian cells, the central and peripheral nervous system are particularly sensitive to deficiency of PI(3,5)P₂, resulting in extensive spongiform neurodegeneration (Chow et al, 2007; Zhang et al 2007; Jin, Chow et al 2008; Zolov et al 2012). This insight from mouse models led to the identification of patient mutations in human neurological disorders including Charcot-Marie-Tooth disease (Chow et al, 2007; Nicholson et al, 2011), Yunis-Varon Syndrome (Campeau et al, 2013), and polymicrogyria (GML and MHM, manuscript submitted). Another important outcome from mouse models was elucidation of the pathogenic mechanism of the common human pathogenic variant *FIG4*-I41T found in patients with CMT4J (Lenk et al, 2011). Overexpression of the I41T variant rescued lethality and neurodegeneration of *Fig4* null mice, indicating that increased expression of this allele could be therapeutic. A third important observation was the discovery that loss of *Fig4* in neurons has a secondary effect on myelination (Winters et al, 2012). Analysis of mouse models has also provided evidence for genetic interaction between genes regulating PI(3,5)P₂ biosynthesis (Vacarri et al, 2011). This review will focus on mouse models of PI(3,5)P₂ deficiency caused by the mutations of the genes *Fig4*, *Vac14*, *Pikfyve* (*Fab1*) and *Mtmr2* (Table 1).

2. Design of mouse models

The earliest mouse models of human disorders were spontaneous mutants that were detected by their visible phenotypes (Paigen, 2003). The spontaneous mutations of *Fig4* and *Vac14* were identified by their visible neurological dysfunction and early lethality. These mutants exhibit global expression of the mutated gene in all tissues, as is the case for patients with human inherited disorders. In addition to the global mutants, the design of tissue-specific mutations can provide unique biological information, especially when the global mutant is causes early lethality. Tissue-specific models including transgenic, conditional null, and gene-trap alleles have been used to study the genes regulating the PI(3,5)P₂ pathway.

Choices among these alternative technologies are dictated by both practical and theoretical considerations.

Classically, “transgenic” mice are generated by the addition of a cloned transgene to the germline of a wildtype mouse via microinjection of fertilized eggs followed by random chromosomal insertion of multiple copies of the transgene. The transgene typically contains a previously characterized tissue-specific promoter fragment, between a few hundred bp and a few kb in length, fused upstream of a mutant or wildtype cDNA, to achieve tissue-specific expression. A broad range of tissue-specific promoter fragments have been characterized for this purpose (Donahue et al, 2012). It is important to characterize at least two independent transgenic lines to control for the unanticipated effects of chromosomal insertion site on transgene expression, and to compare the effects of different quantitative levels of transgene expression. Other practical issues include leaky expression of tissue-specific promoters in non-targeted tissues, and incomplete expression of the transgene in the targeted cells.

Gene-trap mice are generated by infection of ES cells with transposons that insert at random sites in the genome (Nord et al, 2006). The precise location of the insertion in each ES cell clone is then determined by PCR. Libraries of ES cells carrying gene-trap alleles at known positions are available. Most gene trap insertions cause “hypomorphic alleles” with reduced gene expression, while some completely abolish expression and generate null alleles. The level of residual gene expression must be determined for each gene-trap allele after the mouse is generated from the mutated ES cells via chimeric embryos.

Targeted knock-out lines are produced by inserting CRE recombinase recognition sites (loxP sites) flanking an exon using homologous recombination in ES cells. Mice carrying the ‘floxed’ allele are crossed with transgenic mice that express the CRE recombinase under the regulation of a global or tissue-specific promoter (Murray et al, 2012). The use of floxed alleles requires access to CRE transgenic lines with the desired tissue specificity. The international mouse knock-out project, KOMP, is generating targeted alleles every gene in the mouse genome for distribution to investigators on request (Ayida et al, 2012; Saunders, 2010). Potential limitations of this approach include off-target expression of the CRE recombinase and incomplete deletion of the floxed allele in the targeted tissue. Combining one null allele with one floxed allele is a popular approach towards increasing the extent of deletion in the targeted tissue.

