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Fig4 Deficiency: A Newly Emerged Lysosomal Storage Disorder?

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Abstract

FIG4 (Sac3 in mammals) is a 5'-phosphoinositide phosphatase that coordinates the turnover of phosphatidylinositol-3,5-bisphosphate (PI(3,5)P₂), a very low abundance phosphoinositide. Deficiency of FIG4 severely affects the human and mouse nervous systems by causing two distinct forms of abnormal lysosomal storage. The first form occurs in spinal sensory neurons, where vacuolated endolysosomes accumulate in perinuclear regions. A second form occurs in cortical/spinal motor neurons and glia, in which enlarged endolysosomes become filled with electron dense materials in a manner indistinguishable from other lysosomal storage disorders. Humans with a deficiency of FIG4 (known as Charcot-Marie-Tooth disease type 4J or CMT4J) present with clinical and pathophysiological phenotypes indicative of spinal motor neuron degeneration and segmental demyelination. These findings reveal a signaling pathway involving FIG4 that appears to be important for lysosomal function. In this review, we discuss the biology of FIG4 and describe how the deficiency of FIG4 results in lysosomal phenotypes. We also discuss the implications of FIG4/PI(3,5)P₂ signaling in understanding other lysosomal storage diseases, neuropathies, and acquired demyelinating diseases.

Keywords

Fig4; CMT4J; lysosomal storage; PI(3,5)P₂; peripheral neuropathy; segmental demyelination

Introduction

Phosphatidylinositol 3,5-bisphosphate (PI(3,5)P₂) is one of seven known phosphoinositides that are involved in the regulation of intracellular organelle trafficking in mammalian cells (Figure 1A). One of the key proteins that regulates the concentration of PI(3,5)P₂ is known as the FIG4 phosphatase (alternatively known as SAC3 in mammals). Recent discoveries of recessive *FIG4/SAC3* gene mutations causal for neurodegeneration, abnormal lysosomal storage, and segmental demyelination in humans have substantiated its importance in

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neuronal functions and myelination. These pathological findings during FIG4 deficiency are reminiscent to what has been described in other lysosomal storage diseases, supporting the role of endolysosomal pathway dysregulation in the neurological disease. In this review, we will first discuss the clinical presentation, electrophysiological alterations, and endolysosomal pathology in patients and rodents with Fig4 deficiency. We will then describe how FIG4/SAC3 controls PI(3,5)P₂ abundance and endolysosomal trafficking in tandem with other PI(3,5)P₂ regulatory proteins. Finally, we will discuss how these molecular events might involve in the abnormal lysosomal accumulation in this neurological disease. For in-depth reviews of Fig4 and PI(3,5)P₂, refer to (Dove *et al.*, 2009a; Ho *et al.*, 2012).

Clinical Presentation of CMT4J

In humans, the *FIG4/SAC3* gene is localized to chromosome 6q21 and it encodes a protein of approximately 103 kD comprised of 907 amino acids (Chow *et al.*, 2007). CMT4J is an inherited peripheral neuropathy that is caused by autosomal recessive mutations of the *FIG4* gene. Typically, the disease is caused by the combination of a *FIG4* null allele and a missense *FIG4*^{I41T} mutation in the other allele (Chow *et al.*, 2007; Nicholson *et al.*, 2011; Zhang *et al.*, 2008). These recessive mutations result in a loss of function of FIG4/SAC3 and a deficiency of PI3,5P₂. While the missense mutant *FIG4*^{I41T} allele still produces a partially functional phosphatase, the mutation results in an increase of FIG4/SAC3 cytosolic degradation (Ikononov *et al.*, 2010). Thus, the *I41T* point mutation consequently exerts a loss of function effect (Lenk *et al.*, 2011).

The deficiency of FIG4/SAC3 in CMT4J patients manifests in rapidly progressive asymmetric muscle weakness with minimal or absent sensory symptoms. This renders CMT4J as a special subtype of CMT since most forms of CMT manifest as slowly progressive, symmetric and length-dependent polyneuropathies (Patzko and Shy, 2011). Symptomatic onset of CMT4J can range from early childhood to adulthood. Similarly, the severity of the CMT4J phenotype can vary from mild neurological impairment to fatal outcomes (Nicholson *et al.*, 2011). It is currently unknown what genetic factors, if any, modify the onset and severity of CMT4J. While early onset patients can develop gait abnormalities with asymmetric limb involvement that appear as soon as the patients begin walking in childhood, late onset patients may develop subtle abnormalities for years that are insufficient to prompt them to seek medical attention (Nicholson *et al.*, 2011; Zhang *et al.*, 2008).

The most prominent clinical finding in CMT4J patients is typically muscle weakness with atrophy. In almost all cases, the severity of the weakness/atrophy is conspicuously asymmetric. There are mild sensory abnormalities, such as reduced sensation to touch and/or pin-prick in distal limbs. Deep tendon reflex may also be mildly decreased. Interestingly, however, central nervous system abnormalities have not been observed in patients with CMT4J (Nicholson *et al.*, 2011; Zhang *et al.*, 2008). This issue will be discussed further in the next section. On neurological examination, dysfunction of the cranial nerves is uncommon and these patients usually display normal cognitive function. Their brain MRI scans show no abnormalities (Nicholson *et al.*, 2011; Zhang *et al.*, 2008).

Most CMT4J patients have been examined by electrophysiological studies. Nerve conduction studies show prolonged distal latencies and reduced conduction velocities indicative of demyelination. Unlike most CMT1 patients, who have uniform slowing of conduction velocities with little variation from one nerve to another or from one limb to another, CMT4J patients have decreased conduction velocities that are often non-uniform or asymmetric. Temporal dispersion occurs in some cases. These findings closely resemble the

electrophysiological features of acquired demyelinating diseases such as chronic inflammatory demyelinating polyneuropathy and Guillain-Barre syndrome.

This demyelinating change is further supported by analyses of human sural nerve biopsies from patients with CMT4J. Myelin thickness appears reduced in some myelinated nerve fibers and onion bulb is occasionally observed. There is also a severe loss of large myelinated nerve fibers (Nicholson *et al.*, 2011; Zhang *et al.*, 2008). In a previous study, we analyzed a sural nerve biopsy that was taken nine years prior to the onset of severe weakness and muscle denervation. A teased nerve fiber study on the sural nerve biopsy demonstrated segmental demyelination in 60% of the myelinated nerve fibers (Zhang *et al.*, 2008). Thus, it is possible that patients with CMT4J may develop a primary demyelinating neuropathy or a neuropathy with a predominant pathology of demyelination in the early phase of its clinical course.

