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What we know about TMEM106B in neurodegeneration

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Abstract

Frontotemporal lobar degeneration is a neurodegenerative disorder affecting over 50,000 people in the United States alone. The most common pathological subtype of FTLN is the presence of ubiquitinated TAR DNA binding protein 43 (TDP-43) accumulations in frontal and temporal brain regions at autopsy. While some cases of FTLN-TDP can be attributed to the inheritance of disease-causing mutations, the majority of cases arise with no known genetic cause. In 2010, the first genome-wide association study (GWAS) was conducted in patients with FTLN-TDP to determine potential genetic risk factors for this homogenous subgroup of dementia patients, leading to the identification of the *TMEM106B* locus on chromosome 7. In this manuscript, we review the initial discovery and replication studies describing *TMEM106B* variants as disease risk factors and modifiers in TDP-43 proteinopathies, such as FTLN-TDP caused by progranulin (*GRN*) or *C9orf72* mutations, as well as Alzheimer's disease and hippocampal sclerosis. We further summarize what is currently known about the previously uncharacterized TMEM106B protein and its role as a potential regulator of lysosomal function, and we discuss how modifying TMEM106B levels might uncover promising therapeutic strategies for individuals suffering from TDP-43 proteinopathy.

Keywords

TMEM106B; frontotemporal dementia; TDP-43; lysosomes; genetic association

Introduction

Frontotemporal lobar degeneration (FTLD) is the second leading cause of pre-senile neurodegeneration after Alzheimer's disease (AD) and is characterized by the preferential atrophy of the frontal and temporal lobes of the brain. Since the development of this disease can occur across a large brain area, the symptoms at disease onset differ among individuals with this disorder. The term frontotemporal dementia (FTD) is most often used as an umbrella term for the spectrum of clinical manifestations. The most common of these subtypes is behavioral variant frontotemporal dementia (bvFTD) in which reported symptoms include abnormal behaviors such as poor hygiene, social disinhibition, or hoarding [7, 57]. Primary progressive aphasia (PPA) is another, less common, variety of FTD classified by language deterioration. PPA can be further sub-classified into three

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different presentations: semantic variant of PPA (svPPA) in which the patient finds it difficult to remember the meaning of words, non-fluent variant of PPA (nfvPPA) in which the patient is unable to adequately form words, or logopenic variant of PPA (lvPPA) in which the patient's ability to retrieve words has deteriorated [25]. All FTD clinical subtypes can present with the motor neuron disease, amyotrophic lateral sclerosis (ALS; termed FTD-ALS), or with other movement disorders such as corticobasal syndrome, progressive supranuclear palsy, or parkinsonism [5].

Nearly 30–50% of FTD cases report a family history of dementia [24, 57, 58, 62, 85], suggesting a strong genetic component for this disease. The first gene discovered to cause FTLT was the microtubule-associated protein tau (*MAPT*) located on chromosome 17, in which patients exhibit intraneuronal aggregates of hyperphosphorylated tau in the affected brain regions at autopsy (FTLD-tau) [30]. However, *MAPT* mutations only account for a small percentage of familial FTLT cases. In contrast, the majority of FTLT patients display intranuclear and/or cytoplasmic accumulations of ubiquitinated proteins (FTLD-U) [32, 33, 40, 44]. The primary ubiquitinated protein was identified as the transcription factor TAR DNA binding protein 43 (TDP-43) [4, 51], now classified as FTLT-TDP. Mutations in the gene encoding progranulin (*GRN*) were found to be a frequent cause of FTLT-TDP [6, 16, 22, 72]. Biochemical and pathological assessments have determined that FTLT-causing *GRN* mutations invariably cause progranulin protein (PGRN) haploinsufficiency and subsequent TDP-43 proteinopathy [6, 16, 22, 41, 43]. Since this discovery in 2006, over 60 *GRN* mutations in approximately 230 families have been identified [13, 16, 22, 72]. However, *GRN* mutations do not account for all patients with TDP-43 inclusions. It was not until 2011 that two independent groups discovered the most common genetic cause of FTLT-TDP. This mutation was identified as a hexanucleotide repeat expansion, (GGGGCC)_n, in the chromosome 9 open reading frame 72 (*C9orf72*) gene and mutation carriers can present with FTLT, FTLT-ALS, or ALS alone, yet they all have TDP-43 pathology [17, 48, 59]. The identification of a common genetic cause and subsequent pathological manifestation in both FTLT and ALS provided concrete evidence that these two diseases exist as a part of a common disease spectrum. *C9orf72* and *GRN* mutations likely account for over half of all familial occurrences of FTLT-TDP; however, mutations in the genes encoding tank-binding kinase 1 (*TBK1*) and the valosin containing protein (*VCP*) have also been discovered as less common genetic causes in patients with FTLT-TDP [20, 23, 46, 54, 77, 84]. In rare cases of FTLT-U, the ubiquitinated protein is a TDP-43 protein family member known as fused in sarcoma (FUS) [50, 67], but there is no known genetic cause for this pathological subtype. Finally, FTLT-U is also observed in patients with causal mutations in the charged multivesicular body protein 2B (*CHMP2B*) gene [70]; however, the ubiquitinated protein in these individuals has not been identified. In summary, while genetic mutations can account for a large number of individuals suffering from FTLT-TDP, the cause of the majority of cases remains unknown.

