Impaired eIF2B activity in oligodendrocytes contributes to VWMD pathogenesis

Vanishing white matter disease (VWMD, MIM #603896), also known as childhood ataxia with central nervous system (CNS) hypomyelination (CACH), is an inherited autosomal-recessive leukodystrophy (van der Knaap et al., 2006). The neurological signs of VWMD, such as cerebellar ataxia and spasticity, usually begin in late infancy or early childhood. VWMD is chronic and progressive, with additional episodes of rapid deterioration after febrile infection or minor head trauma. Most patients die within a few years of disease onset. VWMD patients display cystic cavitations of the white matter in the CNS. In contrast, the CNS gray matter and the other organs are generally spared. The grossly affected white matter shows profound myelin loss. Oligodendrocytes are predominantly affected in VWMD, which exhibit characteristic foamy morphology. Although oligodendrocyte numbers are decreased in the severely affected white matter, the numbers are increased in the less affected areas. Moreover, apoptotic oligodendrocytes are observed in the white matter of VWMD patients. Additionally, a recent study suggests that the myelinating function of oligodendrocytes is suppressed in VWMD patients (Bugiani et al., 2013).

Mutations in the ubiquitously-expressed genes encoding the five subunits of eukaryotic translation initiation factor 2B (eIF2B) are believed to be the cause of VWMD (van der Knaap et al., 2006). To date, hundreds of distinct mutations have been identified in these genes in VWMD patients. Greater than 80% of eIF2B mutations are missense mutations that cause a single amino acid to be altered to another amino acid (Pavitt and Proud, 2009). eIF2B functions in protein translation as a guanine nucleotide exchange factor and is essential for all translation initiation events. eIF2B mutations in VWMD patients are considered to be loss-of-function mutations. A large number of studies show that VWMD mutations reduce the guanine nucleotide exchange factor activity of eIF2B, the deficit varying from 20–80%; however, the correlation between the degree of attenuated eIF2B activity and the severity of disease is unclear. Moreover, although knockout mice with eIF2B mutations show attenuated eIF2B activity in cells, these mice appear healthy and do not display obvious myelin abnormalities under normal conditions (Geva et al., 2010). Thus, the mechanisms by which eIF2B mutations selectively affect the white matter in most cases of VWMD remain unknown. Oligodendrocytes produce a vast amount of myelin sheath wrapping around axons as an extension of their plasma membrane. During the active phase of myelination, each oligodendrocyte must synthesize an enormous amount of myelin membrane proteins and lipids to assemble myelin sheath. Not surprisingly, oligodendrocytes are highly sensitive to the disruption of protein translation (Lin and Popko, 2009). Therefore, there is a possibility that the inhibitory effects of eIF2B mutations on protein biosynthesis preferentially cause oligodendrocyte dysfunction and contribute to the pathogenesis of VWMD.

The major challenge in VWMD research is to generate animal models that resemble the pathology in human patients. Despite considerable effort, attempts to recapitulate fundamental aspects of VWMD pathology in a relevant model system had been unsuccessful. The guanine nucleotide exchange activity of eIF2B is regulated by phosphorylation of eIF2α, with the two proteins forming a nonproductive phosphorylated-eIF2α-eIF2B complex. Four distinct kinases are known to phosphorylate eIF2α, including pancreatic endoplasmic reticulum kinase (PERK) that is activated by endoplasmic reticulum (ER) stress (Lin and Popko, 2009). Using a powerful chemical genetic tool, we had generated a mouse model system, PLP/Fv2E-PERK transgenic mice, which allows for pharmacological temporally-controlled activation of PERK specifically in oligodendrocytes, in the absence of ER stress (Lin et al., 2013). PLP/Fv2E-PERK mice express Fv2E-PERK, an artificial PERK derivative that is generated by fusing the eIF2α kinase effector domain of PERK to a polypeptide containing tandem modified FK506 binding domains (Fv2E), under the control of the mouse proteolipid protein (PLP) transcriptional control region. These mice express the Fv2E-PERK transgene exclusively in CC1-positive oligodendrocytes in the CNS, but not in platelet-derived growth factor receptor (PDGFrα)-positive oligodendrocyte precursor cells (OPCs). Importantly, treatment of PLP/Fv2E-PERK mice with Fv2E ligand AP20187 enhances the activity of PERK signaling specifically in oligodendrocytes in a dose-dependent manner without causing ER stress. Therefore, using this unique mouse model system, our study was designed to test the hypothesis that impaired eIF2B activity in oligodendrocytes cell-autonomously causes the cell dysfunction, resulting in the selective white matter pathology in VWMD (Lin et al., 2014b).

