HHS Public Access

Author manuscript

N Engl J Med. Author manuscript; available in PMC 2021 July 15.

Published in final edited form as:

N Engl J Med. 2020 October 08; 383(15): 1489–1491. doi:10.1056/NEJMcibr2025332.

Editing the Mitochondrial Genome

Maria Falkenberg, Ph.D., **Michio Hirano, M.D.**

Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden (M.F.), and the H. Houston Merritt Neuromuscular Research Center, Columbia University Medical Center, New York (M.H.).

Mitochondrial diseases are a heterogeneous group of disorders with varying clinical features caused by impaired function of the mitochondrial respiratory chain. The underlying cause can be gene mutations affecting either the nuclear DNA or the mitochondrial DNA (mtDNA), both of which contain genes encoding components of the oxidative phosphorylation machinery required to generate ATP, the currency of cellular energy.[1] For most patients, only symptomatic treatment is available, and there is a need for new therapies that directly target the underlying disease mechanisms. A spectrum of more than 250 distinct mitochondrial diseases have been linked to a variety of pathogenic variants occurring in the nuclear or mitochondrial genomes; these diseases typically manifest as severe and often lethal multisystemic disorders. Although each individual disease is ultrarare, in the aggregate, mitochondrial diseases affect 5 to 15 persons per 100,000 population. Pathogenic mutations in mtDNA more commonly cause mitochondrial disease in adults than do mutations in nuclear genes. More than 270 pathogenic variants of mtDNA have been reported.

The clinical features and inheritance of mtDNA mutation disorders are distinct from those of autosomal disorders. A key concept in this regard is heteroplasmy: the coexistence of normal and pathogenic variants within cells affected by mtDNA diseases. The small, circular mitochondrial genome is present in thousands of mtDNA copies in a normal cell, and the number of mutant genomes must reach a critical heteroplasmy level, or threshold, in order to cause cellular dysfunction and mitochondrial disease (Fig. 1A). In previous work, efforts have been made to develop therapies that can specifically eliminate pathogenic mtDNA variants until their numbers are below threshold levels. To this end, DNA nucleases, such as transcription activator–like effector nucleases (TALENs) and zinc-finger nucleases, have been genetically engineered to target mutant copies of mtDNA.[2,3] After linearization, mutant mtDNA copies are degraded, and a downward shift in heteroplasmy levels has been observed in both cell and mouse models. An alternative approach would be to specifically correct, or edit, mutated base pairs. Recently, the CRISPR-Cas (clustered regularly interspaced short palindromic repeats associated with a Cas endonuclease) technologies have revolutionized our ability to change the nuclear genome at will. However, CRISPR-Cas requires a guide RNA, and since long DNA and RNA strands cannot be imported into mammalian mitochondria, the technology cannot be used to edit mtDNA.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

In a recent publication, however, Mok et al. describe new tools to enable precise manipulation of mtDNA sequences.[4] To this end, the authors creatively harnessed a cytidine deaminase to efficiently convert specific sites in mtDNA. The key that unlocked this new technique was the identification of a *Burkholderia cenocepacia* cytidine deaminase, DddA. This enzyme functions as an interbacterial toxin that deaminates cytosine (C) preceded by a thymine (T) residue in the double-stranded DNA of other bacteria. The authors split the DddA domain that causes toxicity ($DddA_{tox}$) into two inactive halves, which were linked to programmable DNA-binding proteins. When those fusion proteins bind to different but adjacent sites on mtDNA, the split $DddA_{tox}$ halves are brought together, and the deaminase becomes active (Fig. 1B). The DNA-binding proteins are programmed to target sites so that the reactivated $DddA_{tox}$ straddles the double-stranded mtDNA at the target C (preceded by T). Deamination converts C into uracil (U), a nucleobase that normally pairs with adenine (A). As a consequence, when the edited DNA is used as a template for mtDNA replication, A is introduced opposite U. In this manner, the original CG base pair is converted into a UA base pair after a round of mtDNA replication. Finally, the cellular repair pathways replace the U with T, forming TA and completing base editing. The authors showed the efficiency of this system in cell lines, where it could convert CG to TA at specific target sequences in 5 to 50% of the mtDNA sequences tested, with no off-target alterations in nuclear DNA and only low off-target effects on mtDNA.

The DddA-derived cytosine base editor (DdCBE) enables the direct editing of mtDNA. As shown by Mok et al., this base-editing tool can also be used to generate mtDNA mutations in cell lines and potentially in animal models. Clinically, the technique holds the promise of editing mutant CG mtDNA base-pairs, which, the authors note, could correct nearly half of the known pathogenic mtDNA variants. Nevertheless, the current version of DdCBE can edit only cytosines preceded by thymine, and gene-delivery systems will need to overcome the challenges of widespread tissue delivery of two DdCBEs in order to treat most of the mtDNA diseases, which are multisystemic. In addition, efficient base editing requires active mtDNA replication, and many mtDNA disease mutations cause phenotypes in postmitotic tissues, such as brain and muscle. It remains unclear whether the low levels of mtDNA replication that continuously take place in these cells are sufficient for efficient base editing. Further refinements of DdCBEs will surely follow the initial work by Mok et al. and lead to a better understanding of mtDNA variants in human mtDNA-related diseases and conditions such as aging, not to mention the treatment or prevention of primary mtDNA mutation disorders.