By the time that severe weakness and even paralysis develops in CMT4J patients, needle electromyography demonstrates abundant and diffuse abnormal spontaneous activities such as fibrillations and positive waves in muscles. Motor unit action potentials are complex in their morphology with reduced recruitment. These findings, along with the asymmetric progression and minimal sensory symptoms noted earlier, support a phenotype of spinal motor neuron degeneration or motor neuron disease in the later phase of CMT4J (Zhang *et al.*, 2008). For a more complete evaluation of CMT4J pathology, autopsies of CMT4J patients may be necessary in order to understand the extent of spinal motor neuron degeneration and lysosomal storage pathology (discussed in the following sections).

The Pale Tremor Mouse

The characterization of a spontaneous mouse mutant of *Fig4/Sac3* deficiency, the pale tremor (*plt*) mouse, has allowed scientists to gain a deeper insight into the neurodegenerative pathology observed in CMT4J patients. *Plt* mice are homozygous null for *Fig4* due to a truncation mutation that is produced through transposon insertion and they display a 40–71% reduction of fibroblast PI(3,5)P₂ levels (Chow *et al.*, 2007; Vaccari *et al.*, 2011). Much like that observed in CMT4J patients, *plt* mice exhibit muscle weakness, severe loss of myelinated large diameter nerve fibers in the sciatic nerve and dorsal/ventral roots, loss of dorsal root ganglion sensory neurons, and segmental demyelination. Unlike patients with CMT4J, however, *plt* mice undergo extensive neuronal degeneration in cortical and subcortical regions (Chow *et al.*, 2007; Zhang *et al.*, 2008), and display reduced numbers of myelinating oligodendrocytes (Winters *et al.*, 2011). When compared with CMT4J patients who can live well into adulthood, *plt* mice die relatively early in their lifespans at around 4–6 weeks. The absence of major CNS pathology in CMT4J patients is presumed to stem from the residual activity of the FIG4^{I41T} protein, which *plt* mice do not possess (Lenk *et al.*, 2011; Zhang *et al.*, 2008).

Additional variations of the *plt* mouse model have been recently developed, such as *Fig4* null mice that express *Fig4/Sac3* specifically in neurons. Using this model, it was shown that neuronspecific expression of *Fig4/Sac3* is able to rescue CNS neurodegeneration and sciatic nerve demyelination (Ferguson *et al.*, 2012). Such findings have established the *plt* mouse and its variations as valuable models for investigating how *Fig4* affects neuronal survival and myelination.

The Lysosomal Pathology of Fig4 Deficiency: Implications for Lysosomal Storage Diseases

Fig4/Sac3 deficiency causes major pathological changes to lysosomes in the nervous system. There are two types of abnormal lysosomal storage in Fig4 deficient cells that likely stem from the dysregulation of endolysosomal trafficking, a process in which Fig4/Sac3 function is critically involved. The first occurs in Fig4/Sac3 deficient sensory neurons, which accumulate numerous “bubble-like” vacuoles, which are derived from dilated endolysosomes. These vacuoles accumulate in perinuclear regions and interfere with intracellular organelle trafficking processes (Katona *et al.*, 2011; Zhang *et al.*, 2008). The second occurs in Fig4 deficient cortical/spinal motor neurons and glia, in which dysfunctional lysosomes abnormally store large quantities of lipids and proteins (Katona *et al.*, 2011). These cellular phenotypes are reminiscent to those of other lysosomal storage diseases such as Niemann Pick C, Tay-Sachs, and Mucopolidosis type IV. Thus, Fig4/Sac3 deficiency may become a new member of lysosomal storage disorders. Although it is still unclear as to how these pathological phenotypes contribute to neurodegeneration during Fig4/Sac3 deficiency, the study of Fig4/Sac3 deficient pathogenesis may potentially reveal disease mechanisms implicated in various lysosomal storage disorders. In the following sections, we will summarize the pathological data regarding endolysosomal defects in the Fig4-deficient *plt* mouse model and also examine the implications of these pathological changes in diseases similar to CMT4J.

a. Abnormal accumulation of vacuolated endolysosomes in sensory neurons

Accumulation of vacuoles occurs in 42–82% of *plt* dorsal root ganglion (DRG) sensory neurons *in vitro* and *in vivo* as early as postnatal day 4 (Figure 2). This observation is consistent with the fact that Fig4 expression is persistently high in wild-type DRG sensory neurons throughout development and adulthood, but is only transiently high in spinal motor neurons during early development. By adulthood, Fig4/Sac3 expression is hardly detectable in wild-type spinal motor neurons (Guo *et al.*, 2012). One might wonder how severe vacuolization can occur predominantly in sensory neurons, yet CMT4J patients typically do not complain about sensory symptoms. A possible explanation may be that there is an inhomogeneous degeneration of sensory neurons that preserves non-myelinated smaller nerve fibers responsible for pain and pinprick sensations.

The abnormal storage of cytoplasmic vacuoles in Fig4/Sac3 deficient sensory neurons has been suggested to occur as a result of dysfunctional lysosomal trafficking processes. In Fig4-deficient sensory neurons, the vacuoles become clustered in perinuclear regions and block intracellular organelle trafficking (Zhang *et al.*, 2008). These vacuoles arise from the late endosomes and lysosomes of *plt* mice and CMT4J patients, as revealed by immunohistochemistry in which vacuoles co-localize with a late endosome/lysosome marker, but not an early endosome marker (Figure 2) (Chow *et al.*, 2007; Zhang *et al.*, 2008). Such localization is interesting, given that Fig4/PI3,5P₂ are thought to aid in the maturation of early endosomes to late endosomes.

b. Abnormal endolysosomal storage of high electron dense materials in spinal motor neurons and glia

The lysosome is a critical cellular compartment that mediates the degradation of cellular debris and other materials from the phagocytic, endocytic, and autophagic pathways (Luzio *et al.*, 2007). Failure of lysosomes to degrade and/or properly recycle their contents has been increasingly implicated in a variety of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (Nixon *et al.*, 2008; Zhang *et al.*, 2009). Ultrastructural studies of the *plt* brain and spinal cord have

documented an excessive accumulation of large endolysosomes containing electron-dense granules in up to 80% of spinal motor/cortical neurons and glia (Figure 3). These granules localize to perinuclear regions in a manner similar to the vacuolated endolysosomes of Fig4-deficient sensory neurons. Other neurodegenerative lysosomal storage disorders, such as lipofuscinosis and Tay-Sachs disease, also possess perinuclear vacuole accumulation (Katona *et al.*, 2011; Myerowitz *et al.*, 2002; Sulzer *et al.*, 2008). Additionally, the abnormal accumulation of lysosomal proteins LAMP1, LAMP-2, and NPC1 has been documented in the brains of *plt* mice (Ferguson *et al.*, 2009; Ferguson *et al.*, 2012; Katona *et al.*, 2011). In wild-type samples, only 40% of neurons and glia possess granules, and these granules are less abundant, smaller in size, less electron-dense, and more dispersed throughout the cytoplasm than those of *plt* mice (Katona *et al.*, 2011). This accumulation of high-electron dense materials has also been documented in *plt* spleen cells as well, consistent with a lysosomal storage disorder. These electron-dense granules are thought to accumulate in dysfunctional lysosomes that lack luminal acidification and degradative abilities (Katona *et al.*, 2011). Thus, there is a lack of vacuolar accumulation in spinal motor neurons until the end stages of disease progression (~postnatal week 5 in *plt* mice) (Katona *et al.*, 2011). Even at later stages, vacuolar accumulation in spinal motor neurons is comparatively less than that observed in the DRG sensory neurons (Chow *et al.*, 2007; Katona *et al.*, 2011). The potential cause for the two different forms of lysosomal storage will be discussed further later.

