One Transgene, Two Myopathies: Investigating the Possibility of an MTM1 'Cross Gene Therapy' for BIN1 Deficiency

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This scientific commentary refers to 'MTM1 Overexpression Prevents and Reverts BIN1-Related Centronuclear Myopathy' by Giraud *et al.* (https://doi.org/10.1093/brain/awad251)

Centronuclear myopathies (CNMs) are monogenic myotubule disorders characterized by muscular atrophy and hypotonia. CNMs can be caused by mutations in multiple genes, including *MTM1*, *BIN1* and *DNM2*, which encode membrane-associated proteins implicated in the regulation of transverse tubules (T-tubules) in muscle fibers. Indeed, histopathological hallmarks of CNMs include aberrations in T-tubule formation, as well as in triadic assembly, organelle and nuclear localization, endosomal recycling, and excitation-contraction coupling. Gene therapy has been investigated for the most common form of CNM, X-linked myotubular myopathy (XLMTM). In this issue of Brain, Giraud and colleagues present an interesting preclinical study in which *MTM1* gene therapy is investigated as a potential treatment for *BIN1* related CNM, using relevant mouse models [1]. This work highlights an intriguing relationship between these important players in muscle cell biology, as well as raising the prospect of applying a single gene therapy product to more than one form of CNM.

The gene products of *MTM1*, *BIN1*, and *DNM2* are known to have important roles in muscle fibers. *MTM1* encodes the endosomal lipid phosphatase myotubularin 1 (MTM1) which modulates membrane trafficking by regulating phosphoinositide (PI) dephosphorylation and contributes to the structural organization of muscle fibers. *BIN1* is implicated in autosomal-recessive CNM and encodes bridging integrator-1 (BIN1), a structural protein involved in membrane trafficking, T-tubule organization, and endosomal recycling. *DNM2* encodes Dynamin-2 (DNM2), a GTPase mechanoenzyme that regulates actin filament assembly, as well as the formation of endosomes and autophagosomes [2].

Given the importance of interactions between MTM1, BIN1, and DNM2, Giraud *et al* investigated the possibility of whether adeno-associated virus (AAV) mediated overexpression of MTM1 could correct aberrations in skeletal muscle function and structure in mouse models of BIN1 or DNM2 deficiency. This strategy was unable to rescue a DNM2-deficient mouse model. This follows previously published work from the same group, who reported that DNM2 expression can have a surprising therapeutic effect in mice lacking MTM1 or BIN1 expression [3,4].

However, the authors did find that AAV-MTM1 gene therapy exhibited therapeutic potential for treatment of BIN1-deficient mice. The animal model used was the *Bin1*$^{mck-/-}$ mouse, which presents the skeletal hallmarks of BIN1-related CNM starting from three weeks of age [4]. The vector

employed was based on AAV serotype 9 (AAV9). Intraperitoneal delivery of AAV-MTM1 to neonatal *Bin1*$^{mck-/-}$ mice restored skeletal muscle force and function as assessed by hanging time, spontaneous activity, quadriceps and tibialis anterior atrophy, specific maximal force, and force-frequency response. Furthermore, it corrected ultrastructural myofiber organization in terms of myofiber hypotrophy, mitochondrial localization, sarcomere organization, and T-tubule depth and number per sarcomere. Intervention at week 8, in the form of tibialis anterior intramuscular injection, also ameliorated the phenotype, albeit to a lower extent than neonatal intraperitoneal injection.

