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Considerations for Cardiac CRISPR

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The recently identified two-component CRISPR/Cas9 system has revolutionized the ease with which genome editing can be performed, greatly simplifying the process of generating knockout animal models[1, 2]. However, in spite of the great advances made, fewer studies have focused on the efficacy of the system for editing postnatal or adult tissues as a means to model adult onset diseases or to provide potential therapeutic intervention. In particular, the utility of the system to perform genome editing in a tissue specific manner remains to be thoroughly evaluated, especially for tissues that are less amenable to viral delivery of the CRISPR/Cas9 components, a common strategy for delivery in adult tissues[3]. As the heart is responsible for a large disease burden, it represents a particularly attractive tissue to target for therapeutic purposes[4]. While several studies have shown that cardiomyocytes can be edited in the postnatal murine heart using viral delivery of one or more of the CRISPR/Cas9 system components[5–7], the efficiency of this phenomenon has been less well characterized.

A study in this issue by Johansen *et al*[8] from Eva van Rooij's group performed an in-depth analysis of CRIPSR/Cas9-mediated gene editing in postnatal mouse myocytes. Cardiac-restricted expression of Cas9 was induced using mice in which a cassette encoding Cas9 linked to a GFP reporter was expressed from the Rosa26 locus, crossed to αMHC-Cre mice. Consistent with other models that have expressed Cas9 in either a broad or tissue restricted manner[5, 6, 9], no changes in baseline cardiac function were observed following Cas9 overexpression, suggesting that Cas9 alone has no adverse impact on the heart. To test the utility of these mice for gene editing, the authors chose to target *Tbx20*, which is essential for cardiac function, and *Sav1*, a component of the Hippo signaling pathway, which governs cardiac growth. Single guide RNAs (sgRNAs) were cloned into an adeno-associated virus 9 (AAV9) vector, which displays cardiac tropism, and postnatal pups were injected with a single dose of AAV9. Importantly, gene editing by T7 Endonuclease I assay was observed as was a reduction in mRNA transcript levels for both *Tbx20* and *Sav1* after sgRNA knockdown, suggesting that these loci were edited by the administration of their respective

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Conflicts of Interest

The authors declare no conflict of interest.

sgRNAs. However, only a modest decrease in total heart Tbx20 protein level was noted by Western blot analysis while no changes in total Sav1 protein level were observed, suggesting only partial editing efficiency.

Using deep sequencing on whole heart tissue isolated from edited animals, the level of gene disruption was quantified. As Cas9 is only expressed in cardiomyocytes by this strategy, an estimated degree of myocyte editing was extrapolated from whole-heart deep sequencing, leading to the conclusion that ~31.5% of cells were edited for *Tbx20* and ~38.4% for *Sav1*, with the majority of these reads revealing out-of-frame deletions, predicted to yield truncated proteins. Nevertheless, in spite of the genome editing observed, only very modest changes in cardiac function were noted following knockdown of *Tbx20* with no reported changes in cardiac function after knockdown of *Sav1*. While previous studies have indicated that loss of *Tbx20* in adult cardiomyocytes is lethal[10], no changes in baseline cardiac function following deletion of *Sav1* in cardiomyocytes in postnatal or adult animals have been reported, only increased myocyte proliferation[11]. Therefore, it is unclear if any functional changes should have been expected following *Sav1* deletion. In contrast, consistent with previous studies[5], knockdown of *Myh6* induced a dramatic cardiomyopathy, with significant reduction in cardiac function, despite similar levels of genome editing as observed for both *Tbx20* and *Sav1* knockdown.

To enhance efficiency of the system, two sgRNAs both targeting *Sav1* were cloned into a single AAV9 vector and delivered via systemic injection. This dual guide injection strategy enhanced the efficiency of gene editing, and lead to the induction of a modest but significant cardiac phenotype, with edited animals showing an increased heart weight/tibia length and greater levels of cellular proliferation and hypertrophic markers, as well as an upregulation of *Sav1* target genes. Together, these finding suggest that dual sgRNA injections can enhance the efficiency of this system for targeting the postnatal cardiac genome.