c. The lysosomal storage pathology of neurons and glia is likely pathogenic to neurodegeneration during Fig4 deficiency

Lysosomal diseases were initially defined as a group of diseases with inborn errors of metabolism that displayed pathological inclusions (abnormal lysosomal storage) formed by intravacuolar storage of heterogeneous materials in cells (Hers, 1965). The cause for these diseases was originally thought to be a result of a deficiency in certain enzymatic activity. Examples of this type would include mucopolysaccharidoses (such as Hurler-I or Sanfilippo-III disease), sphingolipidoses (such as Tay-Sachs, Gaucher or Krabe's disease) and glycoproteinoses (α -Fucosidosis or α -Mannosidosis). With the advance of molecular genetics and cell biology, this view has proven to be over-simplistic. Defective enzyme activity has also been found to be caused by genetic factors that regulate the synthesis or transport of lysosome-related proteins (Schultz *et al.*, 2011). Thus, these defects emerge as a fourth group (miscellaneous group) of lysosomal diseases in addition to the three aforementioned groups of lysosomal diseases. For instance, loss of LAMP-2 function in patients with Danon disease develops a lysosomal disorder that predominantly affects cardiac and skeletal muscles with profound accumulation of autophagosomes in the muscle cells (Saftig *et al.*, 2010). LAMP-2 is not a lysosomal enzyme, but rather a lysosomal membrane protein that appears critical for certain substrates to be delivered into lysosomes via a chaperone-mediated autophagic pathway (Orenstein and Cuervo, 2010; Saftig *et al.*, 2010). We believe that it is reasonable to suggest that Fig4 deficiency belongs in this fourth group of lysosomal storage disorders due to the following reasons.

First, our study has provided pathological evidence showing an accumulation of high electron dense organelles containing heterogeneous materials in Fig4/Sac3 deficient cells, a morphological finding consistent with abnormal lysosomal storage. This lysosomal accumulation is also accompanied by high levels of lysosomal protein LAMP-2 (Katona *et al.*, 2011). While such accumulation has not been shown in human neurons, however, vacuoles derived from dilated endolysosomes have been demonstrated to accumulate in fibroblasts isolated from humans with CMT4J (Zhang *et al.*, 2008). It is important to be reminded that vacuolated lysosomal storage has been observed in other lysosomal storage diseases, such as Mucopolysaccharidosis, and can be interchangeable with high electron dense lysosomal storage under certain conditions (Kogot-Levin *et al.*, 2009). Nevertheless, it

would be helpful if the electron dense form of lysosomal storage could also be demonstrated in human neurons or glia when autopsy materials become available. *Second*, there are multifaceted features in *Fig4*^{-/-} mice that are consistent with lysosomal disease, including diluted coat color, high electron dense lysosomal storage in the spleen, growth retardation, and hydrocephalus. *Third*, patients with CMT4J mainly show abnormalities in the peripheral nerves and spinal cord, but their CNS is spared. This should not be viewed as evidence against lysosomal disease since other classical lysosomal diseases can be confined exclusively to the peripheral nerves and/or spinal cord. For instance, Adrenomyeloneuropathy (AMN) is a well-known variant of Adrenoleukodystrophy that mainly affects spinal cord and peripheral nerves. Clinical cerebral abnormalities may develop in patients with AMN, but are later features and only occur in 45% of patients with AMN (Edwin *et al.*, 1990; Moser, 1997). Moreover, an isolated peripheral neuropathy has also been observed in another lysosomal disease known as Krabbe's disease (Marks *et al.*, 1997). *Finally*, PI3,5P₂ is decreased in *Fig4* deficient cells (Chow *et al.*, 2007; Vaccari *et al.*, 2011). This phosphoinositide is well documented to play a significant role in regulating the trafficking of lysosomal membrane. Loss of function of proteins in complex with *Fig4*/*Sac3*, such as *Fab1*/*PIKfyve* and *Vac14*/*ArPIKfyve*, all result in abnormal lysosomes (Di Paolo and De Camilli, 2006; Ho *et al.*, 2012; Zhang *et al.*, 2007). While vacuolated lysosomes have been reported in *Fab1*/*PIKfyve* or *VAC14*/*ArPIKfyve* deficient rodents (Ikonomov *et al.*, 2011; Zhang *et al.*, 2007), it would be interesting to examine whether the abnormal storage of high electron dense lysosomes is also present in the neurons of these animals.

Abnormal lysosomal storage in *Fig4* deficient motor neurons and glia appears to be closely associated with neurodegeneration. Although *Sac3*/*Fig4* deficiency causes lysosomal defects in both sensory and motor neurons/glia (Katona *et al.*, 2011; Zhang *et al.*, 2008), the clinical manifestations appear to correlate with the degeneration of spinal motor neurons and demyelination of the peripheral nerves. This suggests that abnormal lysosomal storage of protein and lipids in spinal motor neurons and glia is the major factor responsible for the CMT4J clinical phenotype. Such a pathogenic mechanism is not particularly uncommon, since spinal motor neuron degeneration and de-/dysmyelination of peripheral nerves have also been observed in a number of lysosomal storage disorders. For example, in a group of lysosomal storage disorders known as the GM2 gangliosidoses, patients may have a deficiency of a lysosomal hydrolase (β -hexosaminidase). These patients present with clinical features of spinal muscular atrophy that primarily result from the degeneration of spinal motor neurons (Navon *et al.*, 1997). Furthermore, the knockout of β -hexosaminidase in mice also results in the abnormal lysosomal storage of glycosphingolipid that accompanies neuronal degeneration, including damage to spinal motor neurons (Liu *et al.*, 1999). Removal of lysosomal glycosphingolipid accumulation significantly improves life span, neurological function, and reduces the degeneration of neurons (Liu *et al.*, 1999). These findings suggest that abnormal lysosomal storage can be causal for neuronal degeneration, and that it could be the primary pathogenic mechanism resulting in the degeneration of *Fig4* deficient spinal motor neurons.

How does the *Fig4* deficiency lead to abnormal lysosomal storage (either electron lucent or dense form)?