The use of targeted mutations often involves crosses between mice with different strain backgrounds. For example, floxed alleles generated by targeting in an ES cell line from strain 129 may be bred with CRE alleles maintained on strain C57BL/6J. The resulting segregation of genetic variation from the two background strains can produce phenotypic variation among the mutant mice. Other issues include variable susceptibility of different floxed alleles to the CRE recombinase, depending upon chromosomal location, and unanticipated expression of transgenes in the male or female germ line, which can subvert the intended tissue specificity (e.g. Rempe et al, 2006).

3. A spontaneous null mutation of *Fig4*: the *pale tremor* mouse

The first PI(3,5)P₂ deficient mouse to be identified was the spontaneous mutant *pale tremor*. The recessive mutant was recognized in our mouse colony by its diluted pigmentation and resting tremor. The loss-of-function mutation was caused by insertion of a 5.5 kb ETn2b retrotransposon into intron 18 of *Fig4*, preventing processing of the full length mRNA (Chow et al, 2007) and resulting in lack of FIG4 protein (Lenk et al, 2011). Homozygous *Fig4^{plt/plt}* mice do not survive beyond 6 weeks of age. The cellular phenotype of PI(3,5)P₂ deficiency is evident in cultured fibroblasts, which accumulate large empty vacuoles, as shown in Figure 1A. The vacuole membranes contain the lysosomal proteins LAMP1 and LAMP2 (Chow et al, 2007; Ferg et al, 2009). These vacuoles do not stain for lipid or carbohydrate and also appear empty by electron microscopy. Measurement of PI(3,5)P₂ level by HPLC detected an overall two-fold reduction compared with wildtype cells (Chow et al, 2007). Vacuolization is also visible in tissue sections from brain and spleen, and primary cultures of neurons, consistent with a basic housekeeping role for PI(3,5)P₂. The appearance of vacuolated cells in *Fig4* null tissues is reminiscent of the enlargement of the primary vacuole in PI(3,5)P₂ deficient yeast strains (Bonangelino et al 2002; Gary et al 2002; Mitchell et al 2006). It has recently been demonstrated that PI(3,5)P₂ is an activator of several cation channels in the lysosomal membrane that permit the exit of calcium and other cations (Dong et al, 2010; Wang et al, 2012). Thus the enlarged vesicles may result from increased osmotic pressure within the lysosome, and the osmotic swelling may trap the lysosomes in an inactive form and prevent the normal regeneration process.

Backup in the autophagic pathway that feeds into lysosomal degradation was first identified in tissue splices of *Fig4* null brain, which contain numerous inclusion bodies containing p62 and ubiquitinated proteins (Ferguson et al, 2009). Two types of vesicles accumulate in mutant brain: small electron dense bodies with the appearance of autolysosomes, and large empty vesicles similar to those shown in cultured fibroblasts above (Chow et al, 2007; Zhang et al 2008; Ferguson et al 2009; Katona et al, 2011; unpublished observations). Both types of vesicles appear to be derived from the late endo-lysosomal system since they contain lysosomal but not endosomal markers. Co-staining with GFAP demonstrated that the dense inclusion bodies contain p62 and ubiquitinated proteins, and are localized to astrocytes rather than neurons in the mutant brain.

Another pathological characteristic discovered in the null mouse is defective myelination in CNS and PNS, resulting in major reduction of myelin proteins in the brain, dysplasia of the corpus callosum and white tracts of the cerebellum, thinning of the myelin sheath of optic nerve and sciatic nerve, and reduction in nerve conduction velocity (Chow et al, 2007; Winters et al, 2011). The spongiform degeneration of *Fig4* deficient brain thus results in abnormalities of neurons, astrocytes and oligodendrocytes. To elucidate the relationships between these cellular phenotypes, we generated cell-type-specific transgenic lines and a floxed allele of *Fig4*, as described in the following sections.