We found that heterozygous PLP/Fv2E-PERK (PLP/Fv2E-PERK Tg/+). mice treated with a low dose of AP20187 (0.5–2 mg/kg) appear healthy and do not exhibit any abnormalities of oligodendrocytes or myelin in the CNS of young, developing mice or adult mice (Lin et al., 2013, 2014a). We also showed that treatment with the low dose of AP20187 only slightly reduces protein biosynthesis in oligodendrocytes of PLP/Fv2E-PERK Tg/+ mice, indicating moderate PERK activation in the cells. Interestingly, we further demonstrated that moderate PERK activation protects mature oligodendrocytes and myelin against immune attack during the demyelination phase of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, and promotes remyelinating oligodendrocyte survival and remyelination during the remyelination phase of EAE (Lin et al., 2013, 2014a).

To mimic impaired eIF2B activity in oligodendrocytes of VWMD, we treated homozygous PLP/Fv2E-PERK (PLP/Fv2E-PERK Tg/Tg) mice with the high dose of AP20187 (5 mg/kg) daily starting on postnatal day (P) 10 (Lin et al., 2014b). The high dose of AP20187 treatment significantly reduces protein biosynthesis in oligodendrocytes of PLP/Fv2E-PERK Tg/Tg mice, indicating strong PERK activation and impaired eIF2B activity in the cells. Importantly, all PLP/Fv2E-PERK Tg/Tg mice treated with 5 mg/kg AP20187 during the active phase of developmental myelination exhibit a severe tremoring phenotype starting around P14, and die by P24. In contrast, PLP/Fv2E-PERK Tg/Tg mice treated with the high dose of AP20187 display severe myelin loss,
The number of oligodendrocytes in the CNS is not significantly changed in the CNS of these mice. Additionally, the high dose of AP20187 treatment does not alter protein biosynthesis in Schwann cells and has no effect on the myelination process in the peripheral nervous system of PLP/Fv2E-PERK Tg/Tg mice. Thus, these results demonstrate that impaired eIF2B activity induced by strong PERK activation specifically in myelinating oligodendrocytes of young, developing mice is sufficient to reproduce the clinical features and the fundamental pathology of VWMD, including hypomyelinating phenotypes, premature death, myelin loss, and foamy oligodendrocytes (Lin et al., 2014b).

Intriguingly, adult PLP/Fv2E-PERK Tg/Tg mice treated with the high dose of AP20187 (5 mg/kg) for 3 weeks are phenotypically normal (Lin et al., 2014b). The high dose of AP20187 treatment significantly reduces protein biosynthesis in oligodendrocytes of adult PLP/Fv2E-PERK Tg/Tg mice. Nevertheless, we found that 5 mg/kg AP20187 treatment does not alter the number of the morphology of oligodendrocytes in adult PLP/Fv2E-PERK Tg/Tg mice. There are no apoptotic oligodendrocytes in the CNS of adult PLP/Fv2E-PERK Tg/Tg mice treated with the high dose of AP20187. Moreover, minimal myelin abnormalities are observed in the CNS of AP20187-treated adult PLP/Fv2E-PERK Tg/Tg mice. Thus, these results demonstrate that impaired eIF2B activity induced by strong PERK activation specifically in mature oligodendrocytes of adult mice has a minimal effect on the cell viability and myelin integrity in the CNS (Lin et al., 2014b).

Our report showed that impaired eIF2B activity does not affect the viability of myelinating oligodendrocytes in young developing mice or mature oligodendrocytes in adult mice, but suppresses the myelinating function of newly-generated oligodendrocytes (Lin et al., 2014b). Importantly, there is evidence that the myelinating function of oligodendrocytes is compromised in VWMD patients (Bugiani et al., 2013). Thus, our study represents the first experimental demonstration of a link between impaired eIF2B activity in myelinating oligodendrocytes and the fundamental aspects of VWMD pathology, including foamy oligodendrocytes and myelin loss. These data imply that selective disruption of the myelinating function of oligodendrocytes induced by eIF2B impairment contributes to VWMD pathogenesis.