Interestingly, work from Lionello *et al* previously found that AAV-mediated BIN1 overexpression can ameliorate an MTM1-deficient model [5]. This highlights an intriguing synergistic relationship between these proteins in muscle fibers, where overexpression of one can potentially compensate for absence of the other. A collection of research outputs from the Laporte group has revealed how these proteins maintain membrane trafficking, T-tubule structure, contractability and autophagy in muscle fibers [2–5]. This compensatory relationship between MTM1 and BIN1 suggests they have convergent roles in skeletal muscle physiology (Figure 1), which will undoubtedly be a focus of further investigations. The proposition of a 'cross therapy' approach, in which AAV-MTM1 gene therapy is used to treat BIN1-CNM, is particularly intriguing and represents a gene therapy paradigm in which modifier genes can be overexpressed to mediate functional reconstitution of a different gene. This approach has been explored in other contexts, for example Li *et al* demonstrated the efficacy of a single gene therapy, AAV8-*Nr2e3*, in ameliorating retinal degeneration in five models of retinitis pigmentosa [6].

It is important to note that a clinical trial of gene therapy for XLMTM (ASPIRO NCT03199469), was recently halted due to fatalities in patients receiving high doses of AAV vector (>$3x10^{14}$ gc/kg), owing to severe hepatotoxicity [7]. AAV doses tested by Giraud *et al* were reported as $4.9x10^{11}$ gc per neonatal mouse, which translates to a similar dose used in the clinical study, when accounting for the typical weight of neonatal mice (~$3.3x10^{14}$ gc/kg for 1.5g mouse). Toxicity studies included in the paper did not find any significant alterations in potential biomarkers of liver toxicity. Caution, however, is still warranted, as AAV-MTM1 was not found to induce any serious adverse effects in preclinical studies in canine, murine and non-human primate models at doses up to $8x10^{14}$ gc/kg [8]. The pathophysiologic cause of hepatotoxicity in the ASPIRO trial is still being investigated. Proposed mechanisms include the possibility that pre-existing XLMTM-related hepatobiliary complications may have predisposed patients to hepatotoxicity [7,8]. Giraud *et al* highlight that BIN1 deficiency is not associated with hepatic manifestations, in which case the risk of adverse events may not be directly comparable to XLMTM.

Further distinction between this study and XLMTM trials lies in the choice of vector capsid. Giraud *et al* employed AAV9 for MTM1 delivery, whereas the ASPIRO trial utilizes AAV8. Although both AAV8 and AAV9 are capable of transducing skeletal muscle following systemic delivery, their biodistribution is likely to be distinct. However, for both capsids, large doses are generally required to achieve therapeutic skeletal muscle transduction, which consequently results in significant liver transduction thereby increasing the risk of hepatotoxicity. To combat this, in future trials it may be interesting to investigate use of muscle-targeted capsids, such as MyoAAV which may allow reduced systemic doses with liver de-targeting [9].

Beyond translational considerations relating to viral vector toxicity, further work may be required to validate the benefit of an AAV-MTM1 gene therapy for BIN1-CNM. For example, the mouse model used in the current study has *Bin1* knocked out specifically in skeletal muscles, and thus may not fully represent the pathophysiology of a patient with bodywide loss of function mutations.

The authors opted to use this model because constitutive *Bin1* knockout is associated with perinatal fatality, thereby precluding meaningful experimentation. The resulting limitation however is that AAV-MTM1 cannot be assessed for multisystemic rectification of the BIN1-CNM phenotype. Furthermore, the study lacks a direct comparison of AAV-MTM1 versus AAV-BIN1 gene therapy. Such evaluation could be relevant as MTM1 overexpression neither completely corrected CAV3 levels nor affected mTOR phosphorylation.

In conclusion, this work highlights MTM1 as an important player in myology, capable of influencing various cellular metabolic processes via its enzymatic activity and structural interactions. The intriguing relationship with BIN1 seems to suggest that overexpression of either protein could compensate for lack of the other, whereas the other player in this group, DNM2, seems to have a more complex relationship. The possibility of repurposing AAV-MTM1 to treat BIN1-CNM warrants further investigation, particularly if there is scope for accelerated deployment of the gene therapy already in trials, applying it to an orphan indication with severely limited treatment options. It is crucial however to exercise caution in advancing this approach whilst specific mechanisms for adverse events in MTM1 gene therapy trials remain to be elucidated.