In 2010, single nucleotide polymorphisms (SNPs) identified on chromosome 7 led to the discovery of the first genetic risk factor for FTLT-TDP. The SNPs were in a genomic region encoding the transmembrane protein 106B (*TMEM106B*) [76]. Research efforts have since focused on both the genetic correlations as well as the functional properties of this relatively uncharacterized transmembrane protein. In this review we focus on the key findings related

to TMEM106B and its role in FTLN risk. Moreover, we discuss how TMEM106B levels are hypothesized to be the critical factor linking TMEM106B to disease pathogenesis, revealing TMEM106B as a potential drug target in FTLN and related TDP-43 proteinopathies.

Identification of *TMEM106B* as a risk factor for FTLN-TDP

A subset of FTLN patients can be explained by inherited genetic mutations; however, a large portion of cases remain unexplained. In an attempt to find genetic risk factors for FTLN, Van Deerlin and colleagues focused on the homogenous population of cases with FTLN-TDP pathology and conducted a GWAS in 2509 control subjects and 515 subjects with pathologically-confirmed FTLN-TDP, some of which contained a *GRN* mutation [76]. This GWAS led to the discovery of 3 SNPs within a 68 kb region on chromosome 7p21.3 that associated with the occurrence of FTLN-TDP with genome-wide significance (rs6966915, rs102004, and top marker SNP rs1990622; p-value range = 5.00×10^{-11} to 1.08×10^{-11}) (Fig. 1) [76]. The 3 significantly-associated SNPs were in the same linkage disequilibrium (LD) block as 9 other SNPs of nominal association, and all spanned the *TMEM106B* gene locus. In this case-control cohort, the minor allele of each significant SNP was greatly underrepresented in FTLN-TDP patients (32.1% versus 43.6% in controls for rs1990622 minor C-allele; p-value = 1.08×10^{-11}), suggesting that individuals expressing the minor *TMEM106B* alleles are less likely to develop disease (odds ratio (OR) = 0.61 for the rs1990622 minor allele). For simplicity, we refer to the common *TMEM106B* variants as the risk alleles and the minor variants as the protective alleles throughout this manuscript. Van Deerlin and colleagues replicated their findings in a second pathologically-confirmed FTLN-TDP case-control cohort, but were unable to observe significant differences in a small third cohort containing living FTD patients of unspecified pathology. Of particular interest, Van Deerlin and colleagues stratified their initial GWAS cohort by *GRN* mutation status and found that the association of the top 3 SNPs remained significant in both groups, but was greatest in people with *GRN*-related FTLN-TDP [rs1990622 p-value = 1.34×10^{-9} ; OR = 0.34] in *GRN* carriers versus in non-*GRN* carriers (rs1990622 p-value = 6.90×10^{-7} ; OR = 0.68). This suggested that *TMEM106B* SNPs might have the greatest effect in people with *GRN* mutations, a rather unexpected finding given that *GRN* mutations had been considered near fully penetrant previous to this discovery [76]. In fact, since this initial report, while several groups tried to replicate the associations of *TMEM106B* in clinical and pathological cohorts of FTLN with variable success, all studies confirmed the highly significant effect of *TMEM106B* SNPs in modifying risk in *GRN* mutation carriers. Of note, the 12 *TMEM106B* SNPs included in the Van Deerlin et al. publication are in high linkage disequilibrium with only one coding SNP (rs3173615) located within the *TMEM106B* gene that results in a threonine to serine change at amino acid 185 (Figure 1). The potential functional implication of this *TMEM106B* variant and others on TMEM106B-related mechanisms is described starting on page 13.

Validation of *TMEM106B* SNPs as risk factors across the genetic FTL-D-TDP spectrum

A number of independent clinical and pathological cohorts of FTL-D cases with and without *GRN* mutations have now been studied in an attempt to replicate the association between *TMEM106B* SNPs and FTL-D risk (results described in Table 1; *TMEM106B* association studies are listed and summarized in Online Resource Tables 1 and 2). The first independent replication was published by Finch and colleagues in which all 3 initially identified *TMEM106B* SNPs (rs1990622, rs1020004, and rs6966915) were nominally associated with FTL-D risk in a cohort containing 822 controls and 640 FTD cases, the majority of which were living and, thus, of unknown pathologic subtype. However, when only people with *GRN* mutations were considered, the frequency of the minor alleles for all 3 analyzed SNPs was significantly reduced as compared to healthy individuals (p-value range = 0.006 to 0.0009) [19]. Genotyping data revealed that only the frequency of people homozygous, not heterozygous, for the minor alleles of each of the 3 SNPs was significantly reduced in *GRN* mutation carriers versus controls (2.6% versus 19.1%, respectively; p-value = 0.009 for rs1990622) [19]. These findings suggested a recessive disease model where an individual must carry two copies of the minor allele in order for *TMEM106B* SNPs to confer protection against *GRN*-related FTL-D-TDP. The extremely low frequency of homozygous *TMEM106B* minor allele carriers in *GRN* mutants further indicated that a significant number of individuals with *GRN* mutations and two minor *TMEM106B* alleles may remain without symptoms throughout life and simply do not show up in the clinic to participate in research studies. It is noteworthy to also consider that only one publication to date has identified a significant association in a largely clinical FTD cohort of unknown pathology [78], while 4 others have not [19, 29, 61, 76]. The inconsistent nature of these findings is likely a result of differences in the number of individuals who will present with TDP-43 pathology on autopsy. Thus, depending on the diagnostic inclusion criteria and relative contribution of patient ascertainment from Memory, Movement Disorder or ALS Clinics, a different percentage of patients will likely have an underlying TDP-43 proteinopathy.