4. Tissue-specific *Fig4* transgenes: neurons vs. astrocytes

Both neurons and astrocytes are morphologically abnormal in *Fig4* null mice (Ferguson et al, 2009). We evaluated the relative contributions of these two cell types to the neurological disorder by directing *Fig4* cDNA expression specifically in neurons or astrocytes, using a neuron-specific promoter (NSE) or an astrocyte specific promoter (GFAP) (Ferguson et al, 2012). The tissue-specific transgenes were bred to the *Fig4* null background and the phenotypes of the transgene-positive, *Fig4* null mice were determined. Remarkably, the neuron-specific transgene was sufficient to rescue virtually all of the pathogenic effects of *Fig4* deficiency, including juvenile lethality, size, tremor, spongiform degeneration, and accumulation of inclusion bodies in astrocytes. Although expression of *Fig4* in the oligodendrocytes is absent in the NSE-Tg mice, the myelination deficit was rescued in CNS (Winters et al, 2011) and PNS (Ferguson et al 2012). In contrast, *Fig4* expression in astrocytes corrected the accumulation of inclusion bodies but did not extend the lifetime of the *Fig4* null mice (Ferguson et al 2012). This result demonstrated that *Fig4* deficiency is primarily a neuronal disorder, and it will be necessary to treat the neuronal defect in order to alleviate neurodegeneration in patients.

5. Conditional knockout of *Fig4* in neurons

To investigate the requirement for *Fig4* expression in specific tissues, we generated a floxed allele of *Fig4* with loxP sites flanking exon 4 (Ferguson et al 2012). The floxed allele can be combined with a variety of CRE recombinase transgenic mice to examine the effects of *Fig4* deletion at various developmental points as well as in different cell types. In crosses with a neuron-specific synapsin CRE line, mice lacking *Fig4* expression in neurons developed spongiform degeneration, a movement disorder, and tremor. However, these mice survive several months longer than the global null mice, indicating that expression of *Fig4* in other tissues can moderate some of the effects of neuronal *Fig4* deficiency (Ferguson et al 2012). The neuron-specific knockout mouse demonstrates that *Fig4* expression in neurons is *necessary* for survival, while the neuron-specific transgenic rescue demonstrates that expression of *Fig4* in neurons is *sufficient* for survival.

6. The human disease mutation FIG4-I41T in transgenic mice

In order to examine the mechanism of pathogenesis of the human mutant I41T found in patients with Charcot-Marie-Tooth neuropathy type 4J, we generated a transgene with the mutant *Fig4* cDNA driven by the globally expressed chicken β -actin promoter (Lenk et al 2011). The transgene was crossed on to the *Fig4* null background, in order to generate a mouse that produced only mutant FIG4-I41T protein, like CMT4J patients. An interesting effect of expression level of the I41T mutant protein was observed. In mice with 5X overexpression of the mutant transcript, there was complete rescue of the *Fig4* null phenotype, restoration of normal brain morphology, survival beyond 2 years of age. This rescue was obtained despite the very low level of mutant protein in the transgenic mouse tissues, 10% of normal protein level. However, in a second line of transgenic mice with 2X transcript expression, survival was much shorter, only 3 to 4 months. It thus appears that the minimum requirement for the *Fig4* enzyme is approximately one tenth of wildtype

expression level. This work indicates that up-regulation of the I41T allele in CMT4J patients could offer a therapeutic strategy. Further analysis demonstrated that the instability of the I41T protein is a consequence of impaired binding to the VAC14 scaffold protein. The level of FIG4-I41T is also very low in patient fibroblasts, and can be increased by inhibition of degradation by the proteasome pathway (Lenk et al, 2011; Ikonomov et al, 2010).