The prominent lysosomal storage in *Fig4*/*SAC3* deficiency raises an important question: how does the deficiency of *Fig4*/*SAC4* result in lysosomal storage? Based on current literature, we describe several potential pathways below.

Fig4/*SAC3* is a SAC-domain phosphoinositide phosphatase with specificity toward the 5'-phosphate of PI(3,5)P₂. Structural studies of *Fig4*/*SAC3* have not yet been performed, but have been inferred through its homologous protein in *S. cerevisiae* known as *Sac1p*. *Sac1p*

has a catalytic domain containing the conserved catalytic motif CX₅R(T/S) specific for phosphoinositide substrates. The catalytic core shares a similar topology with other phosphoinositide phosphatases like MTMR2 and PTEN (Table 1), but possesses a unique configuration in its P-loop domain which contains the catalytic CX₅R(T/S) motif (Manford *et al.*, 2010).

In order for Fig4/Sac3 to exert its biological function, it typically complexes with a scaffolding protein known as Vac14/ArPIKfyve and a 5'-kinase of PI3P known as Fab1/PIKfyve (Duex *et al.*, 2006; Efe *et al.*, 2005; Efe *et al.*, 2007; Ikononov *et al.*, 2009; Jin *et al.*, 2008). This regulatory complex (PAS) is thought to localize on early endosomal membranes during the transition from early endosomes to late endosomes (Guo *et al.*, 2012; Sbrissa *et al.*, 2007; Yuan *et al.*, 2007). Alongside the myriad of endosomal maturation processes that occur at this time, the PAS complex is suggested to mediate the conversion of early endosomal PI3P to late endosomal PI(3,5)P₂ - a conversion that appears to be essential for protein sorting and the trafficking of late endosomes to the degradative lysosomal compartment (Huotari and Helenius, 2011; Sbrissa *et al.*, 2007). The *I41T* mutation that underlies CMT4J is located in the SacN domain, which is suggested by the structural study to interact with other proteins (Manford *et al.*, 2010). Interestingly, this mutation has been shown to affect the interaction between Fig4 and Vac14/ArPIKfyve, promoting FIG4^{I41T} degradation (Ikononov *et al.*, 2010).

Although the abundance of PI(3,5)P₂ is much lower in comparison to other phosphoinositides (PI3,5P₂ comprises just 0.04–0.08% of total cellular inositol phospholipid), cells are very sensitive to PI(3,5)P₂ loss. Once PI(3,5)P₂ has formed on late endolysosomal membranes, it interacts with a variety of proteins that may mediate endolysosomal acidification (Dove *et al.*, 2009b; Gary *et al.*, 1998; Rusten *et al.*, 2006), the hyperosmotic stress response (Bonangelino *et al.*, 2002; Dove *et al.*, 1997; Sbrissa and Shisheva, 2005), endolysosomal membrane fusion and fission (Gary *et al.*, 1998; Ikononov *et al.*, 2006; Ikononov *et al.*, 2009; Poccia and Larijani, 2009), ryanodine receptor activation (Touchberry *et al.*, 2010), Ca²⁺ regulation (Dong *et al.*, 2010; Shen *et al.*, 2012; Shen *et al.*, 2009; Silswal *et al.*, 2011), exocytosis (Berwick *et al.*, 2004; Dove *et al.*, 2009b), gene expression (Han and Emr, 2011), and trafficking between the endolysosome and Golgi apparatus (Bryant *et al.*, 1998; Dove *et al.*, 2009b; Rutherford *et al.*, 2006). Each component of the Fig4/Vac14/Fab1 complex is dependent on either direct or indirect interactions within the complex for proper function and stability. For example, it has been shown that Fig4 must associate with Vac14/ArPIKfyve before the complex can efficiently bind Fab1/PIKfyve (Botelho *et al.*, 2008; Ikononov *et al.*, 2009; Sbrissa *et al.*, 2007). In this manner, Fig4/Sac3 can decrease PI(3,5)P₂ levels via its phosphatase function *and* also promote PI3,5P₂ synthesis by acting as a secondary scaffold for the Fab1/Vac14 interaction. However, the later function appears dominant. Loss of either component results in a similar outcome – a destabilization of the PAS complex and a reduction of PI(3,5)P₂ that gives rise to greatly dilated endolysosomes (Chow *et al.*, 2007; Gary *et al.*, 1998; Ikononov *et al.*, 2001; Jin *et al.*, 2008).

Our studies have localized the Fig4/SAC3 protein to early endosomal membranes, where it may form the PI(3,5)P₂ regulatory complex with Vac14 and Fab1 in order to regulate early endosome-to-lysosome maturation (Guo *et al.*, 2012). It has also been suggested that PI3P and PI(3,5)P₂ on early endosomal membranes are involved in the recruitment of ESCRT protein machinery that regulates the biogenesis of multi-vesicular bodies (intermediate organelles between early endosomes and late endosomes) (Bonangelino *et al.*, 1997; Ho *et al.*, 2012; Ikononov *et al.*, 2002b). Given these findings, one might speculate that the deficiency of Fig4/SAC3 and PI(3,5)P₂ could impair endolysosomal membrane fusion/scission and protein sorting processes required to properly target proteins and lipids for

lysosomal degradation. Based on studies in yeast, lysosomal fission is more likely affected by Fig4/PI(3,5)P₂ deficiency than lysosomal fusion with other organelles (Dove *et al.*, 2009a). This notion is also supported by our previous finding that showed a decrease of organelle fission once the dilated endolysosomes were fused into the clusters of perinuclearly accumulated vacuoles in human fibroblast cells of CMT4J (Zhang *et al.*, 2008).

One caveat in these proposed mechanisms is that they are all built on an unproven premise – which is that the reduction of PI(3,5)P₂ is directly responsible for the storage of the dilated endolysosomes. In PIKfyve deficient culture cells, endolysosomal vacuoles can be eliminated by injecting PI(3,5)P₂ into these cells, suggesting that PI(3,5)P₂ deficiency is causal for the vacuolation (Ikonomov *et al.*, 2002a). However, this causal role of PI(3,5)P₂ in Fig4 deficient cells has yet to be established. Moreover, fibroblasts from *p/t* mice were recently reported to have ~40% less PI(3,5)P₂ than wild-type (Vaccari *et al.*, 2011), which is in contrast to a previous finding of a 71% decrease in PI(3,5)P₂ reported by Chow *et al.* (Chow *et al.*, 2007). This may be problematic, since a ~40% decrease in PI(3,5)P₂ was found in fibroblasts from mice with a single *PIKfyve* allele knockout that did not show a phenotype (Ikonomov *et al.*, 2011). Such a discrepancy could be a result of technical difficulties in the measurement of PI(3,5)P₂ levels due to its extremely low abundance. Alternatively, there could be a PI(3,5)P₂-independent molecular mechanism causing vacuolated endolysosomal accumulation. It would be important to compare the anatomical localizations of Fig4 and other proteins in the PAS complex (PIKfyve and ArPIKfyve) along with the pathological changes between *p/t* mice and animal models with loss of function of other proteins in the PAS complex. Identical localization and pathological changes between these proteins would support a shared common mechanism, such as PI(3,5)P₂ deficiency.

