Gene therapy

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CRISPR gene-editing therapies for hypertrophic cardiomyopathy

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Pre-symptomatic gene editing in preclinical models of hypertrophic cardiomyopathy shows therapeutic promise; clinical studies are now needed to assess safety and efficacy in humans.

Hypertrophic cardiomyopathy (HCM) is a primary cardiac disorder characterized by abnormal heart muscle thickening and caused by heterozygous pathogenic variants in genes encoding sarcomeric proteins[1]. HCM often presents during young adulthood and can progress to heart failure, arrhythmia and sudden cardiac death[2]. Treatment options are limited, and include anti-arrhythmic drugs, implantable defibrillators and heart transplant[3]. Precision-based therapies have also emerged, most notably the cardiac myosin inhibitor mavacamten, which targets the pathological hypercontractility thought to drive HCM but also confers increased risk of heart failure[4]. In vivo CRISPR gene editing is emerging as a potential treatment option for human disease, most notably transthyretin amyloidosis, cancer, hematological disorders and blindness syndromes[5,6]. In paired papers published in this issue of *Nature Medicine*, Reichart et al.[7] and Chai et al.[8] report preclinical studies of new gene-editing-based precision therapies to pre-symptomatically correct a HCM pathogenic variant (Fig. 1).

The two groups use distinct but complementary approaches involving CRISPR–Cas9 adenine base editing (ABE) to target a well-studied missense variant in the *MYH7* gene (c.1208G>A; p.Arg403Gln). This pathogenic variant increases sarcomere contractility via a dominant-negative mechanism; genetic treatment therefore requires allele correction or deletion. Although ABE can achieve highly efficient and precise correction, it is limited to scenarios where editing adenine to guanine reverts the gene to wild-type function, and where the target adenine lies within a 'window' at a defined distance upstream of the protospacer adjacent motif (PAM) used by the base editor to engage the genomic site. Importantly, non-target adenines within this region are subject to bystander editing, which can introduce new pathogenic variants[9].

Chai et al.[8] identified a variant ABE and guide RNA (gRNA) that maximized on-target editing of the p.Arg403Gln variant, minimized bystander editing when tested in vitro, and rescued many of the pathological changes seen within in vitro HCM systems. To test editing