The initial studies assessing *TMEM106B* SNPs as disease risk factors in FTL-D-TDP were conducted prior to the discovery of *C9orf72* repeat expansion in 2012. Since this discovery, two independent groups found *TMEM106B* SNPs to also associate with one's risk of developing FTL-D and/or ALS caused by *C9orf72* mutations [21, 75] (results described in Table 1). In these *C9orf72* mutant cohorts, the frequency of individuals carrying the minor allele of *TMEM106B* SNPs [rs1990622 and/or rs3173615 in LD with rs1990622 (Fig. 1)] was significantly reduced, although not as markedly as in cohorts of *GRN* mutation carriers [21, 75]. Interestingly, when van Blitterswijk et al. analyzed *C9orf72* repeat expansion carriers in groups based on their predominant disease presentation as either FTL-D, FTL-D-ALS, or ALS, it was shown that *TMEM106B* SNPs specifically protect against the development of FTL-D but not ALS, with less TDP-43 burden in the brains of *C9orf72* expansion carriers homozygote for the protective *TMEM106B* alleles as compared to risk allele carriers [75]. These findings are in agreement with an earlier examination of *TMEM106B* SNPs in a clinical cohort of ALS patients in which *TMEM106B* SNPs rs1990622 and rs1020004 were not associated with disease risk, but did significantly

associate with cognitive function in ALS patients, with individuals homozygous for the rs1990622 minor allele having better cognitive performance than individuals heterozygous or homozygous for the major risk alleles [79]. The second study of *TMEM106B* in *C9orf72* expansion carriers (of all clinical presentations) surprisingly reported an earlier age at onset and death in *C9orf72* expansion carriers with the protective *TMEM106B* allele, which may seem counterintuitive at first glance. However, this finding may be in line with the van Blitterswijk et al. study (thoroughly summarized by Deming and Cruchaga [18]), and a consequence of the different effect of *TMEM106B* on ALS versus FTLT presentations of the disease. For example, *TMEM106B* may protect against the development of FTLT but not ALS, leading to an overall earlier age at onset and death associated with the ALS phenotype in the group of protective allele carriers. A third examination of rs1990622 in a French cohort of *C9orf72* mutation carriers with FTD and FTD-ALS did not report a significant association in their *C9orf72* mutant cohort, even though the association in *GRN* mutation carriers was found to be significant [38] (Table 1). This suggests that the association of *TMEM106B* risk variants might be different in individual *C9orf72* populations. Importantly, despite the variable composition of genetically defined FTLT-TDP patient cohorts, *TMEM106B* SNP frequencies remained significantly underrepresented in a pathologically-confirmed cohort of FTLT-TDP patients even after excluding all carriers of *GRN* mutations and *C9orf72* expansions [75], signifying an effect of *TMEM106B* on all FTLT-TDP patients irrespective of the underlying genetic defect. The selective impact of *TMEM106B* SNPs on TDP-43 pathology in cortical brain areas (as seen in FTLT-TDP) as compared to the spinal cord (as seen in ALS) remains unexplained at present, but may potentially provide insight into *TMEM106B* expression and function in future studies. To better understand this discrepancy, future studies could potentially focus on the differences in *TMEM106B* levels and/or distribution in the brain versus the spinal cord. Additionally, lysosomal function has been suggested to play a role in TDP-43 aggregation [8, 81, 82]; thus, it is also possible that *TMEM106B*-related lysosomal phenotypes are different between cortical and motor neurons.

***TMEM106B* variants associate with disease risk in other TDP-43 proteinopathies**

Research related to *TMEM106B* SNPs in disease has repeatedly concluded that *TMEM106B* variants only associate with disease risk in FTLT cohorts with confirmed or mutation-predicted TDP-43 pathology. However, a number of other neurodegenerative diseases also present with TDP-43 pathology, including AD, Lewy body dementia (LBD), and hippocampal sclerosis (HpScl) [2, 87]. Thus, research has been conducted to determine whether *TMEM106B* variants might be associated with risk of general TDP-43 proteinopathy not specific to an FTLT presentation (study groups and outcome measures are summarized in Online Reference Tables 1 and 2). A study performed by Rutherford et al. in 2012 was the first to report that *TMEM106B* SNPs might be important for the pathological presentation of AD [64]. More specifically, this research group examined the association of rs1990622 in a pathologically-confirmed AD cohort of over 900 subjects, comparing rs1990622 genotypic frequencies in AD patients with and without TDP-43 pathology. The percentage of individuals homozygous for the rare allele in AD patients without TDP-43

pathology was similar to previously published non-demented cohorts (approximately 21%) indicating no significant association of rs1990622 in this subgroup. However, in AD patients with TDP-43 pathology, this percentage was significantly less, with only 13% of individuals homozygous for the minor *TMEM106B* allele, suggesting *TMEM106B* rare variants protected against the development of TDP-43 even in the setting of AD [64]. HpScl is also common in elderly patients with dementia, including FTLN-TDP and AD, and TDP-43 pathology accompanies over 70% of those individuals [2]. The Rutherford et al. study indicated that *TMEM106B* SNPs strongly associate with one's risk of developing HpScl in AD, with 6% of individuals with AD-HpScl homozygous for the rs190622 minor allele [64]. This strong association was also found by Murray and colleagues who reported no individuals homozygous for the minor allele of rs1990622 in neither an AD-HpScl cohort of 132 subjects, nor in a 30 person cohort of pure HpScl, revealing *TMEM106B* as the strongest genetic indicator of AD-HpScl and HpScl known to date [47]. Later studies conducted by Nelson et al. and Yu et al. also reported a significant underrepresentation of *TMEM106B* protective alleles in patients with HpScl [49, 86], with further assessments indicating lower TDP-43 pathological burden in patients homozygous for the protective *TMEM106B* alleles [86]. Genetic assessments were also conducted in a cohort of LBD in which the minor *TMEM106B* allele of rs1990622 was 17% less represented in people with accompanying HpScl than those without HpScl, although this did not reach statistical significance possibly due to a smaller sample size (n=35 versus 109, respectively) [3]. Finally, in a GWAS study conducted this year, SNPs in *TMEM106B* showed a near genome-wide significant interaction with *APOE* genotype, providing further evidence that *TMEM106B* alleles might modify AD risk [34]. These studies collectively suggest that *TMEM106B* SNPs are risk factors for the development and severity of TDP-43 proteinopathy in non-FTLD disorders such as AD and HpScl.

