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Commentary

Edging closer to successful gene therapy for Wilson disease

Thomas Damgaard Sandahl[1] and Valentina Medici[2]

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In a recent article in *Molecular Therapy – Methods and Clinical Development*, Padula and co-authors present pre-clinical data for a new approach to gene therapy in Wilson disease (WD).[1] By using dual adeno-associated viral (AAV) vectors combined with split-intein technology to catalyze ligation of two separate peptides, the authors manage to transfect a full-size ATP7B protein to a mouse model of WD. The pre-clinical data from the present study are encouraging and pave the way for future clinical studies.

WD is an autosomal recessive disorder caused by *ATP7B* gene mutations leading to pathologic accumulation of copper in the liver and brain.[2] While current treatments are generally effective, WD is still associated with considerable morbidity, and patients must be compliant with a long-term and strict medication regimen.[2] After decades of minimal progress in the development of new treatments, the tide has changed. In recent years, advances in both diagnostic procedures, basic and pre-clinical science, and drug discovery have led to promising advances in the field with the progress to clinical trials of new therapeutic approaches.[3,4] One of the most exciting outcomes has been the development of gene therapy where vector-based delivery of *ATP7B* variants has proven efficient in mice and is currently moving forward to clinical application in patients with WD (ClinicalTrials.gov: NCT04537377 and NCT04884815). Currently, one of the challenges with AAV-vector-based gene therapy is the limited amount of genetic information that can be packed into the virus vector, which in turn limits the size of the transfected protein. In the case of WD, the *ATP7B* gene is too large for optimal AAV vector delivery.[1] Therefore, the technologies currently under investigation in clinical trials have addressed this problem by implementing a truncated version of the ATP7B protein with a reduced number of copper-binding domains (two or three) instead of the normal six. While still functional, the protein is less effective in transporting copper, with potential impaired effectiveness of the gene therapy.[1]

In the current study, Padula and colleagues use two viral vectors, each containing a part of the *ATP7B* gene, combined with split-intein technology to reassemble and deliver the full-length ATP7B protein. The authors present comprehensive data showing that the intein-reconstituted ATP7B is functional and displays normal intracellular trafficking in ATP7B knockout cells. Furthermore, the authors administer the vector to a mouse model of WD, demonstrating hepatic protein expression, improvement in copper metabolism parameters, and clinical improvement. The findings indicate a favorable safety profile, with the WD mice having improvement in their liver enzymes after treatment as well as reduced inflammation and necrosis in the liver pathology staining.

While patients with WD and clinicians are eagerly awaiting the results of the ongoing clinical gene therapy trials, the present study shows that the field is still in its early phases but rapidly evolving.

The current trials necessitate intensive immune suppression regimens during the vector infusions and for weeks post treatment to secure that the gene is delivered by the vector and to hinder hepatic inflammation. At this stage, any severe hepatic inflammatory reaction either during vector therapy or after (e.g., viral hepatitis) will result in hepatocellular turnover and dilution of the transfected protein, as new hepatocytes will not contain the transfected gene. While the dual-vector approach ensures delivery of the entire ATP7B protein, it is expected to have similar immunogenic limitations as the currently investigated therapies. Furthermore, splitting and splicing the target protein may lead to protein misfolding or the production of truncated protein species, thereby reducing efficiency.[5] Another challenge of ongoing clinical trials is the strict subject selection, which often excludes patients with more advanced liver disease and cirrhosis. Future mouse studies on gene therapy should include models of advanced fibrosis to evaluate prevention of fibrosis development or safety and efficacy if chronic liver disease is present. In addition, the fact that WD clinical presentation is different in female and male patients is often overlooked. Women present more frequently with hepatic involvement, whereas men present more severe neurological signs and symptoms. Studies on animal models should include assessment of copper metabolism in both sexes.

In summary, the present study adds to the body of data attesting safety and efficacy of gene therapy in WD. The study is an important step forward as it demonstrates the possibility of large genome delivery and targeted protein expression with improvement of copper metabolism parameters. The ongoing phase I-II-III clinical trials will demonstrate whether the pre-clinical results for vector-based gene therapy can be replicated in a real-world clinical setting and, eventually, if the correction of copper metabolism will be sustained in the long term at the point that anti-copper medications can be stopped, offering a real life-changing solution to patients. Moving forward, more pre-clinical research identifying treatments with less immunogenicity will be key to identifying

[1]Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of California Davis, Davis, CA, USA

Correspondence: Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus, Denmark.
E-mail: thomsand@rm.dk

optimal treatment strategies for patients with WD.

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