The recent discovery of a lysosomal ion channel activated by PI(3,5)P₂ provides an alternative pathogenic route for the abnormal lysosomal storage of Fig4 deficiency. This channel, known as the transient receptor potential mucolipin-1 (TRPML1/MCOLN1) channel, releases Ca²⁺ from the lysosomal lumen into the cytosol when PI(3,5)P₂ binds to its N-terminus (Dong *et al.*, 2010). Mutation or inhibition of this channel results in decreased lysosomal Ca²⁺ release *in vitro*. Mutations in the gene encoding TRPML1/MCOLN1 in humans cause an autosomal recessive lysosomal storage disease; Mucopolidosis type IV (Bargal *et al.*, 2000; Shen *et al.*, 2012). In addition, down-regulation of TRPML1/MCOLN1 activity appears to underlie lysosomal defects in the Niemann Pick C lysosomal storage disorder (Shen *et al.*, 2012). Similar to that observed in CMT4J patients and *p/t* mice, cells that lack TRPML1/MCOLN1 exhibit enlarged endolysosomes and LEL-to-Golgi retrograde trafficking defects (Dong *et al.*, 2010; Shen *et al.*, 2012). Given the role of Fig4/PI3,5P₂ in endolysosomal regulation, one might suggest that the loss of Fig4 could subsequently lead to the inhibition of the TRPML1/MCOLN1 channel. In support of this view, transfection of TRPML1/MCOLN1 into Vac14/ArPIKfyve null fibroblasts with PI3,5P₂ deficiency can rescue the vacuolization phenotype (Shen *et al.*, 2012). One might question this mechanism since a different type of lysosomal storage with membranous inclusions (in contrast to electron dense or translucent inclusions in *Fig4*^{-/-} cells) has been observed in cells, mice, and patients with TRPML1/MCOLN1 deficiency (Folkerth *et al.*, 1995; Miedel *et al.*, 2008; Venugopal *et al.*, 2007). However, Niemann Pick-C develops polymorphic lysosomal storage (membranous inclusions + electron dense + translucent vacuoles). Yet, a decrease of TRPML1/MCOLN1 activity appears responsible for the lysosomal defects in the Niemann Pick- C cell model (Shen *et al.*, 2012). Interestingly, TRPML1/MCOLN1 has been suggested to play a role in neuronal autophagy (Curcio-Morelli *et al.*, 2010), whereas, as to be discussed below, autophagy is not essential for the pathogenesis of CMT4J. Moreover, the clinical phenotype in patients with Mucopolidosis type IV differs from those in patients with CMT4J in multiple aspects (Nicholson *et al.*, 2011; Wakabayashi *et al.*, 2011; Zhang *et al.*

al., 2008). Thus, it is likely that TRPML1/MCOLN1 is involved in additional functions and/or could also be expressed in different tissues/cellular localizations when compared with Fig4. It would be helpful to determine whether TRPML1/MCOLN1 is down-regulated in *Fig4*^{-/-} cells and if over-expression of TRPML1/MCOLN1 can rescue the phenotype in Fig4 deficient cells.

Segmental Demyelination during Fig4 Deficiency

A nerve fiber can increase its action potential conduction velocity by wrapping its axon with Schwann cell membrane (**myelin**) in sequential segments (**internodes**). The segments are separated by punctuate gaps where the axon is denuded of myelin, called **nodes of Ranvier**. This configuration permits the action potentials to be conducted in an efficient **saltatory fashion** from one node to the next (Baker, 2002). Central to the myelination process are glial cells known as Schwann cells (located in the PNS) and oligodendrocytes (located in the CNS). These cells interact with axons and wrap them in electrically insulating myelin sheathing. Demyelination denudes axons and shunts depolarizing current out of the nerve. As a result, nerve conduction of action potentials can be slowed or even eliminated (The latter is also known as conduction block). This change causes neurological disabilities such as sensory loss, vision loss, and limb paralysis in a variety of neurological diseases (Relevant examples include Guillain-Barré Syndrome, chronic inflammatory demyelinating polyneuropathy, multiple sclerosis, certain subtypes of CMT, etc.) (Kaji, 2003).

Fig4 is strongly expressed in both oligodendrocytes and Schwann cells throughout development and adulthood, indicating that FIG4 may play an important role in myelination (Guo *et al.*, 2012). Indeed, the deficiency of FIG4 results in segmental demyelination. Nerve conduction studies of *plt* sciatic nerves and CMT4J nerves demonstrated significantly reduced conduction velocities when compared to wild-type controls (Chow *et al.*, 2007; Zhang *et al.*, 2008). Oftentimes, these changes in conduction velocities were non-uniform. Additionally, segmental demyelination has been morphologically confirmed in >60% of teased CMT4J sural nerve fibers of an adult human and 11% of teased *plt* sciatic nerve fibers at the age of 5–6 weeks (Figure 4). Macrophage infiltration in the peripheral nerves also occurs in *plt* mice (Zhang *et al.*, 2008). When combined with the knowledge that CMT4J manifests as an asymmetric and rapidly progressive weakness, these findings suggest that demyelination in CMT4J shares key features with acquired demyelinating diseases. Understanding how Fig4 deficiency impacts myelination could provide pathogenic insights applicable to other demyelinating diseases.

Reductions in myelinating oligodendrocyte (OL) abundance have recently been observed in the brain and optic nerve of *plt* mice. Additionally, this reduction in OL abundance is accompanied by a decrease in myelin thickness (Winters *et al.*, 2011). These findings implicate Fig4 in the maturation of oligodendrocyte precursor cells (OPCs) to OLs. Exogenous Fig4 in neurons is sufficient to correct this phenotype, suggesting that dysmyelination in the CNS may be secondary to abnormal signaling from *Fig4* deficient neurons. Although sciatic nerve demyelination appears to be rescued during neuron-specific *Fig4* expression (Ferguson *et al.*, 2012), it is still not known whether such a pathogenic mechanism applies to segmental demyelination in *Fig4* deficient peripheral nerves. One might wonder how this mechanism could apply to CMT4J patients, given that they possess a *Fig4*^{L41T} allele that protects their CNS, but not their PNS, from neurodegeneration. Our recent studies of Fig4 expression during the development of wild-type mice indicate that Fig4 expression remains high in the PNS but drastically declines in the CNS with age (Guo *et al.*, 2012). Thus, it is possible that PNS myelin may depend more on Fig4 activity than the myelin of the CNS.