***TMEM106B* variants associate with brain volume and connectivity**

TMEM106B variants have been studied in the context of non-demented individuals either as part of the general population or in asymptomatic individuals carrying *GRN* mutations. Adams and colleagues addressed whether *TMEM106B* variants might be associated with subclinical, structural brain changes in non-demented elderly individuals, possibly leaving a subgroup of subjects more susceptible to the pathophysiology of FTLN or related disorders. This group discovered that the risk allele of rs1990622 accompanied significantly reduced volume of the superior temporal gyrus, most markedly in the left hemisphere [1]. Interestingly, this area of the brain includes structures important for language processing, often affected in FTLN patients [36, 42, 60]. In line with this study, Premi et al. discovered asymptomatic *GRN* mutation carriers portray significantly decreased brain connectivity of the left frontoparietal network as compared to non-demented individuals, and that this phenotype was worsened in people expressing two copies of the risk rs1990622 allele [56]. Taken together, these analyses might suggest that *TMEM106B* SNPs confer risk for TDP-43 proteinopathies by predisposing affected brain regions to FTLN-related pathogenesis.

TMEM106B is a lysosomal protein

TMEM106B belongs to the TMEM106 family of proteins that are between 200–300 amino acids (aa) in length with relatively unknown function, consisting of TMEM106A, TMEM106B, and TMEM106C. Since the identification of TMEM106B as an FTL risk factor, research has been underway to further understand the role of this protein in normal brain and cellular function. Immunohistochemical assessments of TMEM106B expression in human brain tissue have been conducted with newly developed antibodies targeting this protein. These assessments indicate that TMEM106B is expressed in neurons (Fig. 2) [11, 14, 52], with expression also observed in glial and endothelial cells [11, 52]; however, the specificity of these antibodies has not been validated in TMEM106B knockout systems. Within the cell, *in vitro* assays uncovered that TMEM106B is a single-pass, type 2 integral membrane glycoprotein predominantly located in the membranes of endosomes and lysosomes (Fig. 2) [9, 12, 14, 37, 52, 73]. The transmembrane domain is predicted to range from aa 96–118 (http://smart.embl-heidelberg.de/smart/show_motifs.pl?ID=Q9NUM4&), and five sequence motifs of post-translational N-glycosylation (N-X-T/S) span its luminal domain. Simple glycans are added to three of the asparagine (N) residues (N145, N151, and N164), and complex glycans are added to the most C-terminal motifs at N183 and N256 [37]. Mutagenesis experiments have indicated that the first three glycosylation sites are not critical for TMEM106B localization; however, loss of complex glycans on residue N183 impairs TMEM106B forward transport to endosomes/lysosomes and results in ER retention of the mutant protein. Additionally, N256 complex glycosylation is necessary for TMEM106B sorting, since N256 mutants were trafficked from the endoplasmic reticulum (ER) to the plasma membrane instead of endosomal/lysosomal compartments [37]. Once in the lysosomal membrane, TMEM106B undergoes evolutionarily-conserved regulated intramembrane proteolysis (RIP) [10]. In the case of TMEM106B, this mechanism is a two-step process involving the initial release of the luminal domain by an unknown enzyme, leaving an N-terminal fragment (NTF) still anchored in the membrane [10]. The NTF is further cleaved by the aspartyl protease, signal peptide peptidase-like 2a (SLPP2a), to create an intracellular domain (ICD) that is rapidly degraded, followed by intramembrane processing of the transmembrane stump [39]. It is not yet known whether this processing is misregulated in FTL, how RIP affects full-length TMEM106B function, or whether TMEM106B fragments created by RIP have a function of their own.

TMEM106B levels affect lysosomal function

Expression studies in cell culture models have led to several independent reports indicating that TMEM106B levels are critical for lysosome size, acidification, function, and transport in various cell types. The first of these studies was conducted in 2012 by Chen-Plotkin and colleagues who showed that TMEM106B overexpression significantly enhances the size of LAMP-1/TMEM106B-positive structures [14]. This study and others have reported that these large structures derived by TMEM106B overexpression do not acidify properly to create mature and/or functional lysosomes, causing a delay in endolysosomal-dependent degradation and concomitant cytotoxicity [9, 12, 14, 73]. Additional data by Brady et al. showed that in addition to larger lysosomal size, TMEM106B overexpression in cultured N2a cells causes a concomitant reduction in the total number of lysosomes per cell [9]. Later

publications showed that TMEM106B overexpression in primary neurons did not affect cell viability despite the fact that the previously reported effects of TMEM106B overexpression on lysosomal size and/or acidification were replicated [12, 73]. Moreover, overexpression experiments in primary neurons were unable to show that the overall number of lysosomes was reduced [73], which might signify distinct TMEM106B-mediated phenotypes in various cell types. Nonetheless, increased TMEM106B levels in neurons drastically reduced the number of mobile LAMP-1-positive structures upon TMEM106B overexpression, with the majority of LAMP-1-positive structures accumulating in the cell soma [73]. Enhanced TMEM106B expression has also been shown to cause the translocation of transcription factor EB (TFEB) to the nucleus and to upregulate gene expression from the Coordinated Lysosomal Expression and Regulation (CLEAR) gene network [73]. A shift in the location of TFEB from the lysosome to the nucleus has previously been shown to cause increased CLEAR network expression and this phenomenon is commonly used as a marker of lysosomal stress [65, 69]. Taken together, these findings indicate that TMEM106B upregulation results in lysosomal defects, including improper lysosomal formation, poor lysosomal acidification, reduced lysosomal transport, and increased lysosomal stress.