While our report indicates the cell-autonomous role of eIF2B impairment in myelinating oligodendrocytes in the pathogenesis of VWMD, it is important to use other model systems to verify this mechanism. The induced pluripotent stem cells (iPSCs)-derived OPC xenograft model could be an ideal model system to demonstrate the cell-autonomous role of eIF2B mutations in oligodendrocytes in VWMD pathogenesis. Mice homozygous for the shiverer spontaneous mutation of MBP (Shiverer +/− mice) fail to develop MBP or compact myelin in the CNS. It is known that transplanted human iPSCs-derived OPCs can differentiate into mature oligodendrocytes and myelinate axons in the CNS of Shiverer +/− mice (Wang et al., 2013). iPSCs generated from VWMD patients would be differentiated into OPCs, and then these OPCs would be injected into the brains of Shiverer +/− mice. If the transplanted OPCs differentiate into oligodendrocytes but fail to myelinate axons in Shiverer +/− mice, the data would provide direct evidence that eIF2B mutations cell-autonomously cause oligodendrocyte dysfunction in VWMD, resulting in myelin loss in the CNS. Nevertheless, since eIF2B mutations derived from VWMD patients have no significant effect on oligodendrocyte function and myelin integrity in the CNS of knock-in mice (Geva et al., 2010), it would not be a big surprise if the human iPSCs-derived OPCs xenograft model failed to reproduce the pathology of oligodendrocytes and myelin in VWMD.

Another important finding in VWMD pathology is that PERK signaling is activated in oligodendrocytes of VWMD patients (van der Vooroot et al., 2005). Our report showing that strong PERK activation specifically in oligodendrocytes reproduces VWMD pathology in mice suggests a potential role of PERK activation in oligodendrocytes in VWMD pathogenesis. Interestingly, both febrile infection and minor head trauma, the stresses that trigger rapid neurological deterioration of VWMD, can induce ER stress in cells. Therefore, the clinically evident stress sensitivity of VWMD raises the possibility that the synergistic inhibitory effects of eIF2B mutations and PERK activation on protein biosynthesis cause oligodendrocyte dysfunction and contribute to the pathogenesis of VWMD (Figure 1). As mentioned above, both knock-in mice

![Figure 1](image-url)
with eIF2B mutations and mice with moderate PERK activation specifically in oligodendrocytes do not display oligodendrocyte dysfunction or myelin abnormality (Geva et al., 2010; Lin et al., 2014a). To determine whether PERK activation in oligodendrocytes is a precipitating factor in the pathogenesis of VWMD, a straightforward experiment would be to cross the eIF2B mutant mice with PLP/Fv2E-PERK Tg/+ mice, and then treat the compound mice with the low dose of AP20187 (0.5 mg/kg) during the active phase of developmental myelination. If the compound mice treated with the low dose of AP20187 show significant reduction of protein biosynthesis in oligodendrocytes and the fundamental aspects of VWMD, including hypomyelinating phenotype, foamy oligodendrocytes, and myelin loss, this result would define the importance of PERK activation in oligodendrocytes in VWMD and provide additional evidence that impaired eIF2B activity in oligodendrocytes contributes to VWMD pathogenesis.

In summary, using a powerful genetic tool for selectively impairing eIF2B activity in oligodendrocytes, our recent study indicates that impairment of eIF2B cell-autonomously causes myelinating oligodendrocyte dysfunction during developmental myelination, resulting in VWMD pathology (Lin et al., 2014b). Nevertheless, our mouse model system has its limits; additional studies are essential and necessary to verify the cell autonomous role of eIF2B impairment in oligodendrocytes in VWMD pathogenesis. Additionally, there are many other open questions regarding VWMD pathogenesis: 1) how impaired eIF2B activity suppresses the myelinating function of oligodendrocytes without affecting their viability; 2) how impaired eIF2B activity in myelinating oligodendrocytes results in foamy oligodendrocytes; 3) the molecular mechanisms for ER stress activation in oligodendrocytes; 4) the contributions of other cell types, such as astrocytes and neurons, to VWMD pathogenesis; 5) the mechanisms for the adult onset forms of VWMD.

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