7. A spontaneous missense mutation of *Vac14* in the *ingls* mouse

The spontaneous *ingls* mutation (infantile gliosis) was identified at the Jackson Laboratory in 1991 by its juvenile lethality, and mapped to a chromosomal location close to *Vac14*, the scaffold protein in the PI(3,5)P₂ biosynthetic protein complex (Bronson et al 2003). Homozygous *ingls* mutants exhibit neurodegeneration and gliosis that closely resemble the phenotype of the *Fig4* null mutant. We therefore tested *Vac14* as a positional candidate gene for *ingls*. Sequencing the exons of *Vac14* identified the mutation Leu156Arg in the fourth heat repeat domain of the protein (Jin et al, 2008). The mutation prevents binding of VAC14 to the kinase protein PIKFYVE, resulting in a 50% reduction in the level of PI(3,5)P₂ in cultured fibroblasts. Tissues of homozygous *ingls* mutants contain normal levels of FIG4, VAC14 and PIKFYVE proteins, but the impaired formation of the protein complex leads to deficiency of PI(3,5)P₂. The similarity in pathology between the *ingls* mutant and the *Fig4* null mouse, including vacuolization of fibroblasts, neurodegeneration, astrocystosis and formation of inclusion bodies in astrocytes containing p62 and ubiquitinated protein, supports the conclusion that reduced PI(3,5)P₂ levels are responsible for the pathology in both mutants (Jin et al, 2008; Ferguson et al 2009).

8. A null gene-trap allele of *Vac14*

Mice null for *Vac14*, the scaffold protein in the biosynthetic complex for PI(3,5)P₂, were generated in the KOMP project by random insertion of the β -geo gene-trap vector into intron 1 of *Vac14*. This insertion ablated detectable protein from mice homozygous for the “trapped” allele, resulting in earlier lethality than the Leu156Pro mutation in the *ingls* mouse (Zhang et al 2007). Similar to the *Fig4* null mice described above, the *Vac14* null mice exhibit spongiform degeneration of the brain, extensive vacuolization of primary cultured fibroblasts, and a 50% reduction of PI(3,5)P₂ in fibroblasts (Zhang et al 2007). Mis-localization of the cation-independent mannose-6-phosphate receptor (CI-MPR) and CI-MPR cargo cathepsin D in this mutant implicates PI(3,5)P₂ in retrograde transport from the endo-lysosomal system to the trans-golgi network (Zhang et al 2007). Synaptic localization of VAC14 in hippocampal neurons and impaired turnover of the post-synaptic AMPA receptor have also been observed in *Vac14* null mice (Zhang et al, 2012). The similarity of the pathological changes in mutant mice suggest that mutations of human *VAC14*, like *FIG4*, could cause peripheral neuropathy or Yunis-Varon syndrome, but to date patient mutations have not been identified.

9. A hypomorphic gene trap allele of *Pikfyve* (*Fab1*)

A global null mutant of *Pikfyve*, the 5-kinase of the PI(3,5)P₂ synthetic complex, was recently described. Deletion of floxed exon 6 by CRE recombinase resulted in preimplantation lethality, possibly as early as E3.5 (Ikonomov et al 2011). This is much

earlier than the lethality caused by lack of *Fig4* or *Vac14*, suggesting that loss of *Pikfyve* may result in complete deficiency of PI(3,5)P₂. Transfection of CRE recombinase into homozygous *Pikfyve*^{flax/flax} fibroblasts also resulted in vacuolization and arrested cell division, demonstrating the cell autonomy of the defect in fibroblasts (Ikononov et al 2011).

10. A conditional knockout of *Pikfyve*

A gene-trap allele of *Pikfyve* generated from a KOMP ES cell line resulted in 85% reduction of transcript level (Zolov et al, 2012). The residual 15% expression was sufficient for completion of embryonic development, leading to postnatal lethality during the first 3 weeks after birth. The PI(3,5)P₂ levels in cultured fibroblasts of these mice was approximately 50% of normal, comparable to the *Fig4* and *Vac14* mutants with similar postnatal survival (Zolov et al 2012). Spongiform degeneration of the brain and vacuolization of several other tissues including lung and heart was observed (Zolov et al 2012).