A study by Schwenk et al. knocked down the expression of TMEM106B in HeLa cells and found that reduced TMEM106B expression leads to a clustering of lysosomes near the nucleus that can be rescued upon reintroducing TMEM106B [66]. In primary rat hippocampal neurons, the clustering of lysosomes in response to Tmem106b reduction was not as pronounced, nor did it affect cell viability; however, loss of neuronal Tmem106b expression significantly enhanced retrograde lysosomal motility in dendrites and reduced dendritic branching [66, 73], leaving the remaining, principle dendrite longer in length with reduced expression of synaptic proteins [66]. Interestingly, restoring the balance between retrograde and anterograde lysosomal trafficking rescued the dendritic branching phenotype observed in neurons [66], suggesting that the reduced dendritic branching observed with Tmem106b loss was due to the Tmem106b-mediated changes in lysosomal trafficking. Importantly, TMEM106B knockdown in primary neurons did not trigger TFEB translocation [73], nor did it affect the pH-dependent proteolytic processing of cathepsin B [66] signifying that TMEM106B loss does not induce lysosomal stress or changes in lysosomal pH. However, Stagi and colleagues did report significantly reduced lysosomal size in neurons upon TMEM106B depletion [73]. These findings could indicate that proper TMEM106B levels are critical to maintain proper lysosomal function.

It should be noted that many of the studies of TMEM106B function and/or levels involve biochemical analyses of TMEM106B by Western blotting. The molecular weight of the TMEM106B protein is predicted at approximately 31 kilodaltons (kDa); however, reports have rarely observed TMEM106B at this molecular weight. In fact, the majority of functional studies on the TMEM106B protein report a molecular weight at approximately 40 or 80 kDa depending on the denaturing conditions, the antibody used, and the cellular system utilized for analysis [8, 9, 14, 37, 52]. Since most initial reports on TMEM106B function have required the generation of previously uncharacterized TMEM106B antibodies for protein detection, this may have contributed to the inconsistent TMEM106B detection patterns. It is likely, however, that the protein detected at 40 kDa is the glycosylated monomeric form of TMEM106B, whereas the band observed at 80 kDa is likely dimeric

TMEM106B protein. Some groups report a loss of dimeric and only slightly increased monomeric TMEM106B signal when protein denaturation is conducted at temperatures higher than 4 °C [9, 14], likely because the antibody used in these studies has higher affinity for the dimeric form as compared to monomeric TMEM106B [37, 52]. Thus, it is important to consider these discrepancies when comparing functional studies of this relatively uncharacterized protein, especially when the nature of the biologically relevant form (or forms) of TMEM106B are unknown.

TMEM106B binding partners

To identify proteins that might interact with TMEM106B, Stagi and colleagues conducted a yeast two-hybrid screen using either the TMEM106B cytoplasmic N-terminus (aa 1-96) or luminal C-terminus (aa 118-274) for bait and a prey library consisting of human adult brain proteins. Using this technique in combination with immunoprecipitation experiments, Stagi and colleagues determined that the N-terminus of TMEM106B is involved in interactions with itself and its family member, TMEM106C [73]. The data regarding whether TMEM106C resides in lysosomal structures has been discrepant, with one group showing at least partial localization in LAMP-1-positive structures and another showing no TMEM106C and LAMP-1 overlap [10, 73]. Therefore, additional analyses would be required to determine whether TMEM106 family members B and C might have similar function. The N-terminus of TMEM106B was also found to interact with endocytic adaptor proteins such as clathrin heavy chain (CLTC) and the $\mu 1$ subunit of adipocyte protein 2 (AP2M1) [73]. These protein interactions together with TMEM106B's endolysosomal localization would imply that the TMEM106B cytoplasmic domain possibly participates in the delivery of endocytic cargos to the lysosome. In agreement with this hypothesis, Jun and colleagues reported that TMEM106B also directly binds to CHMP2B [35], known to be a member of the endosomal sorting complexes required for transport III (ESCRT-III) complex that regulates endolysosomal protein trafficking and autophagic structure formation [27, 28]. One additional report identified the C-terminus of microtubule-associated protein 6 (Map6) to also directly bind the N-terminus of Tmem106b by utilizing mass spectrometry analysis of rat brain Tmem106b immunoprecipitation samples [66]. Interestingly, Map6 was found to have a function reciprocal to Tmem106b since Map6 knockdown was shown to rescue the dendritic branching phenotype caused by reduced Tmem106b expression, and overexpression of Map6 mimics Tmem106b loss [66]. Neither study identified proteins that interact with the luminal C-terminus of TMEM106B. This could be explained by the fact that all procedures were performed at cytoplasmic pH, which is significantly higher than the pH of the lysosomal lumen. Thus additional analyses are necessary to determine TMEM106B C-terminal interactions.

