Progranulin and TMEM106B: when two become wan

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Mutations in *GRN*, which encodes progranulin, are a common cause of familial frontotemporal dementia (FTD). FTD is a devastating disease characterised by neuronal loss in the frontal and temporal lobes that leads to changes in personality, behaviour and language. There are no effective treatments for this complex condition. *TMEM106B* is a well-recognised risk factor for FTD caused by *GRN* mutation. While the specific relationship between progranulin and TMEM106B is unclear, it is well established that they are both required for correct lysosome function and trafficking. Elegant experiments have suggested that increased risk is due to elevated levels of TMEM106B (Gallagher *et al.*, 2017;Nicholson *et al.*, 2013). Therefore, recent work has explored the therapeutic potential of reducing TMEM106B levels, with initial results looking encouraging, as crossing a *Grn* deficient mouse to a *Tmem106b* knockout showed a rescue in FTD-related behavioural defects and specific aspects of lysosome dysfunction (Klein *et al.*, 2017). However, three independent studies in this issue report that completely removing *Tmem106b* from *Grn* knockout mice leads to clear exacerbation of phenotypes, causing severe motor deficits, neurodegeneration and enhanced lysosome abnormalities and gliosis. Remarkably, the double knockout mice also develop TDP-43 pathology – a hallmark of FTD patients with *GRN* mutations that has not been consistently observed in either of the single knockouts. These concurrent publications, which all reach the same surprising but definitive conclusion, are a cautionary tale in careful control of TMEM106B levels as a potential therapeutic for FTD. They also re-ignite the debate as to whether loss, or gain of TMEM106B function is critical for altering FTD risk.

Hu et al used CRISPR/Cas9 to remove *Tmem106b* in their double cross. The resultant ataxia and hindlimb phenotypes were severe and reached experiment endpoint by 5.5 months. Defects were most pronounced in the spinal cord, with a decrease in neurons and marked gliosis. At 5 months old these mice exhibited enlarged lysosomal vacuoles at the axon initial segment of motor neurons. Werner et al report strikingly similar results, with severe motor defects at 4-5 months of age in their double knockouts. Zhou et al reported progressive motor defects in their mouse models at the slightly later age of 8 months. However their initial model retained 5–10 % Tmem106b protein; when they used CRISPR to completely ablate *Tmem106b*, onset of motor deficits was significantly advanced. Like the Hu paper, Zhou et al report significant loss of motor neurons at 12 months, and in addition a reduction in myelin density, with disorganised myelin fibers and degenerated myelin debris. All papers report significantly enhanced gliosis in the double knockouts, as well as the presence of phosphorylated TDP-43. These reports clearly show a significant exacerbation of phenotype in $Grn^{-/-}$ mice upon removal of *Tmem106b*.

Why these seemingly disparate results between the original double knockout data from Klein et al and these new papers? Well on closer inspection these conclusions are not entirely different- Klein et al reported that accumulation of autofluorescent material – a sign of impaired lysosome function, was not rescued, and neither was gliosis. However they did show correction of the increased levels and activity of lysosomal proteins observed in $Grn^{-/-}$ single knockouts, phenotypes that were exacerbated in these new reports. An early rescue, at 4 months of age, of two behavioural phenotypes (hyperactivity and disinhibition) was also reported. Interestingly, the Zhou et al paper used the same *Tmem106b* knockout model as Klein et al. A key piece of information in the Zhou paper may help explain the different phenotypic reports from the different labs. Zhou et al show here that this model is an imperfect knockout of *Tmem106b*, with 5-10 % protein levels remaining in the mice. It is abundantly clear from these new papers that neurons are delicately tuned to the correct levels of Tmem106b. The residual levels of Tmem106b in the cortex may have been enough to correct the behavioural phenotype at 4 months of age, prior to the age-related motor deficits first

observed at 6 months of age in the Zhou paper; although undoubtedly the long-term consequence of loss of the majority of Tmem106b is detrimental.