Myelination in both the CNS and PNS appears to be sensitive to the levels of Fig4, displaying abnormalities even during *Fig4*^{+/-} heterozygosity. Recent studies of young *Fig4*^{+/-} mice have demonstrated transient reductions in CNS myelin thickness that are visible at P21 but absent by P90 (Winters *et al.*, 2011). Our study found transiently reduced conduction velocities in the PNS of young *Fig4*^{+/-} mice which also became normal with age (Yan *et al.*, 2012). Given that *FIG4* heterozygosity has been documented in several patients with sporadic ALS, one might ask if the myelination abnormalities of heterozygous *Fig4*^{+/-} mice are relevant to ALS pathology (Chow *et al.*, 2009). However, this speculation requires much evidence to substantiate since myelinspecific abnormalities have not been shown to be causal for ALS.

Given the endolysosomal abnormalities in Fig4 deficiency, it is reasonable to speculate that lysosomal dysfunction is involved in the myelination defects of CMT4J patients and *plt* mice. It should be emphasized that myelin abnormalities are a common feature in many lysosomal diseases (Folkerth *et al.*, 1995; Marks *et al.*, 1997; Moser, 1997). Another shared feature of many lysosomal storage diseases is an imbalance of intracellular calcium (Kiselyov *et al.*, 2010). Excess intracellular calcium is known to cause demyelination (Smith *et al.*, 1985; Smith *et al.*, 2001; Smith and Hall, 1988). Considering that PI3,5P₂ is known to stimulate the lysosomal TRPML1/MCOLN1 channel to release calcium into the cytosol (Dong *et al.*, 2010), and that PIKfyve is known to regulate Ca_v1.2 ion channel internalization (Tsuruta *et al.*, 2009), a deficiency of Fig4 could lead to intracellular calcium abnormalities and subsequent demyelination.

In many lysosomal diseases, lysosomal storage in neurons is striking morphologically. In contrast, this storage is often much milder or even absent in myelin. This difference is also conspicuous in CMT4J. Given the high plasticity of Schwann cells (repetitive differentiation or dedifferentiation); one might wonder if accumulated lysosomal materials are efficiently discarded during demyelination.

Interestingly, an increase in PI(3,5)P₂ level is linked to autosomal recessive disorders such as CMT4B1 and CMT4B2, all of which display de-/dysmyelination. However, the increase of PI(3,5)P₂ level is associated with a very different type of myelin abnormality when compared with that in CMT4J. There are numerous enlarged paranodes in CMT4B1/2 myelinated nerve fibers. They are formed by excessive myelin folding primarily in the longitudinal direction of axons (Bolino *et al.*, 2000; Bolino *et al.*, 2004; Previtali *et al.*, 2003; Quattrone *et al.*, 1996; Vaccari *et al.*, 2011). This myelin alteration is remarkably different from the typical segmental demyelination in Fig4 deficient myelinated nerve fibers. Together, these findings suggest that levels of PI(3,5)P₂ may have to be regulated within a tight range in order to maintain normal myelination.

Autophagy does not play a primary pathogenic role in Fig4 deficiency

Autophagy is essential for maintaining cell viability as it encompasses the lysosomal degradation of damaged organelles and the elimination of large protein aggregates (Ferguson *et al.*, 2009; Klionsky, 2007). Progressive accumulation of protein aggregates has been found in numerous neurodegenerative diseases and can eventually become cytotoxic (Ross and Poirier, 2004). Mutations of *Fig4* have been shown to result in the accumulation of autophagic intermediates (p62, LC3-II) in the astrocytes of *plt* mice. This accumulation is suggested to occur as a result of PI(3,5)P₂ deficiency that would normally coordinate the fusion of autophagosomal compartments (autophagosome to late endosome) and/or recycling of lysosomal membranes from mature autolysosomes (Ferguson *et al.*, 2009). These findings raise the question of whether the loss of function of Fig4 impairs autophagy, thus playing a pathogenic role in CMT4J neurodegeneration.

This initial impression has since been challenged by several recent observations suggesting that autophagy is not a primary pathogenic mechanism in *Fig4* deficiency. First, our studies of *Fig4* expression throughout development found no expression of *Fig4* in the astrocytes of wild-type rodents. Even after nerve injury which induced *Fig4* up-regulation in many cells of the neural tissues, *Fig4* expression was still absent in astrocytes (Guo *et al.*, 2012). These results suggest that the accumulation of autophagic intermediates in *plt* astrocytes is a secondary result occurring downstream of another pathogenic mechanism, such as astrocytic reaction to nearby neuronal degeneration. Moreover, we found that autophagosomes were either absent or only rarely detectable in *Fig4*^{-/-} brain and/or spinal cord by EM studies (Katona *et al.*, 2011).

Second, a recent study evaluated the impact of *Fig4* on the phenotype of mice null for *Mtmr2* (a 3'-phosphoinositide phosphatase of PI3P and PI(3,5)P₂). Deficiency of *Mtmr2* increases PI3,5P₂, however, no changes in the level of p62 or LC3-II/I ratio were observed in *Mtmr2*-null mice. Conversely, the double-knockout of *Fig4* and *Mtmr2* decreases PI3,5P₂ levels more than those in *Fig4*^{-/-} tissues, but does not increase p62 levels relative to *plt* mice (Vaccari *et al.*, 2011). These findings question the effect of PI3,5P₂ fluctuations on autophagic intermediate accumulation.

Third, additional research has shown that mice null for the PI3P kinase PIK3C3 display sensory neuron vacuolation with no observable impairment of neuronal autophagy. PIK3C3 catalyzes the production of PI(3)P and the loss of PIK3C3 would ultimately deplete PI3,5P₂. However, autophagy is not impaired in mice with PIK3C3 deficiency. Further knockout of a molecule necessary for canonical autophagy, Atg7, does not recapitulate the pathology of *plt* neurons (Chow *et al.*, 2007; Katona *et al.*, 2011; Zhou *et al.*, 2010). Interestingly, while the *Fig4*/PI3,5P₂ pathway is highly conserved among different species, yeast does not require PI3,5P₂ for autophagy (Dove *et al.*, 2009a; Gary *et al.*, 1998).

Finally, a recent publication by Ferguson *et al.*, 2012 further clarifies the autophagy issue. The authors utilized a global *Fig4* null mouse with neuron-specific expression of *Fig4* and demonstrated that neuron-specific expression of *Fig4* is sufficient to rescue neuronal degeneration. In contrast, astrocyte-specific *Fig4* expression did not rescue the phenotype, but restored the levels of autophagic intermediates. Although teased nerve fiber studies were not performed in this study, it also appears that demyelination in mouse sciatic nerves is rescued during neuron-specific *Fig4* expression since slowed conduction velocities were reversed and g-ratios became comparable with wild-type mice. Together, these data strongly suggest that abnormal autophagy does not play a significant role in the pathogenesis of *Fig4* deficiency. The accumulation of autophagic intermediates is mild and likely a secondary phenomenon.

