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SCIENTIFIC COMMENTARIES

TMEM106B and myelination: rare leukodystrophy families reveal unexpected connections

This scientific commentary refers to 'A recurrent *de novo* mutation in *TMEM106B* causes hypomyelinating leukodystrophy', by Simons *et al.* (doi:10.1093/brain/awx314).

Leukodystrophies are a group of rare genetic disorders that affect the CNS by disrupting the growth or maintenance of the myelin sheath that insulates nerve cells. A classification system based on the pathological mechanisms responsible for the white matter pathology was recently proposed, reserving the term hypomyelinating leukodystrophies (HLDs) for those diseases with a primary or predominant involvement of oligodendrocytes and/or myelin and a permanent deficiency in the formation or deposition of myelin (in contrast to demyelinating leukodystrophies in which the integrity of myelin is disrupted after its formation) (van der Knaap and Bugiani, 2017). HLDs are genetically and clinically diverse; however, most patients present in the neonatal or infantile period with a combination of hypotonia and nystagmus and a range of possible additional symptoms including developmental delay, ataxia, spasticity intellectual disability. Pelizaeus-Merzbacher disease (PMD) is the archetypical HLD caused by mutations in the gene encoding proteolipid protein 1 (PLP1), a primary constituent of myelin. However, more than a dozen additional HLD genes have been identified with a wide range of functions involved in different cellular processes: from RNA and protein synthesis to endolysosomal trafficking (Fig. 1) (Baskin *et al.*, 2016; Charzewska *et al.*, 2016; Edvardson *et al.*, 2016). In this issue of *Brain*, Simons and co-workers reveal an intriguing connection between TMEM106B, a relatively unknown transmembrane protein localized to the lysosomal membrane, and HLD through the identification of a recurrent *de novo TMEM106B* mutation in four families (Simons *et al.*, 2017).

Two unrelated patients recruited and studied on different continents by independent research groups formed the basis for the discovery. Researchers from the Care4Rare Canada Consortium and the Amsterdam Database of Unclassified Leukoencephalopathies in The Netherlands each diagnosed a patient with PMD-like disease based on the presence of nystagmus and hypotonia shortly after birth, delayed motor development, and prominent hypomyelination on brain MRI; however, genetic testing excluded mutations in *PLP1*. As a result of the childhood onset of disease and absence of family history, trio exome sequencing was performed in both families and, remarkably, the same *de novo* mutation c.754G>A (p.Asp252Asn) in *TMEM106B* was identified in both patients. Through effective use of the GeneMatcher website, which enables connections between researchers dealing with 'unsolved exomes', the researchers noted the strong overlap in clinical presentation and identical gene mutation, suggesting a potential causal role for this mutation in their patients. The study of exome data from 10 additional trios from The Netherlands and one unrelated patient from Canada, identified another two unrelated patients carrying the same c.754G>A mutation. Each of the four unrelated patients had the classical clinical presentation of hypomyelination with early-onset nystagmus, hypotonia and delayed motor development with variable degrees of intellectual disability and epilepsy. In one family the mutation was found to be transmitted from the father, who is a mosaic for the p.Asp252Asn mutation, to the affected child. The father expresses approximately 25% mutant TMEM106B, according to quantification of expression in leucocytes. While this presumably led to nystagmus and developmental delay in infancy, the currently 65-year-old male has normal cognition and no obvious neurological abnormalities.

TMEM106B was first reported in 2010 as a genetic risk factor for frontotemporal lobar degeneration with pathologically confirmed TDP-43 pathology (FTLD-TDP), a neurodegenerative disease characterized by the preferential atrophy of the frontal and temporal lobes (Nicholson and Rademakers, 2016). Subsequent studies provided strong support for *TMEM106B* as a disease modifier,