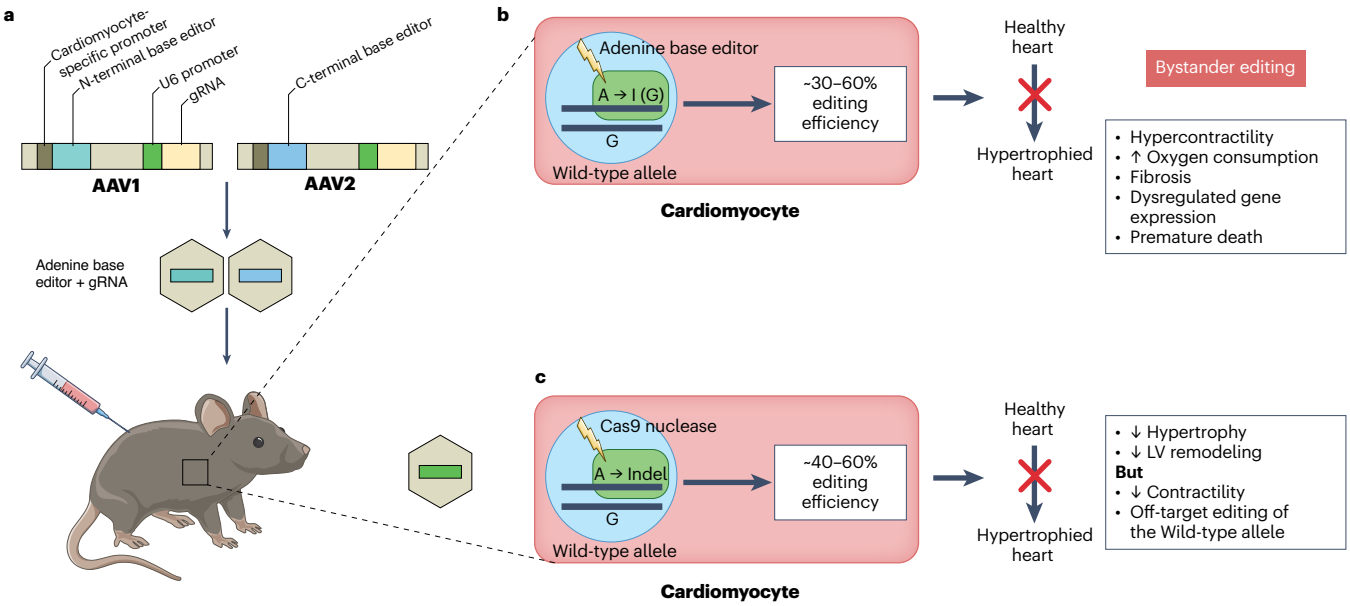

Fig. 1 | CRISPR gene-editing strategies for HCM. a, Researchers used an AAV9 delivery system with an ABE and a gRNA, driven by a cardiomyocyte-specific promoter and a U6 promoter, respectively, and divided between two vectors due to AAV capacity limitations. AAVs were delivered by intrathoracic injection into mouse models of the well-studied *MYH7* pathogenic variant p.Arg403Gln. **b**, This led to 30% editing efficiency with minimal bystander edits (Chai et al.[8]) and 60% editing efficiency with appreciable bystander editing (Reichart et al.[7]). Both studies reported amelioration of cardiac hypertrophy and fibrosis. A, adenine; I, inosine; G, guanine. **c**, As an alternate approach, Reichart et al.[7] used the *S. aureus* Cas9 nuclease and a p.Arg403Gln variant specific gRNA to selectively disrupt the pathogenic allele and demonstrated 40–60% editing efficiency, but with significant off-target editing of the wild-type allele at high AAV doses and consequent compromised cardiac contractility. LV, left ventricle.

efficiency and phenotypic rescue in vivo, the group developed a novel humanized mouse model of the *MYH7* p.Arg403Gln variant, allowing for direct testing of gRNAs developed for human application. In this model, heterozygous humanized mice develop ventricular hypertrophy and fibrosis by 9 months of age, while homozygous mice develop significant cardiomyopathy and fibrosis and die at around 1 week of life. Chai et al.[8] used the adeno-associated viral vector (AAV) AAV9 to deliver the ABE and gRNA, restricting expression to cardiomyocytes through use of a cardiac troponin T (cTnT) promoter. Intrathoracic injection of a high-dose vector into homozygous mice on the first day of life led to approximately 35% correction at the transcript level and extended the lifespan to 2 weeks. In heterozygous mice, the treatment achieved similar correction and rescued left ventricular hypertrophy and remodeling at up to 16 weeks of life, compared with untreated mice. There was no detectable bystander or off-target RNA editing.

Reichart et al.[7] used a similar AAV-based strategy to deliver an ABE and gRNA driven by the cardiomyocyte-specific chicken *TNNT2* promoter to correct the same pathogenic variant in pre-symptomatic mice. In contrast to Chai et al.[8], they used a well-established (but non-humanized) mouse model in which the pathogenic variant is present in the orthologous position in the *Myh6* mouse locus – which did not allow the testing of human-specific gRNAs. Against the 129SvEv genetic background, in which male mice develop cardiomyopathy between 20 and 25 weeks, treatment (using mouse-specific gRNAs) at 10–13 days of life resulted in approximately 68% transcript correction in ventricular cardiomyocytes and 26–39% transcript correction in atrial cardiomyocytes. Tests at 32–34 weeks of life demonstrated rescue of cardiac hypertrophy, amelioration of cardiac fibrosis and normalization of dysregulated gene expression. Bystander editing was detected and increased with sequential AAV injections, which may be in part due to the choice of an ABE with a wide editing window and a less-specific PAM that promotes engagement with many sites in the genome.

As an alternate approach, Reichart et al. [7] used *Staphylococcus aureus* Cas9, which introduces double-stranded DNA breaks (in contrast to ABEs) to selectively inactivate the pathogenic p.Arg403Gln allele. Functional testing demonstrated efficient editing and rescue of hypertrophy phenotypes; however, there was also reduced contractile function with higher doses, reflecting unintentional editing of the wild-type allele in cardiomyocytes and highlighting the narrow therapeutic window if one were to pursue this approach.

The findings of Chai et al.[8] and Reichart et al.[7] provide promising proof of concept of successful gene editing in HCM models; however, translation to human patients will require overcoming a variety of challenges. Neither study definitively addressed the ability of ABE to halt or reverse cardiomyopathy phenotypes after clinical onset of disease (Chai et al.[8] addressed this peripherally in homozygous humanized mice, but this genotype is not representative of human disease). This could be addressed by treating mice after cardiomyopathy develops and assessing editing efficiency and phenotypic rescue. Many remaining questions require human clinical trials; for example, to establish the editing threshold needed for therapeutic efficacy, whether there will be adverse effects (such as arrhythmogenesis) from incomplete editing and functional 'cardiac mosaicism', and to evaluate the toxicity and immunogenicity of high viral vector dosing and long-term expression of the ABE. The latter in particular has proven to be an issue in large animals treated with AAV CRISPR therapy[10].

In vivo genome editing for a genetically heterogenous disease such as HCM has additional challenges, such as the limitations and base pair restrictions of adenine and cytosine base editors, the need for characterization of a new gRNA for each causal variant, limited availability of appropriately positioned PAMs for each variant and, perhaps most importantly, the potential for bystander editing and for off-target editing. Fortuitously, the sequence context of the *MYH7* p.Arg403Gln variant lent itself to efficient correction with existing ABEs and to acceptable levels of bystander editing, but not all HCM variants will be so straightforward to address.

These challenges notwithstanding, the promise of single-dose definitive treatments not only for HCM, but also for the multitudinous other genetic syndromes not amenable to gene knockout or overexpression strategies, make them a goal well worth pursuing. Most immediately, phase 1 clinical trials in adults with advanced disease would help clarify vector and editing safety, toxicity, immunogenicity and editing efficiency, and determine phenotype reversibility and rescue.

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Competing interests

The author declares no competing interests.