11. Genetic interactions: *Fig4*, *Vac14* and *Mtmr2*

The availability of multiple mouse mutants in a pathway of interest makes it possible to examine genetic interactions using crosses between mutant lines. Myotubularin-related 2 (MTMR2) is a phosphatase that removes the 3-phosphate from PI(3,5)P₂, while FIG4 removes the 5-phosphate. Mice lacking MTMR2 are models of peripheral neuropathy CMT4B1 (Bolino et al, 2004), and are thought to accumulate excess PI(3,5)P₂. To test the hypothesis that *Mtmr2* and *Fig4* act on the same subcellular pool of PI(3,5)P₂, mice with mutations at both loci were generated. Interestingly, heterozygosity for the null allele of *Fig4* reduced the severity of neurodegeneration and myelin outfolding in *Mtmr2* null mice, demonstrating that the two phosphatases can access the same substrate pool and that MTMR2 does hydrolyze PI(3,5)P₂ *in vivo* (Vaccari et al 2011).

Null heterozygotes for either *Fig4* or *Vac14* do not exhibit visible abnormalities. To evaluate the possibility that double heterozygosity for *Fig4* and *Vac14* might cause a visible defect, we generated *Fig4*^{+/-}, *Vac14*^{+/-} mice. In this case, no interaction was observed and the mice were viable and fertile, with normal lifespan.

The stability of the FIG4 protein is reduced by the human pathogenic mutation I41T mutation that impairs binding to VAC14 (Lenk et al, 2011). To evaluate the dependence of the wildtype FIG4 protein on interaction with VAC14, we carried out Western blotting of VAC14 null tissues. We observed a complete absence of FIG4 protein in the VAC14 mutant, clearly demonstrating that the stability of wildtype FIG4 is dependent on interaction with VAC14 (Lenk et al 2011).

These examples demonstrate the utility of testing genetic interactions using mutant mice. The availability of both global and conditional alleles for the major components of the PI(3,5)P₂ biosynthetic pathway will be useful for further analysis of *in vivo* gene interactions in this pathway.

12. Genetic effects of strain background

During the generation of transgenic and conditional knock-out mice, it is often difficult to avoid mixing the genetic backgrounds of different inbred strains of mice. While the segregation of modifier variants in different inbred backgrounds can complicate the characterization of mutant phenotypes, the positive aspect of interstrain variation is the potential to identify the critical differences between strains and to better understand the underlying pathogenic mechanisms. For example, analysis of strain differences in the phenotypes of sodium channel mutations led to identification of the *Scnm1* splice factor affecting the *Scn8a* transcript (Buchner et al, 2003), and the modifying effect of potassium channel *Kcnv2* on seizures caused by mutation of sodium channel *Scn2a* (Jorge et al, 2011). To examine the effect of inbred strain backgrounds on *Fig4* null lethality, we crossed the spontaneous null allele *plt* onto strains C57BL/6J and C3H, by repeated backcrossing of *Fig4*^{+/-} heterozygotes to wildtype mice of each strain for more than ten generations. A significant difference in survival was observed, with neonatal lethality on the congenic strain B6. *plt/plt* (Figure 2). Interestingly, null homozygotes on the hybrid F1 background survive longer than either inbred strain, suggesting that there is interaction between multiple loci affecting dependence on *Fig4*. Identification of these modifier loci could provide novel targets for treatment of *Fig4* deficiency.

13. Future applications of mouse models of PI(3,5)P₂ deficiency

Mouse models provide tools for evaluation of the rare variants that are being rapidly discovered in patient populations by exome sequencing. For example, primary fibroblasts cultured from *Fig4* null mice exhibit extensive cytoplasmic vacuolization that can be rescued by transfection of wildtype *Fig4* cDNA (Figure 1C). The functional effects of human variants can be tested by transfection of mutated *Fig4* cDNAs into the null fibroblasts and comparison of rescue efficiency with the wildtype cDNA (e.g. Campeau et al, 2013). Pathogenic mechanisms can also be investigated by expression of the mutated cDNA in transgenic mice (e.g. Lenk et al, 2011).