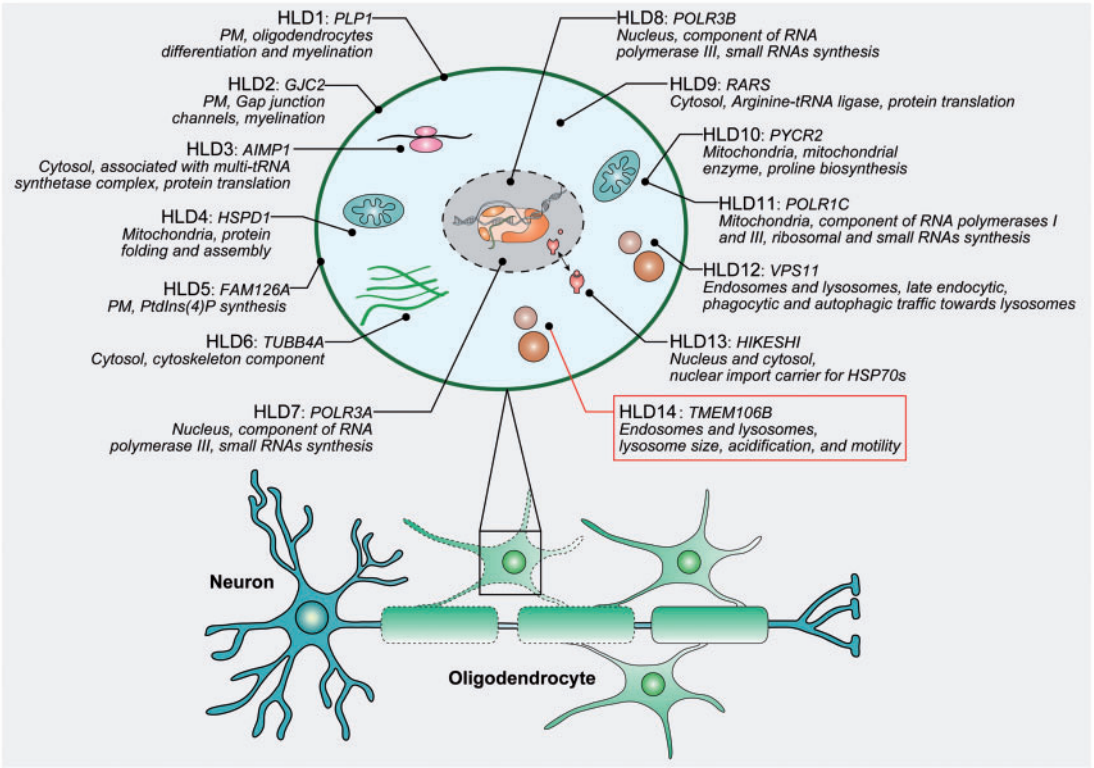

Figure 1 Overview of known disease genes for classical hypomyelinating leukodystrophies (HLDs). For each HLD-associated protein, the primary subcellular localization is reported as well as its primary known function(s) (Baskin *et al.*, 2016; Charzewska *et al.*, 2016; Edvardson *et al.*, 2016). Nomenclature and numbering of HLD1 through HLD13 is in accordance with the OMIM database (https://www.omim.org/), while the newly identified HLD gene, *TMEM106B*, was temporarily assigned the acronym HLD14. PM = plasma membrane.

especially in patients with FTLD-TDP with loss-of-function mutations in progranulin (*GRN*), a neurotrophic growth factor that is processed into possibly functionally active granulin peptides within lysosomes. While the basis for the risk/protective effect of *TMEM 106B* is still being studied, available data suggest that an increase in TMEM106B levels is cytotoxic and is associated with increases in lysosomal size and reduced lysosomal acidification, leading to the disruption of endolysosomal- and autophagic-lysosomal degradation (Nicholson and Rademakers, 2016). Lowering TMEM106B levels has therefore been suggested as a potential therapeutic avenue in patients with *GRN* mutations, and it was recently reported that *Tmem106b* deletion can normalize lysosomal protein levels in $Grn^{-/-}$ mice and rescue FTLD-related behavioural abnormalities and retinal degeneration in this model (Klein *et al.*, 2017). However, TMEM106B knockdown may not be completely without consequences. Studies in neuronal cultures suggested mild effects on lysosomal trafficking, and the activity of several lysosomal enzymes was reduced in $Tmem106b^{-/-}$ mice, arguing for a tight regulation of TMEM106B *in vivo* (Nicholson and Rademakers, 2016; Klein *et al.*, 2017). In line with these observations, relatively mild effects on the expression levels of *TMEM106B* were observed in individuals carrying the *TMEM106B* risk (increased expression) or *TMEM106B* protective (decreased expression) alleles (Nicholson and Rademakers, 2016). Intriguingly, the same *TMEM106B* variant(s) implicated in FTLD-TDP were recently identified in an unbiased screen for genetic modifiers of healthy brain ageing, with increased inflammation, neuronal loss, and cognitive deficits in brain specimens of *TMEM106B* risk allele carriers (Rhinn and Abeliovich, 2017). This study suggested an inappropriate polarization of microglia and other innate immune myeloid cells toward a pro-inflammatory state in *TMEM106B* risk allele carriers, yet the authors did not rule out a function for TMEM106B in neurons.

