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Commentary

# Toward a translational gene therapy for mucolipidosis IV

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A study by Sangster et al., published in the June 2024 issue of *Molecular Therapy Methods and Clinical Development*, describes a novel gene therapy approach for the rare disorder mucolipidosis IV (MLIV), where they evaluated systemic delivery of a newer-generation adeno-associated virus (AAV) capsid, AAV-CPP16.[1,2] The authors' prior work demonstrated the therapeutic potential of AAV9-based *MCOLN1* gene transfer in the mouse model, although translation to the clinic was limited due to the vector properties.[3] An effective patient treatment would necessitate delivery to deep brain regions, so in their new study, Sangster et al. optimized delivery to the brain using a recently described vector (AAV.CPP.16) that is capable of crossing the blood-brain barrier (BBB) across multiple species.[1]

MLIV is a rare pediatric autosomal recessive neurological disorder caused by loss-of-function mutations in the *MCOLN1* gene.[4] *MCOLN1* encodes a lysosomal cation channel, transient receptor potential mucolipin 1 (TRPML1), which mediates lysosomal calcium trafficking, lysosomal fusion and fission, iron homeostasis, and exocytosis.[5] Loss of TRPML1 *in vitro* leads to lysosomal substrate accumulation, and impaired lipid transport is observed in fibroblasts from patients with MLIV. Several loss-of-function mutations have been identified within the *MCOLN1* gene, which contribute to disease severity and age of onset. Affected children are reported to have corneal clouding and developmental delays in their first year of life.[6] Motor dysfunction, such as hypotonia and pyramidal and extrapyramidal symptoms, leads to an inability to self-ambulate. White matter abnormalities and symptom progression are associated with blindness and subcortical volume loss within the second decade of life.[6] Life expectancy in patients with MLIV is reduced due to secondary organ failure, which occurs during adolescence and up to their fourth decade of life. MLIV has an extremely high unmet need, and there have been no investigational clinical trials for this patient population yet.

Previously, this group evaluated systemic AAV-PHP.B as well as systemic and cerebrospinal fluid (CSF)-directed AAV9-mediated MCOLN1 delivery in an MLIV mouse model.[3] AAV-PHP.B is an engineered variant of AAV9 that can result in 40-fold greater CNS transduction in certain mouse strains than AAV9.[7] The *Mcoln1* knockout mouse (*Mcoln1*$^{-/-}$) develops motor, neurological, and visual impairments and early lethality, similar to patients with MLIV.[7] When the *MCOLN1* coding sequence was systemically transferred using 1e12 vg ssAAV-CMV-PHP.B, DeRosa et al. showed full reversal of neurological dysfunction when administered to symptomatic 2-month-old *MLIV*$^{-/-}$ mice. Additionally, they found that intra-cerebrovascular injection of 2e10 vg scAAV9-JeT-MCOLN1 in neonatal pups (PND1) facilitated wide brain transduction of MCOLN1 and was also efficacious. However, when 5e11 vg scAAV9-JeT-MCOLN1 was delivered by tail vein injection at 2 months of age, it was insufficient to alleviate the motor deficits of *Mcoln1*$^{-/-}$ mice, and AAV9 transduction was very low in the central nervous system (CNS). While the results from this prior work were promising, AAV-PHP.B's enhanced ability to cross the BBB does not translate to other species, including non-human primates, limiting its clinical application.[8] The authors also concluded that while AAV9-mediated *MCOLN1* gene transfer was a potentially viable approach for treating young patients with MLIV, AAV9 delivery in older patients was unlikely to provide sufficient benefit. These studies demonstrated the potential for providing a working copy of *MCOLN1* to alter the course of MLIV when administered as an early preventative or after the onset of symptoms in a mouse model of MLIV but underscore that therapeutic benefit later in the disease course was contingent upon efficient CNS delivery.[3]

In their new study, to achieve efficient and broad distribution of their vector, Sangster et al. assessed treatment of adult *Mcoln1*$^{-/-}$ mice using an alternative AAV9 variant, the AAV.CPP.16 capsid.[1] Systemic delivery is an attractive route for achieving broad distribution of AAV vectors in the brain and spinal cord because of the high density of vasculature in the CNS, and AAV9 can cross the BBB. Using intravenous (i.v.) administration in 2-month-old *Mcoln1*$^{-/-}$ mice, an age relevant to clinical diagnosis following symptom onset in patients,[7] the authors demonstrate that AAV.CPP.16 has higher CNS penetrance compared to AAV9 and improved delivery of MCOLN1 using either 5e11 or 1e12 vg scAAV-CPP.16-MCOLN1 via tail vein injection. Both doses used in this study were able to significantly increase survival. Additionally, the high-dose-MCOLN1-treated group exhibited improvement in motor function and did not develop hindlimb paralysis distinguishable from their wild-type (WT) littermates at the end of the study. Markers of improved lysosomal function were also observed in MCOLN1-treated mice. Decreased aggregation of LAMP1 was observed within lysosomes of the treated mice along with amelioration of lysosomal protein expression. Although sex differences have not been previously reported in MLIV, female treated mice had significant improvement in motor function and lower RNA markers of glial activation than male mice. Overall, Sangster et al. provided evidence of a gene therapy approach to treat neurological deficits due to MLIV in adult

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animals using i.v. delivery of a newly generated AAV9 variant, the AAV.CPP.16 capsid.