Mice provide a valuable system for testing therapies for inherited disorders *in vivo*. For example, reduced turnover of the *Fig4* I41T variant by proteasome inhibitors could be evaluated in the I41T transgenic mouse. It is an attractive hypothesis that the disrupted vesicle trafficking in PI(3,5)P₂ deficiency cells may be secondary to reduced activity of lysosomal ion channels, since the cation channels *MCOLN1* (TRPML1), *TPC1* and *TPC2* are directly activated by PI(3,5)P₂ (Dong et al 2010; Wang et al 2012). Therapeutic activation of these channels to treat neurodegeneration is an exciting possibility that can be tested in the *Fig4* null mice.

Long lived mutants like the neuron-specific *Fig4* transgenic mouse will be useful for analysis of non-neuronal pathology, such as the recently recognized dysmyelination and bone dysplasia in *Fig4* null mice (Ferguson et al 2012; Campeau et al, 2013). Careful analysis of these mice may reveal additional effects of PI(3,5)P₂ deficiency and thereby suggest new patient populations for screening.

The variable age of onset and clinical diversity in CMT4J patients suggests that genetic modifiers may influence disease severity (Nicholson et al 2011). Mouse models offer a unique resource for identification of genetic modifiers segregating in crosses between inbred strains.

Several human diseases are known to result from defects in the PI(3,5)P₂ pathway, and more are likely to be identified in the coming years through the application of high-throughput genome sequencing. A variety of mouse models will continue to contribute to characterizing the physiological roles of PI(3,5)P₂ signaling and testing therapies for PI(3,5)P₂ deficiency disorders.

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Figure 1. Phenotypes of the spontaneous *Fig4* null mutant 'pale tremor'

A, accumulation of cytoplasmic vacuoles in primary cultured fibroblasts (MEFs).

B. Spongiform degeneration of the brain stem at P21, with neuronal vacuolization.

C. The cytoplasmic vacuoles in primary fibroblasts cultured from *Fig4* null mice are rescued by transfection with wildtype *Fig4* cDNA plus GFP, but not by GFP alone.

Figure 2. Effects of genetic background on survival of homozygous *Fig4* null mice

The congenic lines B6. plt and C3H. plt carrying the *Fig4^{plt}* null mutation were generated by repeated backcrossing of *Fig4^{+/-}* heterozygotes to inbred strains C57BL/6J and C3H, for more than ten generations. Crosses between heterozygotes within each line generated the congenic homozygotes. Crosses between B6 and C3H congenic heterozygotes generated the homozygous *Fig4^{-/-}* F1 mutants. The difference in survival of *Fig4* null homozygotes on each genetic background is indicated. B6.plt/plt, n=50; C3H.plt/plt, n=18; F1.plt/plt, n=30.

Table 1Mouse models with altered metabolism of PI(3,5)P₂

Gene	Geneticmouse model	Reference
<i>Fig4</i>	Spontaneous null (<i>pale tremor</i>)	Chow et al (2007)
	Neuron-specific transgene	Ferguson et al (2012)
	Astrocyte-specific transgene	Ferguson et al (2012)
	Human disease variant transgene	Lenk et al (2011)
	Floxed allele	Ferguson et al (2012)
<i>Vac14</i>	Spontaneous missense allele (<i>ingls</i>)	Jin et al (2008)
	Genetrap null allele	Zhang et al (2007)
<i>Pikfyve</i>	Genetrap hypomorphic allele	Zolov et al (2012)
	Floxed allele	Ikonomov et al (2011)
<i>Mtmr2</i>	<i>Mtmr2</i> null	Bolino et al (2004)