The studies presented in this manuscript demonstrate important technical considerations for the cardiac field as CRISPR/Cas9 technology continues to advance and be implemented in a more widespread manner. Johansen *et al.* suggest this system of cardiac gene editing can result in mosaic gene deletion. In this case, injection of AAV9, while showing broad cardiac tropism, resulted in the infection of only ~70% of cardiomyocytes with the sgRNA. Moreover, even in those cells where indels are induced, some fraction will result in small insertions/deletions that are a multiple of 3 and therefore unlikely to result in a non-functional protein.

While there are inherent limitations to this system as a replacement for knockout animal models in its current form, there are potential ways to enhance efficiency to push the system closer to generating true loss-of-function models. First, one of the major hurdles is the use of AAV as a viral delivery vector for sgRNA components, as the efficiency of gene editing depends on viral titer, as well as levels of expression of Cas9 and sgRNAs[6]. As a result, strategies to enhance AAV penetrance in cardiac tissue, particularly in adult heart, could prove especially critical in generating more widespread infection of cardiomyocytes. Alternatively, non-viral delivery methods for AAV components could also be useful, particularly for clinical investigations[12]. Additionally, as described both here and

previously[5, 6], the use of two sgRNAs appears to enhance efficiency of the system, at least in some settings. Furthermore, careful design of sgRNAs to take advantage of Microhomology Mediated End Joining (MMEJ) could greatly enhance editing efficiency, through the generation of precise, targeted out-of-frame edits[13]. Despite the challenges in using this system in its current form to assess the impact of novel genes on cardiac function, at least one other group has performed elegant studies indicating that a similar system can be used as a screening approach to identify genes essential for cardiomyocyte maturation and function, by taking advantage of the mosaicism induced by this approach[6]. This same study identified frameshifting mutations in >60% of *Jph2* mRNA and substantial protein loss using a modified strategy of a single AAV vector containing an sgRNA and Cre recombinase under control of a cardiac-specific promoter, a significantly greater efficiency than that reported by Johansen *et al.*, suggesting this strategy may already represent a methodological improvement. However, some quantification of gene editing and protein takeout was performed on infected isolated cardiomyocytes in this study[6], as opposed to the use of whole heart tissue by Johansen *et al.*[8], making it difficult to directly compare efficiency.

Finally, it is likely that similar strategies may be especially useful for correction of pathological mutations, as it is expected that correction of only a small fraction of damaging mutations will be sufficient to induce phenotypic rescue, as several studies have recently demonstrated[7, 14]. Other recent work has suggested that, contrary to long-established thought, post-mitotic cardiomyocytes can undergo homology-directed repair *in vitro*[15], opening more avenues for correction of pathogenic mutations and the use of strategies similar to those reported here for *in vivo* investigations of this phenomenon. Together, the studies presented in this paper clearly outline inherent limitations in the use of this tool to generate and perform loss-of-function studies that are prudent for researchers in the field to consider when designing experiments. Nevertheless, as our knowledge of CRISPR/Cas9 continues to grow, and improvements continue to be made, this system will likely become a critical player in the elucidation of novel genes involved in cardiac development, function, and disease. The future of cardio-editing remains open, with many discoveries awaiting us.

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References

1. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014; 157:1262–78. [PubMed: 24906146]
2. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012; 337:816–21. [PubMed: 22745249]
3. Senis E, Fatouros C, Grosse S, Wiedtke E, Niopek D, Mueller AK, Borner K, Grimm D. CRISPR/Cas9-mediated genome engineering: an adeno-associated viral (AAV) vector toolbox. Biotechnol J. 2014; 9:1402–12. [PubMed: 25186301]
4. Strong A, Musunuru K. Genome editing in cardiovascular diseases. Nat Rev Cardiol. 2017; 14:11–20. [PubMed: 27609628]