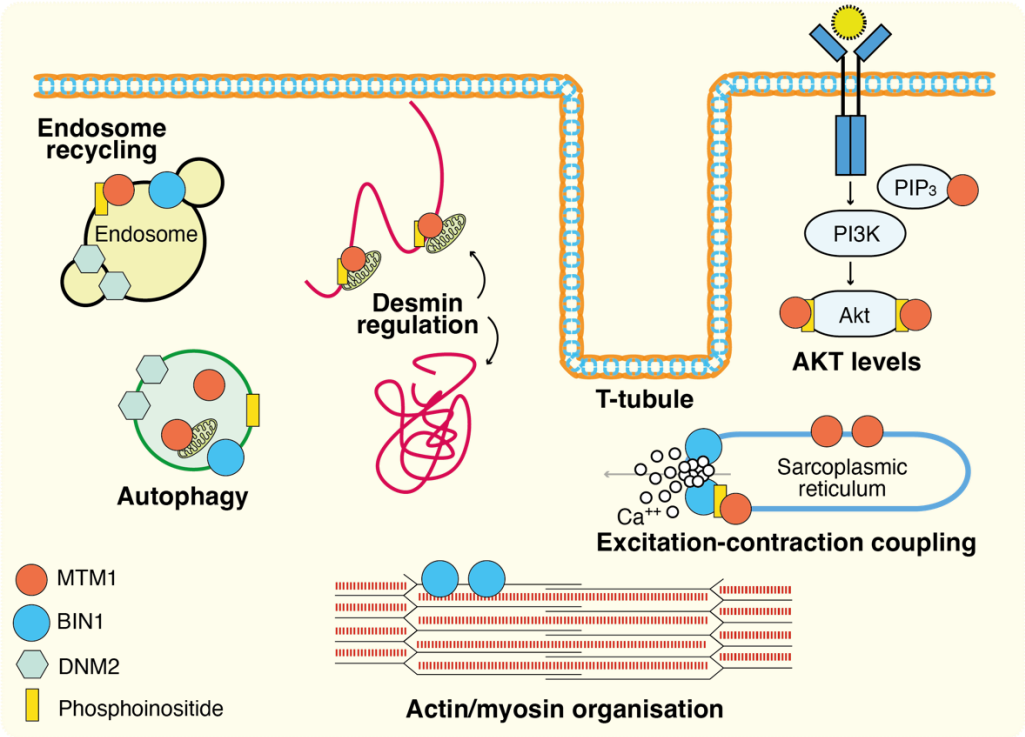

Figure 1. A schematic summarizing muscle functions relevant to the potential compensatory relationship between MTM1 and BIN1. Several aspects of muscle cell biology could potentially be compensated by overexpression of MTM1 or BIN1 [2]. **Excitation-contraction coupling:** BIN1 deficiency is associated with aberrations in T-tubule formation, impaired DHPR current and SR calcium release. MTM1 directly binds BIN1 to enhance its membrane tubulation functionality. It also stabilizes DHPR and RyR1 assembly, thereby enhancing SR calcium release. **Actin regulation:** BIN1 is known to directly bind actin and recruit such actin-regulating proteins as N-WASP, so actin dynamics can be compromised in BIN1-CNM. MTM1 is thought to affect actin

remodelling and could to some extent correct actin-regulator recruitment. **Desmin regulation:** Desmin localization is distorted in BIN1-CNM. MTM1 plays a structural role in restoring desmin assembly, which in turn helps restore organelle localization. **Endosome Recycling:** BIN1 interacts with various endosomal markers. MTM1 enzymatically controls phosphoinositide levels, which are key to regulating membrane recycling and integrin localization. **Autophagy:** BIN1 deficiency is associated with autophagosome accumulation. MTM1 enzymatically controls phosphoinositide levels, which in turn are key regulators of autophagosome dynamics. **AKT Levels:** AKT levels are upregulated to counter hypertrophy in BIN1-defieicny. MTM1 affects phosphoinositide levels, which can directly modulate AKT levels.

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