While promising, several caveats to this study remain to be addressed. Despite being the first study to report that systemic administration of the CPP16 capsid facilitates *MCOLN1* gene transduction in the retina, the eye phenotype was not ameliorated in *Mcoln1*$^{-/-}$ mice[2]. *Mcoln1*$^{-/-}$ mice present retinal thinning along with decreased rhodopsin and impaired rod cell function within the first month of life,[9] and treatment with AAV.CPP.16-MCOLN1 did not improve retinal thickness. The authors note that treatment at 2 months in *Mcoln1*$^{-/-}$ mice may not be an appropriate interventional time point, as retinal thinning occurs at 1 month. These findings warrant further study given that progressive vision loss across two decades is a primary clinical phenotype,[6] which is different from the acute vision loss present in *Mcoln1*$^{-/-}$ mice. The authors also reported sex differences in the rescue of vertical motor activity and glial responses to AAV.CPP.16-MCOLN1 therapy, but those findings are difficult to interpret due to differences in the ages and size of the cohorts used in this study. Female mice were reported for acute, 2-month post-injection outcomes ($n = 12$), whereas longitudinal and dose-dependent outcomes are reported in smaller cohorts of male mice ($n = 4–6$). Future studies are needed to determine if the observed sex differences are in response to treatment and by which mechanism or if they are due to shortcomings of the experimental design. Finally, safety and toxicology were not evaluated in the current study. These will need to be addressed to evaluate the full potential of clinical efficacy, as only the higher dose (1e12 vg, corresponding to ~5e13 vg/kg) demonstrated prevention of neuromuscular impairments. Adverse reactions have been reported in clinical trials using other AAV vectors at high doses,[10] although it remains to be determined if AAV.CPP.16 elicits similar outcomes.

Overall, the study by Sangster et al. provides an assessment of a newly engineered AAV vector that appears superior to AAV9 in the context of a disease model. In addition to reporting promising outcomes for the treatment of MLIV, these findings provide evidence that systemic gene delivery via the AAV.CPP.16 capsid to target neurological disorders may be a viable approach. It is encouraging to note that the authors were able to achieve widespread, effective gene transduction throughout the CNS with lower expression in peripheral tissues than is observed with AAV9.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## REFERENCES

1. Yao, Y., Wang, J., Liu, Y., Qu, Y., Wang, K., Zhang, Y., Chang, Y., Yang, Z., Wan, J., Liu, J., et al. (2022). Variants of the adeno-associated virus serotype 9 with enhanced penetration of the blood-brain barrier in rodents and primates. Nat. Biomed. Eng. *6*, 1257–1271. https://doi.org/10.1038/s41551-022-00938-7.

2. Sangster, M.L., Bishop, M.M., Yao, Y., Feitor, J.F., Shahriar, S., Miller, M.E., Chekuri, A.K., Budnik, B., Bei, F., and Grishchuk, Y. (2024). A blood-brain barrier-penetrant AAV gene therapy improves neurological function in symptomatic mucolipidosis IV mice. Mol. Ther. Methods Clin. Dev. *32*, 101269. https://doi.org/10.1016/j.omtm.2024.101269.

3. DeRosa, S., Salani, M., Smith, S., Sangster, M., Miller-Browne, V., Wassmer, S., Xiao, R., Vandenberghe, L., Slaugenhaupt, S., Misko, A., and Grishchuk, Y. (2021). MCOLN1 gene therapy corrects neurologic dysfunction in the mouse model of mucolipidosis IV. Hum. Mol. Genet. *30*, 908–922. https://doi.org/10.1093/hmg/ddab093.

4. Bargal, R., Avidan, N., Ben-Asher, E., Olender, Z., Zeigler, M., Frumkin, A., Raas-Rothschild, A., Glusman, G., Lancet, D., and Bach, G. (2000). Identification of the gene causing mucolipidosis type IV. Nat. Genet. *26*, 118–123. https://doi.org/10.1038/79095.

5. Di Paola, S., Scotto-Rosato, A., and Medina, D.L. (2018). TRPML1: The Ca(2+)retaker of the lysosome. Cell Calcium *69*, 112–121. https://doi.org/10.1016/j.ceca.2017.06.006.

6. Frei, K.P., Patronas, N.J., Crutchfield, K.E., Altarescu, G., and Schiffmann, R. (1998). Mucolipidosis type IV. Neurology *51*, 565–569. https://doi.org/10.1212/WNL.51.2.565.

7. Deverman, B.E., Pravdo, P.L., Simpson, B.P., Kumar, S.R., Chan, K.Y., Banerjee, A., Wu, W.L., Yang, B., Huber, N., Pasca, S.P., and Gradinaru, V. (2016). Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. Nat. Biotechnol. *34*, 204–209. https://doi.org/10.1038/nbt.3440.

8. Hordeaux, J., Wang, Q., Katz, N., Buza, E.L., Bell, P., and Wilson, J.M. (2018). The Neurotropic Properties of AAV-PHP.B Are Limited to C57BL/6J Mice. Mol. Ther. *26*, 664–668. https://doi.org/10.1016/j.ymthe.2018.01.018.

9. Grishchuk, Y., Stember, K.G., Matsunaga, A., Olivares, A.M., Cruz, N.M., King, V.E., Humphrey, D.M., Wang, S.L., Muzikansky, A., Betensky, R.A., et al. (2016). Retinal Dystrophy and Optic Nerve Pathology in the Mouse Model of Mucolipidosis IV. Am. J. Pathol. *186*, 199–209. https://doi.org/10.1016/j.ajpath.2015.09.017.

10. Wang, J.H., Gessler, D.J., Zhan, W., Gallagher, T.L., and Gao, G. (2024). Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduct. Target. Ther. *9*, 78. https://doi.org/10.1038/s41392-024-01780-w.