References

1. Gorman GS, Chinnery PF, DiMauro S, et al. Mitochondrial diseases. Nat Rev Dis Primers 2016; 2: 16080. [PubMed: 27775730]
2. Bacman SR, Kauppila JHK, Pereira CV, et al. MitoTALEN reduces mutant mtDNA load and restores $tRNA^{Ala}$ levels in a mouse model of heteroplasmic mtDNA mutation. Nat Med 2018; 24: 1696–700. [PubMed: 30250143]
3. Gammage PA, Viscomi C, Simard M-L, et al. Genome editing in mitochondria corrects a pathogenic mtDNA mutation in vivo. Nat Med 2018; 24: 1691–5. [PubMed: 30250142]
4. Mok BY, de Moraes MH, Zeng J, et al. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature 2020; 583: 631–7. [PubMed: 32641830]

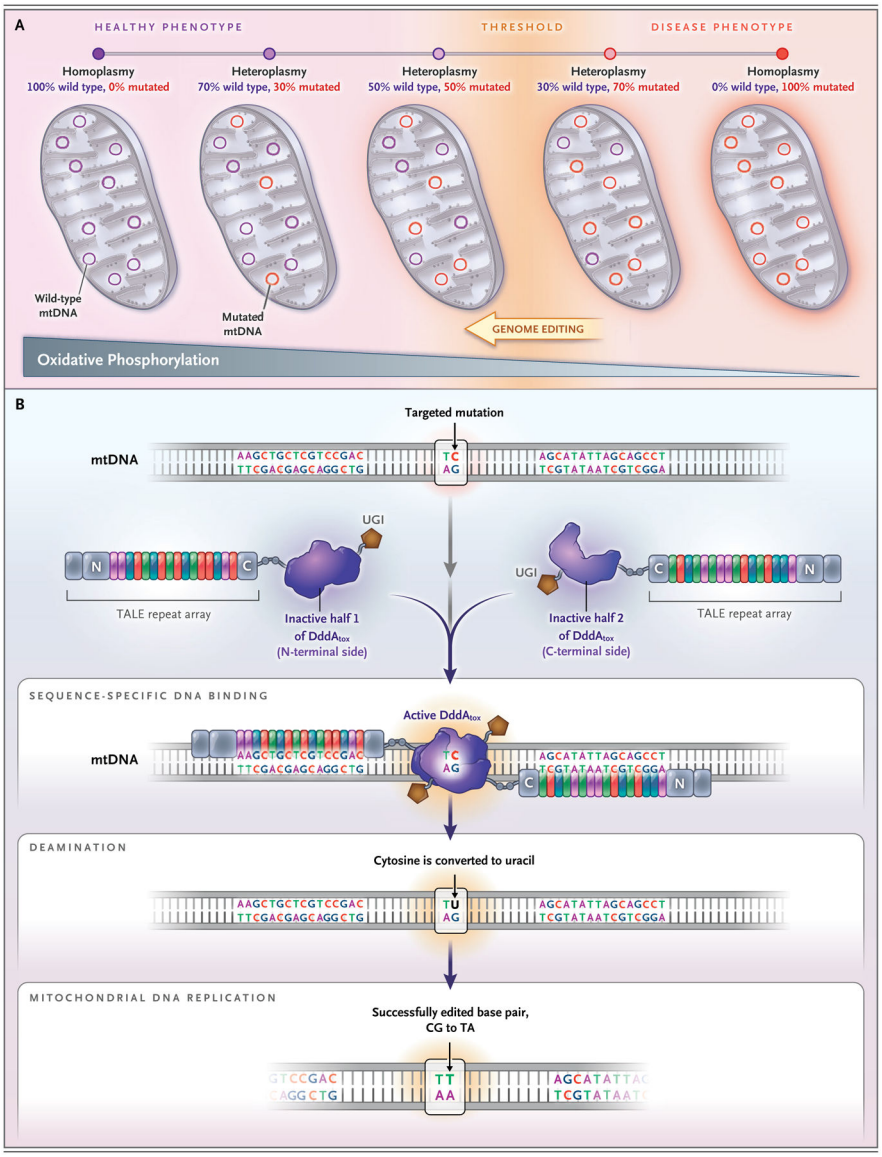

Figure 1. New Tools for Editing the Mitochondrial Genome.

For most disease-causing mutations in mitochondrial DNA (mtDNA), the level and tissue distribution of heteroplasmy determines the phenotypic manifestations (Panel A). A typical human cell contains more than 1000 mtDNA copies, and the presence of mutations in a substantial fraction of these typically has few or no ill effects. Reduced oxidative phosphorylation activity and disease phenotypes are not observed until the level of mutated mtDNA exceeds a specific threshold. Genome editing can correct mtDNA mutations and thereby shift the heteroplasmy levels below the threshold required for disease phenotypes to occur. With the use of a base editor derived from an interbacterial toxin called DddA (DddA-derived cytosine base editor [DdCBE]), mtDNA can be edited directly (Panel B). The cytidine deaminase domain of the toxin ($DddA_{TOX}$) is split into two inactive parts, each fused to an engineered sequence-specific DNA-binding domain (here, a transcription activator–like effector [TALE]). When the TALE constructs bind to adjacent DNA

sequences, the $DddA_{TOX}$ halves align, forming an active deaminase. The deamination of C to U in mtDNA leads to the formation of a UG base pair. Uracil glycosylase inhibitors (UGIs) prevent mtDNA repair machinery from removing the U. During the next round of mtDNA replication, U bonds with A, which eventually leads to the conversion of a CG to a TA base pair.