The current study by Simons and colleagues in this issue of *Brain* (Simons *et al.*, 2017) is the first to link TMEM106B to oligodendrocytes and myelination, unveiling an unexplored area of research into TMEM106B function and disease mechanisms. Unfortunately, the effect of the specific p.Asp252Asn mutation on TMEM106B expression and/or function was not studied *in vitro* or in patient material, and discussion of the potential disease mechanism is consequently speculative at this time. The close vicinity of the p.Asp252Asn mutation to one of the sites requiring complex glycosylation for proper TMEM106B transport, sorting and probably function (Nicholson and Rademakers, 2016), combined with the fact that all patients carried the exact same *de novo* mutation supports

Glossary

Hypomyelinating leukodystrophies (HLD): Genetically determined white matter diseases caused by a primary deficit in myelin deposition. Multiple HLD genes have been identified (Fig. 1).

TMEM106B: Type I transmembrane protein mainly localized to late endosomes and lysosomes. Common variants at the *TMEM106B* locus have been implicated in frontotemporal dementia with TDP-43 pathology.

the hypothesis of a loss-of-function disease mechanism, although a gain of toxic function associated with the specific mutation cannot yet be excluded. Since all patients were heterozygous for the mutation, a dominant-negative disease mechanism may in fact be at play. The majority of HLD genes are transmitted as autosomal recessive disorders or are x-linked (*PLP1*) with the notable exception of *TUBB4A*, in which the heterozygous p.Asp249Asn mutation was shown to cause HLD with atrophy of the basal ganglia and cerebellum. In the latter case, a dominant-negative effect of the mutation presumably led to the loss or inefficient dimerization of microtubules (Simons *et al.*, 2013; Charzewska *et al.*, 2016).

Given that PLP1 is one of the main structural components of the myelin sheath, and that *PLP1* mutations are known to cause PMD with overlapping disease phenotypes to those described in association with the new *TMEM106B* mutation, it is tempting to speculate that mutant TMEM106B could potentially interfere with the highly regulated endocytosis and/or exocytosis of PLP1, thereby affecting its spatial and temporal expression (Saher and Stumpf, 2015). PLP1 is synthesized in oligodendrocytes in the rough endoplasmic reticulum (ER) and then transported to the Golgi and plasma membrane in lipid raft-like membrane domains, where it is integrated into the developing myelin sheet. In the absence of neuronal signals, PLP1 is internalized and stored in late endosomes and lysosomes from where it can be rapidly recruited to the sites of membrane growth when needed (Feldmann *et al.*, 2011). In PMD, point mutations in *PLP1* interfere with its trafficking, resulting in accumulation of PLP1 within the ER/Golgi. By contrast, overexpression of *PLP1* due to duplications leads to excessive PLP1 accumulation in the late endosomes and lysosomes, illustrating that multiple trafficking deficits and sites of PLP1 accumulation can be toxic (Saher and Stumpf, 2015; van der Knaap and Bugiani, 2017).

Regardless of the specific mechanism associated with the recurrent *TMEM106B* mutation, the addition of *TMEM106B* to the list of known HLD genes reinforces the connection between lysosomes and myelination. Currently available data further suggest that TMEM106B levels are tightly regulated and that either too much or too little TMEM106B may have devastating consequences. Future mechanistic studies of this newly discovered p.Asp252Asn mutation will undoubtedly provide much-needed insights into the normal function of TMEM106B within lysosomes. This would appear to be the critical next step towards the development of therapies or disease-modifying treatments not only for HLDs but also for patients with FTLD-TDP with and without *GRN* mutations.