Diverse functions of PI3,5P₂ kinases and phosphatases

Despite the miniscule amount of PI(3,5)P₂ in cells, catalytic enzymes involved in the regulation of phosphatidylinositol 5'-phosphate can form an impressively long list. This list includes the three proteins in the PAS complex (Fab1/PIKfyve, Fig4/SAC3 and Vac14/ArPIKfyve) and ten phosphatidylinositol 5-phosphate phosphatases (Table 1). Thus, it is important to consider these enzymes while investigating the FIG4 functions, since there are potential functional overlaps between FIG4 and these 5-phosphate phosphatases. On the other hand, there have been multiple diseases identified which are caused by the loss of function of these enzymes. The phenotypes of these diseases are highly diverse. Moreover, knockout mice of different enzymes in the list have also revealed a variety of phenotypes *in vivo*. Surprisingly, many of these knockouts result in a lethal phenotype without being compensated by other enzymes that can perform a similar catalytic activity (Table 1). These

findings not only demonstrate diverse functions of these enzymes in biological systems, but also suggest that some of these functions might be PI(3,5)P₂ independent. Alternatively, they share the same preference of certain substrates, but express in different tissues or cells to exert different biological functions. For instance, loss of function of OCRL by autosomal recessive mutations results in the X-linked oculocerebrorenal syndrome of Lowe. Patients with Lowe's syndrome suffer primarily from congenital cataracts, neonatal hypotonia, joint hyper-extensibility, intellectual disability (self-abuse, agitation) and renal proximal tubule dysfunction (Loi, 2006). OCRL has catalytic preference toward PI(4,5)P₂ over PI(3,5)P₂. OCRL deficiency raises the level of PI(4,5)P₂ in cells. OCRL expresses ubiquitously in a variety of tissues and is localized to the Golgi/endosome system that utilizes the clathrin-coated vesicles for bidirectional trafficking between the two organelles. Interestingly, pathological studies have demonstrated single membrane bound, electron lucent vacuoles in OCRL deficient cells. Electron dense or membranous inclusions are only occasionally seen (Wisniewski *et al.*, 1984). However, these inclusions are mainly derived from endosomes (Pirruccello and De, 2012).

A close family member of OCRL, INPP5E, expresses highly in brain and kidney, but within a cell, INPP5E has a localization and catalytic activity similar to that of OCRL. Recessive mutations of *INPP5E* have been found in a subset of patients with MORM syndrome, a ciliopathy affecting multiple organ systems that commonly causes mental impairment, truncal obesity, retinal dystrophy and micropenia (Jacoby *et al.*, 2009). This phenotype is conspicuously different from that in Lowe's syndrome. Moreover, synaptojanin-1 also shows catalytic activity preferentially toward PI(4,5)P₂. This 5-phosphate phosphatase is highly expressed in brain and particularly abundant in presynaptic terminals. It is involved in the regulation of synaptic endocytosis. This localization is consistent with its pathogenic role in Down syndrome and the early onset of Alzheimer's disease (Berman *et al.*, 2008; McPherson *et al.*, 1996; Voronov *et al.*, 2008).

SHIP1 and SHIP2 are homologous 5'-phosphatases which can utilize PI(3,4,5)P₃ or PI(1,3,4,5)P₄ as their substrate. SHIP1 expression is restricted to cells of the hematopoietic lineage. *Ship1*^{-/-} mice develop myeloproliferative syndrome (Helgason *et al.*, 1998), a fact that has implications for immunity and leukemias (Kerr, 2011; Kerr *et al.*, 2011). In contrast, studies of SHIP2 have linked its polymorphism to metabolic syndromes such as insulin resistance and hypertension (Kaisaki *et al.*, 2004; Marciano *et al.*, 2007; Marion *et al.*, 2002). Taken together, diverse functions are expected from these 5'-phosphate enzymes. Detailed data regarding their substrate specificity, anatomical localization, and their interacting protein partners would be very helpful in deciphering these variable functions.

Conclusion

The deficiency of FIG4 in humans and mice likely results in a clinical and pathological phenotype that is consistent with a lysosomal storage disorder. This disorder profoundly affects spinal motor neurons and glia. These findings have thus uncovered a signaling pathway involving FIG4/PI3,5P₂ that appears to be required for the regulation of lysosomal functions. Because lysosomal dysfunction has been implicated in many neurodegenerative disorders and myelin-related diseases, FIG4/PI3,5P₂ signaling may have much broader implications than previously thought.

Despite the remarkable progress that has been made towards understanding the biology of FIG4, there are still many unanswered questions concerning the pathogenesis of FIG4 deficiency. Based on current literature, we propose a hypothetical mechanism in Figure 1B. This figure is by no means a complete story, but it should provide a simplified theoretical platform for future exploration. Early in the endolysosomal pathway, the PAS complex is

assembled and localized to the endosomes prior to their maturation to multi-vesicular bodies (Ho *et al.*, 2012; Sbrissa *et al.*, 2008; Shisheva, 2012). PI(3,5)P₂ begins to be synthesized on endosomal membranes and is additionally required for the activation of lysosomal TRPML1/MCOLN1 channels (Dong *et al.*, 2010; Shen *et al.*, 2012). Thus, a deficiency of FIG4/PI(3,5)P₂ would impair TRPML1/MCOLN1 channel function, leading to the accumulation of calcium in the lysosomes. Alternatively, TRPML1/MCOLN1 might fail to be delivered to the lysosomal membrane secondarily to a mislocalization or expression of other lysosomal membrane proteins. This would decrease calcium release from lysosomes as well, but would occur independent of PI(3,5)P₂. These alterations are speculated to inhibit the fission of cytoplasmic membrane targeted vesicles out of lysosomes (Dove *et al.*, 2009a; Zhang *et al.*, 2008), which would cause lysosomal storage. In cells with a lower lysosomal pH, abnormal lysosomal storage with high electron density forms. In contrast, electron lucent vacuolated lysosomes would form in cells with a higher lysosomal pH (Kogot-Levin *et al.*, 2009). Mechanisms responsible for variable lysosomal pH in different cells are unknown at this point. In addition, the abnormal lysosomal storage would impair the trafficking or degradation of cytoplasmic voltage-gated calcium channels (Kiselyov *et al.*, 2010; Tsuruta *et al.*, 2009), leading an increase of cytoplasmic calcium channels and intracellular calcium concentration. The increase of calcium in myelinating Schwann cells is known to cause segmental demyelination (Smith *et al.*, 2001; Smith and Hall, 1988).