5. Carroll KJ, Makarewich CA, McAnally J, Anderson DM, Zentilin L, Liu N, Giacca M, Bassel-Duby R, Olson EN. A mouse model for adult cardiac-specific gene deletion with CRISPR/Cas9. Proc Natl Acad Sci U S A. 2016; 113:338–43. [PubMed: 26719419]

6. Guo Y, VanDusen NJ, Zhang L, Gu W, Sethi I, Guatimosim S, Ma Q, Jardin BD, Ai Y, Zhang D, Chen B, Guo A, Yuan GC, Song LS, Pu WT. Analysis of Cardiac Myocyte Maturation Using CASAAV, a Platform for Rapid Dissection of Cardiac Myocyte Gene Function In Vivo. Circ Res. 2017; 120:1874–1888. [PubMed: 28356340]

7. Xie C, Zhang YP, Song L, Luo J, Qi W, Hu J, Lu D, Yang Z, Zhang J, Xiao J, Zhou B, Du JL, Jing N, Liu Y, Wang Y, Li BL, Song BL, Yan Y. Genome editing with CRISPR/Cas9 in postnatal mice corrects PRKAG2 cardiac syndrome. Cell Res. 2016; 26:1099–1111. [PubMed: 27573176]

8. Johansen AK, Molenaar B, Versteeg D, Leitoguinho AR, Demkes CJ, Spanjaard B, de Ruiter H, Akbari Moqadam FA, Kooijman L, Zentilin L, Giacca M, van Rooij E. Postnatal Cardiac Gene-Editing Using CRISPR/Cas9 with AAV9-Mediated Delivery of Short Guide RNAs Results in Mosaic Gene Disruption. Circ Res. 2017

9. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, Dahlman JE, Parnas O, Eisenhaure TM, Jovanovic M, Graham DB, Jhunjhunwala S, Heidenreich M, Xavier RJ, Langer R, Anderson DG, Hacohen N, Regev A, Feng G, Sharp PA, Zhang F. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. Cell. 2014; 159:440–55. [PubMed: 25263330]

10. Shen T, Aneas I, Sakabe N, Dirschinger RJ, Wang G, Smemo S, Westlund JM, Cheng H, Dalton N, Gu Y, Boogerd CJ, Cai CL, Peterson K, Chen J, Nobrega MA, Evans SM. Tbx20 regulates a genetic program essential to adult mouse cardiomyocyte function. J Clin Invest. 2011; 121:4640–54. [PubMed: 22080862]

11. Heallen T, Morikawa Y, Leach J, Tao G, Willerson JT, Johnson RL, Martin JF. Hippo signaling impedes adult heart regeneration. Development. 2013; 140:4683–90. [PubMed: 24255096]

12. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, Park A, Yang J, Suresh S, Bizhanova A, Gupta A, Bolukbasi MF, Walsh S, Bogorad RL, Gao G, Weng Z, Dong Y, Koteliansky V, Wolfe SA, Langer R, Xue W, Anderson DG. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat Biotechnol. 2016; 34:328–33. [PubMed: 26829318]

13. van Overbeek M, Capurso D, Carter MM, Thompson MS, Frias E, Russ C, Reece-Hoyes JS, Nye C, Gradia S, Vidal B, Zheng J, Hoffman GR, Fuller CK, May AP. DNA Repair Profiling Reveals Nonrandom Outcomes at Cas9-Mediated Breaks. Mol Cell. 2016; 63:633–46. [PubMed: 27499295]

14. Long C, Amoasii L, Mireault AA, McAnally JR, Li H, Sanchez-Ortiz E, Bhattacharyya S, Shelton JM, Bassel-Duby R, Olson EN. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science. 2016; 351:400–3. [PubMed: 26721683]

15. Ishizu T, Higo S, Masumura Y, Kohama Y, Shiba M, Higo T, Shibamoto M, Nakagawa A, Morimoto S, Takashima S, Hikoso S, Sakata Y. Targeted Genome Replacement via Homology-directed Repair in Non-dividing Cardiomyocytes. Sci Rep. 2017; 7:9363. [PubMed: 28839205]