Does this mean that Tmem106b manipulation no longer harbours viable therapeutic potential for disease? Not necessarily. There are several caveats that preclude drawing this conclusion. Although the homozygous *Grn* knockout mouse model is commonly used as a model of FTD, it is not a completely accurate genetic model. In humans, haploinsufficiency due to heterozygous loss of function of *GRN* drives disease, while rare homozygous mutations instead result in lysosomal storage disorder. Therefore effects may be exaggerated in *Grn* homozygous knockout mice. A more genetically accurate model of partial reduction of *Tmem106b* in a heterozygous *Grn* knockout mouse has already been reported, and appeared neutral, as neither rescue nor exacerbation of social deficits or lysosomal abnormalities was observed (Arrant *et al.*, 2018). This highlights that partial loss of Tmem106b is likely to have distinct effects to its complete ablation. Partial reduction of Tmem106b using antisense oligonucleotides in primary cortical neuronal cultures rescued in vitro lysosomal trafficking defects caused by a rare FTD-causing mutation in CHMP2B (Clayton *et al.*, 2018), indicating partial reduction can be benefical in some contexts.

A striking result from all three reports is that complete loss of *Tmem106b* leads to the accumulation of phosphorylated TDP-43, as it has been a long-standing puzzle as to why even homozygous *Grn* knockout mice do not reliably develop TDP-43 inclusions, even though they are present in all *Grn* mutation patients. This raises the possibility that loss of function of TMEM106B could contribute to FTD risk, rather than the gain of function previously suggested. However, opposing this argument, overexpression of TMEM106B in $Grn^{-/-}$ mice also enhances lysosomal abnormalities in aged mice (Zhou *et al.*, 2017), indicating that either too little or too much TMEM106B is detrimental; the effects of the risk allele may then be cell-type specific, or modify specific lysosomal functions of TMEM106B in addition to changing its levels. Intriguingly, another just published paper reports the accumlation of cytoplasmic TDP-43 in *Grn* homozyogus knockout mouse thalamic neurons from 12 months of

age, that could be reduced by blocking microglial-derived complement (Zhang *et al.*, 2020). The role of TMEM106B was not investigated, but given the enhanced microgliosis in $Grn^{-/-}$;$Tmem106b^{-/-}$ double knockouts, the exacerbated TDP-43 pathology that is also observed could be driven at least in part by this mechanism.

The most striking neuronal defects reported herein were observed in motor neurons- thus more closely resembling a motor neuron disease phenotype, even though *GRN* mutations specifically cause FTD and not ALS. Motor neurons contain extraordinarily long axons, which may render them more susceptible to subtle perturbation in Tmem106b levels, given the known involvement of Tmem106b in endolysosomal trafficking (Schwenk *et al.*, 2014). Indeed Tmem106b deficient mice were recently reported to accumulate LAMP-1 positive vacuoles at the axon initial segment (Lüningschrör *et al.*, 2020), while rare human mutations in *TMEM106B* cause a hypomyelination disorder (Simons *et al.*, 2017).

These new papers prove conclusively that TMEM106B levels need tight spatiotemporal regulation. The robust defects in motor neurons indicate that specific subsets of neurons in the brain may be differentially sensitive to loss of *GRN* and *TMEM106B*. Whilst cortical neurons are potentially susceptible to excess TMEM106B (leading to FTD), it appears motor neurons are more susceptible to too little of the protein (leading to motor deficits). Further studies addressing these issues are needed to tease apart the cost benefit analysis of regional modulation of TMEM106B levels in FTD.

Figure Legend

Removing Tmem106b from progranulin null mice exacerbates disease phenotype. 3 publications show that removing the FTD risk factor Tmem106b from FTD model granulin knockout mice severely worsens phenotype. By 4 months, compared to the single knockouts, motor neurons from double knockout mice show exacerbated lysosomal abnormalities, with enlarged lysosomes clustering at the

axon initial segment, ubiquitinated protein deposits, phosphorylated TDP-43 accumulation and enhanced gliosis. This pathology results in severe motor deficits in the double knockouts.

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