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Abbreviation List

PI(3,5)P₂	phosphatidylinositol 3,5-bisphosphate
CMT4J	Charcot-Marie-Tooth Disease type 4J
kD	kilodalton
PI3P	phosphatidylinositol 3-phosphate
<i>plt</i>	pale tremor
CNS	central nervous system
PNS	peripheral nervous system
DRG	dorsal root ganglion
TRPML1	transient receptor potential mucolipin-1
LEL	late endolysosome
EM	electron microscopy
OL	myelinating oligodendrocyte
OPC	oligodendrocyte precursor cell
ALS	amyotrophic lateral sclerosis

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Highlights

- Loss of function of *FIG4* causes severe neuronal degeneration in humans and mice.
- The predominant pathology of FIG4 deficiency is abnormal lysosomal storage.
- We discuss how the abnormal lysosomal storage contributes to pathological changes.

Figure 1.
A. A diagram of phosphatidylinositol metabolism. PI = phosphatidylinositol. Relevant enzymes to this paper are marked. Their related diseases are shown in parenthesis. Notice that all pathways involved in 4-phosphate are simplified since they are not relevant to this review. B. A hypothetical mechanism in Fig4 deficiency is illustrated.

Figure 2.

Vacuoles in *plt* neurons of the DRG. (A) A semithin section was processed from the DRG of a 5-week-old wild-type mouse. The DRG is filled with sensory neurons with a polygon shape and pale nucleus. There are a few myelinated nerve fibers visible between the neurons. (B) The same study was done in the DRG of a 5-week-old *plt* mouse. Many vacuoles are visible in the neurons (arrowhead). The cytoplasm in some neurons is fully occupied by these vacuoles. (C) Two adjacent vacuoles were examined under electron microscopy, and were determined to be single-membrane bound. Vacuoles have a watery appearance. They contain oil-drop-like materials. A few pieces of membranous debris may occasionally be seen in the vacuoles. (D) A semithin section of DRG from a wild-type mouse at P4 was imaged under light microscopy (scale bar = 20 μm). (E) Large vacuoles in *plt* DRG neurons are readily identifiable at P4 (arrows). *Note:* Reprinted with permission from “Distinct pathogenic processes between Fig4- deficient motor and sensory neurons,” by Katona et al., 2011, *European Journal of Neuroscience*, 33, pp. 1401-1410.

Figure 3.

Mouse spinal cord and brain were dissected for electron microscopy. **(A)** A motor neuron was identified from the spinal anterior horn of a wild-type mouse. The sizes of many motor neurons are larger than those in the *plt* anterior horns. This motor neuron contains electron-dense granules (arrow). However, these granules are small and scattered throughout the cytoplasm, rather than clustered in the perinuclear region. **(B)** In contrast, these high electron dense granules are found in many *plt* neurons and/or glia. They are numerous and always clustered in the perinuclear region (arrow). Granules are not detectable in axons (asterisk). **(C)** Granules were inspected under high magnification. They are inhomogeneous. Large granules appear to be fused from several dark vesicles. **(D)** Similar accumulations were observed in cerebral neurons. *Note:* Reprinted with permission from “Distinct pathogenic processes between Fig4-deficient motor and sensory neurons,” by Katona et al., 2011, *European Journal of Neuroscience*, **33**, pp. 1401-1410.

Figure 4.

Segmental demyelination in teased sciatic nerve fibres from *plt* mice. Mouse sciatic nerves were teased into individual nerve fibers and stained via immunohistochemistry. (A) Myelin specific protein MAG is strongly expressed in Schmidt-Lanterman incisures. MAG staining is absent in some segments of myelinated *plt* nerve fibers (e.g. between arrowheads in A,B). Labeling for NFp (phosphorylated neurofilaments) was intact in the same segment (C), demonstrating segmental demyelination. Many nuclei are lined up along this segment of the axon (D) that are derived from invading macrophages or remyelinating Schwann cells. Demyelination was not observed in wild-type nerve fibers. *Note.* Reprinted with permission from “Mutation of *FIG4* causes a rapidly progressive, asymmetric neuronal degeneration,” by Zhang et al., 2008, *Brain*, **131**, pp.1990-2001.

Table 1

Catalytic enzymes for phosphatidylinositol 5-phosphate.

Protein	Substrate	Knockout Mouse	Human Disease
<i>PAS complex</i> PIKfyve	PI, PI3P	embryonic lethal at stage of 32–64 cells; vacuoles in cells (Ikononov et al JBC 2011)	Francois-Weatens-Fleck corneal dystrophy (CFD) by heterozygous loss of function of <i>PIKfyve</i> – white fleck in all layers of stroma (Li et al Am J Hum Genet 2005)
ArPIKfyve		death at P1-2; vacuoles in brain, DRG and fibroblasts (Zhang et al PNAS 2007)	none
SAC3/FIG4	PI3,5P ₂	death at P5-6 weeks; vacuoles in DRG and fibroblasts; electron dense lysosomal storage in neuron, glia; segmental demyelination (Chow et al Nature 2007; Katona et al Euro J Neurosci 2011)	CMT-4J by homozygous loss of function of <i>FIG4</i> – progressive asymmetric weakness, minimal sensory symptoms, segmental demyelination in PNS (Zhang et al Brain 2008; Nicholson et al Brain 2012)
<i>Phosphatidylinositol 5-phosphate phosphatases</i>			
5-phosphatase-1	1I,4,5P ₃ ; 1I,3,4,5P ₄	only for soluble inositol polyphosphates – no further discussion	
5-phosphatase-II /INPP5b	PI4,5P ₂ PI3,4,5P ₃ ; IP ₃ ; IP ₄	male sterility, vacuoles in seminiferous tubule epithelium (Hellstein et al Biol Reprod 2002)	none
OCRL	same as INPP5b	no phenotype (Janne et al JCI 1998)	Lowe syndrome - congenital cataracts, neonatal hypotonia, behavior disorder, renal tubule dysfunction
Synaptojanin-1	PI3P, PI4P, PI5P	weakness, ataxia, convulsion vesicle accumulation in nerve terminals (Cremona et al Cell 1999)	synaptojanin-1 increased in Down's syndrome (Arai et al Brain Dev 2002)
Synaptojanin-2	PI3,5P ₂	none	none
SHIP-1	PI1,3,4,5P ₄ ; PI3,4,5P ₃ ; PI1,2,3,4,5P ₅	none	none
SHIP-2/INPPL1	PI3,5P ₂ ; IP ₃ ; IP ₄	resistant to dietary obesity (Sleeman et al, Nat Med 2005)	genetically linked to patients with diabetes or insulin resistance
SKIP	PI4,5P ₂ PI3,4,5P ₃	embryo lethal in skip-/- mouse; increased glucose tolerance & insulin sensitivity (Ljuin et al Mol Cell Biol 2008)	none
PIPP	PI3,4,5P ₃ PI4,5P ₂ PI1,4,5P ₃	none	none