What is the *TMEM106B* functional variant?

Further insight regarding how this relatively uncharacterized protein leads to risk or protection from developing FTLT-DTP can be obtained by understanding which SNP or SNPs are responsible for the association, and to determine how these SNPs affect TMEM106B regulation and/or function. The top SNPs originally identified to associate with FTLT-DTP were part of a GWAS study that included a panel of 550,000 common SNPs

[76]. The use of these SNP panels is effective for GWAS to identify risk loci; however, the SNP most significantly associated with the development of FTLD-TDP is unlikely to be the actual SNP within the LD block responsible for the genetic association, and any one of the dozens of SNPs in this LD block, or a combination of these SNPs, could be functionally relevant. SNP rs3173615 is the only coding variant in the rs1990622-related LD block, located within exon 6 (of 9 total exons) of *TMEM106B* at codon 185 (Fig. 1). This missense variant leads to a coding change from the more common, highly conserved threonine (T185; risk allele) to a serine (S185; protective allele); however, *in silico* analyses predict only a mild functional effect of this single aa change on TMEM106B function. Alternatively, rs1990622 or other non-coding variants in the LD region might be regulatory variants affecting gene expression or splicing. In fact, *in silico* prediction analyses suggest that rs1990622 affects the binding of the transcription factor known as CCCTC-binding factor (CTCF) (<http://regulomedb.org/snp/chr7/12283786>).

***TMEM106B* associated variants and *TMEM106B* expression levels**

In the original manuscript by Van Deerlin et al., the authors described higher *TMEM106B* mRNA levels in the frontal cortex of individuals expressing the risk *TMEM106B* variant for rs1990622 as compared to individuals homozygous for the protective allele [76]. The authors also reported that people with FTLD-TDP have significantly higher amounts of *TMEM106B* mRNA as compared to non-demented individuals, although this effect was driven by the *GRN* mutation carriers and might have been a result of this disease group having less individuals homozygous for the minor allele in this small cohort (n=25) [76]. Moreover, they queried a publicly-available mRNA-by-SNP database to determine genetic regulators of *TMEM106B* mRNA expression in lymphoblasts cells, and found that rs1990622 was significantly correlated with *TMEM106B* mRNA expression [76]. While an increase in *TMEM106B* mRNA expression provides a plausible pathogenic mechanism supported by functional studies showing increased TMEM106B expression can cause detriment to lysosomal-related structures, the effect of the associated SNPs on *TMEM106B* mRNA levels was not confirmed in other brain mRNA expression studies [15, 78, 86]. However, these studies were all performed using postmortem brain tissue with possibly variable levels of neuronal loss and cell type composition which may have masked possible SNP-expression associations. Future studies on TMEM106B levels in human brain should consider that variability in TMEM106B levels might be a SNP-dependent effect, or could be a consequence of disease-related lysosomal dysfunction. In fact, *in vitro* and *in vivo* models of lysosomal dysfunction showed that Tmem106b protein levels are significantly increased in these model systems [26, 37]; thus, SNP-related changes in post-mortem tissue TMEM106B protein levels might be best addressed using non-diseased tissues.

Evidence also exists to suggest that coding SNP rs3173615 might directly affect TMEM106B protein levels, independent of mRNA expression levels. As previously described, this missense variant is located in codon 185 of the TMEM106B protein (Fig. 2), which is only two aa downstream from a critical site of N-glycosylation at residue 183 (N813) that is part of the N-X-T/S glycosylation consensus sequence [37]. Upon transient overexpression, Nicholson and colleagues found that the T185 isoform of TMEM106B led to a nearly two-fold increase in TMEM106B protein expression as compared to the S185

isoform, despite equal mRNA expression levels. They further showed that the higher levels of TMEM106B T185 protein were due to more rapid lysosomal degradation of the TMEM106B S185 protein, and that this difference in expression was abrogated when a mutation in the N183 glycosylation site was introduced [52]. Potential p.T185S-mediated changes in N183 glycosylation would likely be modest since both variants of *TMEM106B* are found to adequately localize to endo/lysosomal compartments [9, 52]; however, rs3173615-dependent effects on TMEM106B protein levels could contribute to the protective effects observed in FTLN pathogenesis.

Finally, independent of *TMEM106B* SNPs, it has been suggested that *TMEM106B* expression in the brain is regulated by the microRNA-132 cluster of microRNAs previously implicated in neurodevelopment [45, 80]. MicroRNAs regulate gene expression in a sequence-specific manner, and disruption of microRNA production or processing can cause transcriptional imbalances leading to cellular dysfunction. Chen-Plotkin et al. reported that the levels of three microRNAs found in the microRNA-132 cluster are significantly reduced in FTLN brains as compared to healthy controls, with the strongest effects observed in *GRN* mutation carriers [14]. Furthermore, TMEM106B protein levels were also observed as markedly increased in *GRN* mutation carriers [14]. Yu and colleagues later showed that levels of microRNA-132 significantly correlate with TDP-43 burden in elderly individuals with TDP-43 pathology [86]. These reports provide yet another mechanism by which TMEM106B levels might be critical for the onset of TDP-43-related disorders.