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MOG-antibody neuromyelitis optica spectrum disorder: is it a separate disease?

This scientific commentary refers to 'Clinical presentation and prognosis in MOG-antibody disease: a UK study', by Jurynczyk *et al.* (doi:10.1093/brain/awx276).

In this issue of *Brain*, Jurynczyk and co-workers describe the largest cohort of patients to date with myelin oligodendrocyte glycoprotein antibody (MOG-antibody) neuromyelitis optica spectrum disorders (NMOSD) and confirm two important points: (i) the clinical expression of MOG antibody may be included in the broadest definition of NMOSD; and (ii) the clinical profile of MOG-antibody-positive patients differs from that of patients with aquaporin-4 (AQP4)-antibody NMOSD (Juryńczyk *et al.*, 2017*a*).

Neuromyelitis optica (NMO) was long considered to be a subtype of multiple sclerosis. However, in 2004 a specific antibody—known initially as anti-NMO-IgG, and then later as AQP4 antibody—was found to distinguish NMO from multiple sclerosis (Lennon *et al.*, 2004). From that point forward, NMO was considered a separate entity. The main pathogenic characteristic of NMO is the presence of AQP4 antibodies in the serum and/or CSF; this has been shown in patients and also in animal models. However, passive transfer of purified human AQP4 antibodies alone without blood–brain barrier breakdown is not sufficient to induce NMO in animal models (Jones *et al.*, 2012). Furthermore, several experimental autoimmune encephalitis (EAE) models induced by MOG protein appear to mimic the NMO phenotype, especially in Brown Norway rats (Collongues *et al.*, 2012).

Since the discovery of AQP4 antibodies, various clinical features of AQP4-antibody-positive patients have been published, including recurrent optic neuritis and myelitis, posterior reversible encephalopathy, acute demyelinating encephalomyelitis, and brainstem and area postrema syndromes. Because of the high specificity of AQP4 antibodies, these conditions are all considered part of the expanded NMOSD. A panel of experts recently proposed revised criteria for NMO in order to offer the possibility of earlier diagnosis and treatment (Box 1). These criteria are mainly based upon the AQP4-positive status of patients, and allow a diagnosis to be made after the first clinical episode if the symptoms are highly suggestive of NMOSD: severe or bilateral optic neuritis, myelitis, area postrema/brainstem syndrome.

However, a proportion of patients (20–40% depending on the cohort) with a typical phenotype of NMO are found to be negative for AQP4 antibodies (i.e. seronegative). These patients may eventually be diagnosed with NMO, but with a delay owing to the need for two relapses to have occurred in two different regions (Box 1).

Multiple sclerosis research has also focused on biological markers, especially blood or cerebrospinal antibodies, for use in diagnosis and prognosis. In an attempt to translate findings from EAE models to patients, MOG antibodies and antibodies against myelin basic protein (MBP antibodies) were investigated as potential biomarkers for conversion from clinically isolated syndrome (CIS) to multiple sclerosis. However, these studies yielded mixed results even within the same laboratory, mainly owing to the limitations of early antibody detection methods (Berger *et al.*, 2003; Lim *et al.*, 2005). Antibodies against MOG were originally thought to be involved in classic multiple sclerosis based on results from ELISAs using linearized or denatured MOG peptides as antigen. By contrast, more recent studies using next-generation cell-based assays demonstrated a robust association of antibodies against full-length, conformationally intact human MOG protein with (mostly recurrent) optic neuritis, myelitis and brainstem encephalitis, as well as with acute disseminated encephalomyelitis (ADEM)-like presentations, rather than with classic multiple sclerosis. All of these clinical features are currently considered part of NMOSD (Wingerchuk *et al.*, 2015). The role of immunoglobulin G serum antibodies against MOG in patients with CNS demyelination has also been revisited over the past 3 years. The clinical significance of MOG-antibodies in patients with NMOSD remains an open question: it is possible that they are expressed secondary to tissue damage as part of a bystander effect. Notably, a number of studies in adults, including the current paper by Jurynczyk *et al.*, have detected MOG antibodies in the sera of more than 20% of NMO-seronegative patients tested by cell-based assays, but not in the sera of patients with multiple sclerosis.