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EDITORIAL COMMENT

A Valuable Genetically Engineered Nonhuman Primate Model of Dilated Cardiomyopathy*

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Heart failure (HF), a terminal phenotype common to almost all cardiovascular diseases, is a major global health challenge, demanding constant research to better understand the pathogenesis of the disease to develop new therapies. Dilated cardiomyopathy (DCM), characterized by left ventricular dilation and dysfunction of unknown cause, is the leading cause of severe HF, particularly in young adults and adolescents. Pathogenic variants in genes associated with cardiomyopathy contribute significantly to the pathogenesis of DCM. Approximately 40% of patients with DCM have pathogenic variants and have a worse clinical prognosis than patients without pathogenic variants.[1] In addition, the clinical severity of patients with DCM depends on the specific genes involved. Mutations in the *LMNA* gene, which encodes lamin A/C, a major component of the nuclear membrane, are associated with poor prognosis. Unlike many previous studies on the pathophysiology of lamin-related DCM using mouse models,[2] Luo et al[3] report a primate model generated by base-editing technology in this issue of *JACC: Basic to Translational Science*.

Various mouse models have contributed significantly to HF research, but small animal models are very different from humans, so studies using nonhuman primates (NHPs) are important, especially for clinical applications. NHPs are our closest relatives according to genetics, anatomy, organ size, and behavior. Research using NHPs plays a critical role in clarifying the mechanisms of human disease, especially in the research field of neuroscience.[4] For example, frontotemporal dementia requires a highly developed neocortex, which is unique to primates. If we aim to evaluate the efficacy of therapy for neurodegenerative disorders such as dementia, previous models in rodents or other nonprimate species seem to be inadequate. To date, the majority of reports using NHP disease models are studies of neurologic conditions such as Parkinson's disease, autism spectrum disorder, Huntington's disease, and ataxia. In the research field of cardiology, only a small number of NHP disease models, such as diabetic cardiomyopathy and transverse aortic constriction-induced cardiac hypertrophy, have been reported, and all of them have in common that they do not require gene editing. Thus, the cynomolgus monkey with *LMNA*-mutated DCM generated in the present study by Luo et al[3] is the first gene-edited NHP model for HF study. The same research group also generated cynomolgus monkeys with an *LMNA* (1824 C>T, Gly608Gly) mutation that causes Hutchinson-Gilford progeria syndrome by base-editing technology.[5] Genetically edited monkeys expressed progerin, which is the toxic form of lamin A, and showed clinical features of Hutchinson-Gilford progeria syndrome, including growth retardation, skeletal alterations, hair loss, and vascular abnormalities. Compared with previous NHP models of HF, the cynomolgus monkeys generated by Luo et al might be very beneficial to investigate pathogenesis. To date, there are no data on differences in molecular pathogenesis between NHP and rodent models of disease in

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HF. Therefore, a detailed investigation using multiple analytical techniques, such as single-cell multiomics and cryo-electron microscopic imaging, may reveal the species-specific pathogenesis of *LMNA*-mutant DCM. The NHP model of DCM will also play an important role in translational research on HF. Recent advances in gene editing (such as clustered regularly interspaced short palindromic repeats [CRISPR]/Cas9, base editing, and prime editing) are rapidly driving the development of gene therapy. For example, in vivo CRISPR gene editing is emerging as a potential treatment for various diseases, especially transthyretin amyloidosis, cancer, hematological disorders, and blindness syndromes. In the research field of cardiology, recent reports have shown the efficacy of in vivo base-editing therapy in mouse models of myocardial infarction, hypertrophic cardiomyopathy, and DCM. However, all gene-editing therapies need to deliver the editor enzyme gene by viral vectors such as adeno-associated virus, which pose safety concerns regarding ectopic or unregulated expression of the transgene, off-target biodistribution, and immune interactions. Rodent disease models are clearly insufficient to assess these unexpected adverse events. The gene-edited DCM model of NHP created by Luo et al[3] can be used in preclinical studies to screen for unexpected adverse events of gene-edited therapies targeting DCM and serves as an important bridge between small animal models and human clinical trials.

Although the technology of genome engineering is constantly improving, creating an NHP genetic model that faithfully gene-copies and expression-copies human disease is still a daunting task for researchers, as seen in the present study by Luo et al.[3] Compared with rodents, which have a short gestational length, a large number of births, and multiple pregnancies per year, rhesus monkeys and cynomolgus monkeys, which were used in the study by Luo et al, often produce only 1 offspring annually after a long gestational period (approximately 5.5 months). Besides, assisted reproductive methods are inefficient, with wide variation in response to ovarian stimulation and a limited number of oocytes that can be taken from each female monkey, so a large number of oocyte donors are needed to produce viable offspring. Another obstacle is the low efficiency of implanting embryos produced in vitro in NHPs, often resulting in miscarriages or stillbirths and few live offspring. As seen in the study by Luo et al,[3] because of the low birth rates following embryo transfer, resulting in a small number of edited offspring containing diverse mosaic editing patterns, it can be difficult to conclude genotype-phenotype relationships in NHP genetic models. Therefore, improvements in the reproductive efficiency of assisted reproductive technologies and gene editing are critical to reducing the duration of experiments and the number of animals needed. The introduction of next-generation nucleases will accelerate the ability for accurate gene editing in NHP embryos. Base-editing techniques, as used by Luo et al[3] in their study, greatly improve the specificity and targeting efficiency of gene editing because they do not require homologous recombination. Although prime editing, another type of next-generation nuclease that can create any nucleotide change, is also an attractive alternative for introducing precise gene edits, no study has yet used it. Because guide RNA design is critical to the effectiveness of gene editing, NHP's in vitro cell culture system helps screen guide RNAs first and analyze both on-target and off-target edits before introducing gene editing constructs into embryos.

Finally, the close phylogenetic relationship of NHPs to humans as well as their complex behavior and cognition require application of the highest criteria and welfare standards. Therefore, researchers should always take care to ensure ethical and responsible use of NHPs on the basis of the 3R (replace, reduce, and improve) principle. Veterinarians with knowledge of disease- and species-specific care, as well as experts in the care of infants and older NHPs, should be prepared to provide long-term animal care. National and international cooperative efforts are needed to improve the effectiveness of the creation and breeding of transgenic NHPs while minimizing inbreeding within colonies that cause spontaneous mutations. The efficient use of genetically modified NHPs will play a pivotal role in advancing our understanding of HF and facilitating the development of innovative therapeutic strategies.

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