Linking TMEM106B to other FTLN disease proteins

It has been known that the lysosome is a critical player in the pathogenesis of neurodegenerative disorders such as AD and Parkinson's diseases [53, 63]. However, the role of lysosomal function in FTLN-TDP and other TDP-43 proteinopathies was not as evident until the recent discovery that individuals homozygous for the loss-of-function mutations in *GRN* develop a lysosomal storage disorder [71]. Since the effect of *TMEM106B* SNPs is most significant in *GRN* mutation carriers, a key area of interest has been to link *TMEM106B* and its associated variants to PGRN biology. The first report linking *TMEM106B* SNPs to PGRN levels was published by Finch et al. who showed that non-demented individuals expressing the protective, minor allele of *TMEM106B* SNPs had significantly higher levels of PGRN in plasma [19]. However, plasma PGRN levels were only found increased by approximately 3% and this data was not replicated by other groups [19, 68]. A link between *TMEM106B* and *GRN* was later obtained using RNA sequencing (RNAseq) data that showed a negative correlation between *TMEM106B* mRNA and *GRN* mRNA levels, likely due to SNP-dependent changes in *GRN* gene methylation [86].

In vitro assessments have also been conducted to determine whether overexpression of TMEM106B (independent of the associated p.T185S variant) affects PGRN levels or localization. Such endeavors determined that both the TMEM106B S185 and T185 isoforms similarly co-localize with PGRN in LAMP-1-positive compartments and significantly induce PGRN upregulation in response to TMEM106B overexpression [9, 52, 66]. However, given the detrimental effect of TMEM106B overexpression on lysosomal function [9, 12, 52, 66, 73], the increase in PGRN expression observed in these experiments is likely a

consequence of global lysosomal dysfunction and not specific to PGRN. While important, studies focused on the effect of TMEM106B levels on the cellular function of PGRN unfortunately have not been performed.

Similar to PGRN, Busch et al. recently assessed whether TMEM106B interacts either directly or indirectly with C9orf72. Data obtained in this study suggested that TMEM106B overexpression was unable to exert toxic lysosomal phenotypes when C9orf72 was not present; thus, siRNA-mediated reduction of C9orf72 expression in HEK293 and HeLa cells rescued TMEM106B-dependent increases in lysosomal size [12]. Additionally, C9orf72 loss rescued TMEM106B-induced changes in lysosomal acidification and subsequent cytotoxicity [12]. While this study suggests a mechanistic link between TMEM106B and C9orf72, it is more difficult to determine how this might translate in patients with *C9orf72* repeat expansions. Based on these findings, one would expect that loss of *C9orf72*, as observed in mutation carriers, would be protective against TMEM106B-related cellular changes. Further study into TMEM106B and C9orf72-related mechanisms is required to fully understand how these proteins might be functionally related and whether relative expression patterns of these two proteins could explain selective protection in *C9orf72* expansion carriers against FTLN but not ALS in homozygous *TMEM106B* minor allele carriers.

A study conducted by Jun et al. aimed to establish a link between TMEM106B and the FTLN-related protein, CHMP2B. Patients harboring FTLN-causing *CHMP2B* mutations are much rarer than those with *GRN* mutations or *C9orf72* expansions [55]. Nonetheless, one feature found in patients with disease-causing CHMP2B mutations is the presence of large vacuolar structures in the brain that label positive for endosomal proteins, suggesting an endosomal-related disease mechanism [31]. Interestingly, Jun and colleagues found endogenous Tmem106b to partially co-localize to both wild-type CHMP2B- and mutant CHMP2B-positive structures in mouse neurons [35], further highlighting a potential role for TMEM106B in the endolysosomal pathway. Moreover, this study showed that T185 TMEM106B more readily binds to CHMP2B than S185 TMEM106B, and that T185 TMEM106B-CHMP2B binding is enhanced with the mutant form of CHMP2B [35]. Also, overexpression of T185 TMEM106B showed a greater reduction in autophagic flux, commonly used to measure autophagic degradation activity, versus the S185 variant of TMEM106B, and this difference was independent of total TMEM106B levels [35]. Whether these CHMP2B-related differences in *TMEM106B* variants contribute to the observed associations in all forms of FTLN-TDP remains to be determined.

Conclusions and future directions

An overwhelming body of evidence has revealed *TMEM106B* variants as significant contributors to one's risk of developing various TDP-43 proteinopathies, both in patients harboring disease-causing mutations, and in subjects with TDP-43 pathology of unknown cause. The major theme connecting all of these studies is the link between TMEM106B and TDP-43, and more than one study has suggested that *TMEM106B* SNPs can modify TDP-43 pathological burden [75, 86]. Therefore, it is of particular interest that no study to date has functionally linked TMEM106B and TDP-43. In fact, mislocalized TDP-43 can be

cleared through the process of lysosomal degradation and autophagy, and improper TDP-43 clearance has been attributed by several groups to be, in part, due to impairments in these cellular processes [8, 74, 81, 83]. Thus, future studies should be executed to elucidate how TMEM106B might be involved in TDP-43 clearance.

Studies performed in various cellular systems has provided evidence suggesting that TMEM106B levels, at least in part, are likely involved in TMEM106B-related risk for TDP-43 proteinopathies. Furthermore, multiple genetic association studies have suggested that the mechanism by which *TMEM106B* SNPs confer disease risk is by mediating TMEM106B levels, either through transcriptional and/or post translational regulation. Therefore, one possible therapeutic avenue that might be administered for patients with TDP-43-related diseases would be to reduce the levels of TMEM106B in the brain. While several studies describe a relationship between TMEM106B and endolysosomal function, it remains unknown as to what TMEM106B actually does in these cellular compartments. Furthermore, little information exists regarding the potential consequences of brain or systemic TMEM106B loss. *Tmem106b* knockout mice have been generated and are currently being used by several groups to better study this gene in the context of aging-related disorders. In fact, these mice have not yet been shown to have gross pathological deficits or a reduced lifespan, suggesting that *Tmem106b* is not critical for survival (unpublished observations, personal communication). However, based on the few publications that have studied TMEM106B reduction, it is possible that complete loss of TMEM106B might affect lysosomal trafficking and/or neuronal development [66, 73] without affecting survival, indicating that partial reduction of TMEM106B levels might have the greatest clinical promise; yet, these analyses have yet to be conducted in a model organism.

