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Editorial

# Gene therapy for Friedreich ataxia: Too much, too little, or just right?

Friedreich ataxia (FA) is a progressive and devastating disease in which expression of frataxin (FXN) is severely decreased.[1] Frataxin is an ancient and highly conserved protein that is targeted to mitochondria, where it participates in iron-sulfur (Fe-S) cluster formation through binding to a multi-protein core complex.[2] Now, 25 years after identification of the gene defect,[3] the field is moving closer to understanding exactly what FXN does and developing a therapy. Two points are noteworthy from a therapeutic standpoint: (1) FA is a monogenetic disease in which there is low expression of a key protein. There is nothing wrong with the protein itself, i.e., there is not a dominant-negative mutation in the encoded protein, there just is not enough of it. (2) Frataxin is targeted to the mitochondrial matrix, which is one of the most inaccessible organelles within the cell. Although mitochondrial disorders as a class are common, there still are few or no effective therapies for mitochondrial defects. Thus, developing treatments for FA will advance our understanding of how to treat other mitochondrial disorders. This is an important goal.

Early experiments have shown that viral gene therapies to deliver a human FXN gene are a promising approach but may have toxicities.[4–6] In a series of interesting and thoughtful experiments published in *Molecular Therapy: Methods & Clinical Development*, Huichalaf et al.[7] have sought to determine expression limits for FXN toxicity and, more importantly, the mechanism(s) of this toxicity. The authors show that overexpression of native human FXN using an AAV9 vector (AAV9-CAG-FXN) in the MCK-Cre conditional ablation FA mouse model (loss of FXN in heart and skeletal muscle) is not only toxic to heart, a key target organ in FA, but is also toxic to liver, which is an off-target organ. Progressive overexpression of human FXN in the ablation model resulted only in partial and transient correction of cardiac dysfunction. Because AAV9 has tropism for liver, the authors examined hepatic function in these mice. Hepatic toxicity developed rapidly at higher doses, as evidenced by histology and loss of specific Fe-S-cluster-dependent proteins in mitochondria. Importantly, liver toxicity was moderated by accelerated regeneration in liver[8] with loss of hepatocytes containing the AAV-CAG-FXN construct. They concluded that liver dysfunction was the most likely cause for weight loss and premature death in the mice.

Next, the authors overexpressed FXN in wild-type mice using the same AAV9-CAG-FXN construct. They compared these results against a vector containing a known pathogenic mutation, AAV9-CAG-FXN(N146K), that renders the FXN molecule unable to bind to the Fe-S biogenesis core complex. Surprisingly, the authors found that overexpression of the human FXN protein was not toxic in wild-type mouse heart but again was toxic to liver. Possible explanations for this were not clear. Overexpression of the FXN(N146K) mutation was not toxic in either heart or liver, even though expression levels were similar to the therapeutic construct. Thus, one mechanism of toxicity requires binding of FXN to the Fe-S core complex. This experiment also showed that toxicity in the mouse was not due to sequence differences between mouse and human FXN, nor was it due to toxicity from the AAV9 vector itself.

The authors reasonably concluded that overexpression of FXN can result in Fe-S cluster deficit in the same way that FXN depletion does. From the standpoint of therapeutic development, there are several take-home points from this work. First, there appears to be a range of FXN expression that is therapeutic. Based on published data, this range seems to be from ∼20% of normal expression to ∼8-fold overexpression, but admittedly, these are early data with little long-term follow-up.[5,9] Second, there are differences in sensitivity of heart versus liver to overexpression of FXN. This suggests that tissues and cells with high metabolic needs and high mitochondrial count will have a different requirement and sensitivity to FXN expression compared with tissues having lower mitochondrial count and/or metabolism. Third, off-target expression of FXN can and will be toxic. Here, liver was damaged by overexpression of FXN, but it is unknown whether other organ systems were affected. Although the liver showed recovery with regeneration, this may also increase the risk of secondary disease, such as cancer or cirrhosis, over time. Finally, unlike the mouse model, the human disease results from low expression, but not complete loss, of FXN. Defining minimal expression levels for normal cell function in different tissues, including brain, will be needed to develop effective therapies. These experiments are an important contribution to the field of FA, because they define toxicity of FXN overexpression and, more importantly, identify mechanisms underlying this toxicity.

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## DECLARATION OF INTERESTS

R.M.P. is a paid consultant for Larimar Therapeutics, Inc., which had no input or knowledge of this work.

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## REFERENCES

1. Pandolfo, M. (2009). Friedreich ataxia: the clinical picture. J. Neurol. *256*, 3–8. https://doi.org/10.1007/s00415-009-1002-3.

2. Fox, N.G., Yu, X., Feng, X., Bailey, H.J., Martelli, A., Nabhan, J.F., Strain-Damerell, C., Bulawa, C., Yue, W.W., and Han, S. (2019). Structure of the human frataxin-bound iron-sulfur cluster assembly complex provides insight into its activation mechanism. Nat. Commun. *10*, 2210. https://doi.org/10.1038/s41467-019-09989-y.

3. Campuzano, V., Montermini, L., Molto, M.D., Pianese, L., Cossee, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F., Monticelli, A., et al. (1996). Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science *271*, 1423–1427.

4. Perdomini, M., Belbellaa, B., Monassier, L., Reutenauer, L., Messaddeq, N., Cartier, N., Crystal, R.G., Aubourg, P., and Puccio, H. (2014). Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. Nat. Med. *20*, 542–547. https://doi.org/10.1038/nm.3510.

5. Belbellaa, B., Reutenauer, L., Monassier, L., and Puccio, H. (2019). Correction of half the cardiomyocytes fully rescue Friedreich ataxia mitochondrial cardiomyopathy through cell-autonomous mechanisms. Hum. Mol. Genet. *28*, 1274–1285. https://doi.org/10.1093/hmg/ddy427.

6. Belbellaa, B., Reutenauer, L., Messaddeq, N., Monassier, L., and Puccio, H. (2020). High levels of frataxin overexpression lead to mitochondrial and cardiac toxicity in mouse models. Mol. Ther. Methods Clin. Dev. *19*, 120–138. https://doi.org/10.1016/j.omtm.2020.08.018.

7. Huichalaf, C., Perfitt, T.L., Kuperman, A., Gooch, R., Kovi, R.C., Brenneman, K.A., Chen, X., Hirenallur-Shanthappa, D., Ma, T., Assaf, B.T., et al. (2022). In vivo overexpression of frataxin causes toxicity mediated by iron-sulfur cluster deficiency. Mol. Ther. Methods Clin. Dev. https://doi.org/10.1016/j.omtm.2022.02.002.

8. Malato, Y., Naqvi, S., Schurmann, N., Ng, R., Wang, B., Zape, J., Kay, M.A., Grimm, D., and Willenbring, H. (2011). Fate tracing of mature hepatocytes in mouse liver homeostasis and regeneration. J. Clin. Invest. *121*, 4850–4860. https://doi.org/10.1172/JCI59261.

9. Vasquez-Trincado, C., Patel, M., Sivaramakrishnan, A., Bekeova, C., Anderson-Pullinger, L., Wang, N., Tang, H.Y., and Seifert, E.L. (2021). Adaptation of the heart to Frataxin depletion: evidence that integrated stress response can predominate over mTORC1 activation. Hum. Mol. Genet. https://doi.org/10.1093/hmg/ddab216.