In summary, *TMEM106B* is the most significant genetic risk factor for individuals with FTLTDP and related proteinopathies. Since the original discovery six years ago, the field of FTLTDP has gained considerable ground regarding the role of the lysosome in disease pathogenesis. However, research efforts obtained to date have only skimmed the surface of how TMEM106B is related to these disease mechanisms, and more work is urgently needed to evaluate this protein as a promising therapeutic target for patients suffering from these devastating disorders.

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Fig. 1.

TMEM106B genomic region. Schematic representation of the *TMEM106B* genomic region on chromosome 7p21. Introns are drawn in blue while exons are depicted in green, with coding regions drawn taller than non-coding regions. The transcription start site located in exon 3 (E3) is represented by an arrow showing transcriptional direction. Selected FTLTDP-associated SNPs are marked with an asterisk (*) to denote their relative location within the *TMEM106B* locus.

Fig. 2.

TMEM106B is found in the lysosomal membrane of neurons. **(a)** Representation of a neuron and various intracellular compartments, including the lysosome. **(b)** Magnification of the lysosomal membrane in which TMEM106B resides as a type II transmembrane protein, with the relative location of the p.T185S variant and 5 glycosylation sites (N1–N5) depicted in the luminal region. **(c–d)** Immunohistochemical labeling of TMEM106B in human brain shows a punctate TMEM106B-immunoreactive pattern in pyramidal neurons of the hippocampus **(c)** and in granular neurons of the dentate gyrus **(d)**.

Table 1

TMEM106B risk association studies in *GRN* or *C9orf72* mutation carriers

Mutation group	Study	Group 1 (N)	Group 2 (N)	Model	SNP	Minor allele	p-value ^a	Odds Ratio ^b
<i>GRN</i>								
<i>GRN</i>	Van Deerlin et al., 2010	CON (2509)	<i>GRN</i> (89)	Allelic	rs1990622	C	1.34×10 ⁻⁹	0.34
	Finch et al., 2011	CON (822)	<i>GRN</i> (78)	Allelic	rs1990622	C	0.0003	0.51
	Finch et al., 2011	CON (822)	<i>GRN</i> (78)	Additive	rs1990622	C	0.003	0.57
	Finch et al., 2011	CON (822)	<i>GRN</i> (78)	Dominant	rs1990622	C	0.088	0.65
	Finch et al., 2011	CON (822)	<i>GRN</i> (78)	Recessive	rs1990622	C	0.003	0.12
	Nicholson et al., 2013	CON (822)	<i>GRN</i> (29)	Recessive	rs1990622	C	0.03	0.15
	Gallagher et al., 2014	CON (2509)	<i>GRN</i> (116)	Allelic	rs1990622	C	<0.0001	0.37
	Lattante et al., 2014	CON (552)	<i>GRN</i> (76)	Allelic	rs1990622	C	0.0041	0.58
	Lattante et al., 2014	CON (552)	<i>GRN</i> (76)	Recessive	rs1990622	C	9.54×10 ⁻⁶	0
<i>C9orf72</i>								
<i>C9orf72</i>	van Blitterswijk et al., 2014	CON (376)	all <i>C9</i> (260)	Recessive	rs3173615	G	0.014	0.57
	van Blitterswijk et al., 2014	CON (376)	FTD <i>C9</i> (69)	Recessive	rs3173615	G	0.009	0.33
	van Blitterswijk et al., 2014	CON (376)	FTD/ALS <i>C9</i> (71)	Recessive	rs3173615	G	0.017	0.38
	van Blitterswijk et al., 2014	CON (376)	ALS <i>C9</i> (120)	Recessive	rs3173615	G	0.55	0.85
	Gallagher et al., 2014	CON (2509)	FTLD-TDP <i>C9</i> (80)	Allelic	rs1990622	C	0.008	0.64
	Lattante et al., 2014	CON (552)	FTD <i>C9</i> (95)	Allelic	rs1990622	C	0.47	1.12
	Lattante et al., 2014	CON (552)	FTD/ALS <i>C9</i> (50)	Allelic	rs1990622	C	0.28	1.26
	Lattante et al., 2014	CON (552)	all <i>C9</i> (145)	Recessive	rs1990622	C	0.085	1.49
	Lattante et al., 2014	CON (552)	FTD <i>C9</i> (95)	Recessive	rs1990622	C	0.233	1.41
	Lattante et al., 2014	CON (552)	FTD/ALS <i>C9</i> (50)	Recessive	rs1990622	C	0.163	1.67

Results from studies that assessed whether TMEM106B SNPs associate with disease risk in *GRN* or *C9orf72* mutation carriers are summarized. Several publications include analyses of more than one SNP; thus, we summarize the findings of rs1990622 when available.

^aReported p-values for the statistical models are not corrected for multiple testing.

^bOdds ratios are given with respect to the minor allele.

GRN: progranulin mutation carriers, *C9orf72*: chromosome 9 open reading frame 72 expansion carriers, N: number of individuals, FTD: frontotemporal dementia, FTLD-TDP: frontotemporal dementia lobar degeneration with TAR DNA binding protein 43 pathology, ALS: amyotrophic lateral sclerosis, SNP: single nucleotide polymorphism.