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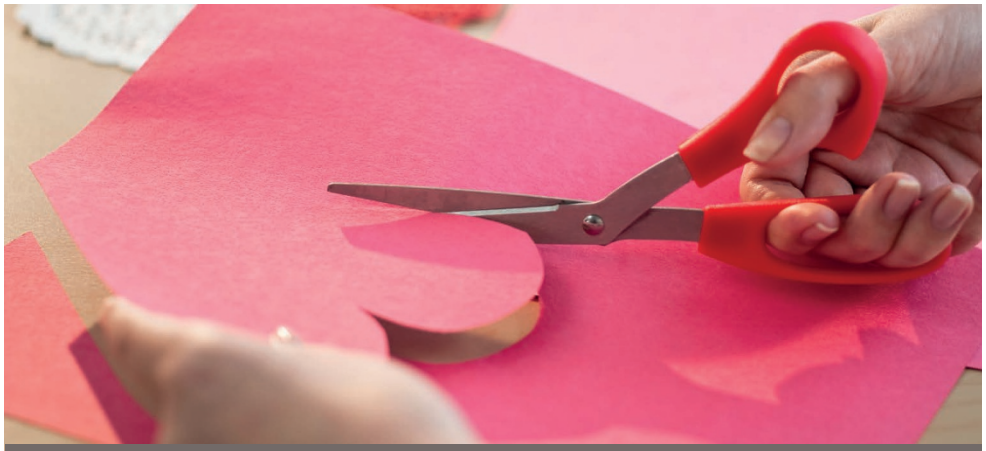

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GENE THERAPY

Human genome editing in heart disease

> "There is now no question that human germline genome editing can be performed"

Human germline genome editing with CRISPR–Cas9 was used with high efficiency, accuracy and safety to correct a heterozygous, autosomal dominant mutation in *MYBPC3* associated with hypertrophic cardiomyopathy, according to a new study in *Nature*.

CRISPR–Cas9 is a versatile tool for recognizing a specific genomic sequence and inducing a double-strand break (DSB) in the DNA. These DSBs are then fixed by endogenous DNA-repair mechanisms: either non-homologous end joining (NHEJ) or homology-directed repair (HDR). In NHEJ, the DSB is repaired by randomly adding or deleting nucleotides, which introduces insertion or deletion mutations ('indels'), making this pathway unsuitable for genome-correction approaches. HDR uses either the nonmutant homologous chromosome or a supplied exogenous DNA molecule as a template to repair the DSB, leading to correction of the mutant allele.

Mitalipov and colleagues sought to demonstrate that heterozygous gene mutations can be corrected in human gametes or early embryos. The investigators used human zygotes produced using sperm from a donor with a heterozygous 4 bp deletion in exon 16 of *MYBPC3*, and oocytes obtained from healthy donors to provide the wild-type allele.

The researchers introduced a mixture of Cas9 protein, single-guide RNA (to target the specific *MYBPC3* deletion), and single-strand oligodeoxynucleotide (encoding the wild-type template) into the zygotes 18 h after fertilization. Injected zygotes and intact controls were cultured for 3 days before each embryonic blastomere was isolated and individually analysed. The overall targeting efficiency in human embryos was high. Of particular interest, the majority of blastomeres resolved the DSB by HDR using the wild-type allele as a template, rather than the exogenous single-strand oligodeoxynucleotide. "Human embryos employ different DNA repair mechanisms than do somatic or pluripotent cells, probably reflecting evolutionary requirements for stringent control over genome fidelity in the germline," suggest the investigators. "Figuring out how that works will be the most interesting next step for this research," comments Eric Olson (UT Southwestern Medical Center, USA), who was not involved in the study.

A major concern with the use of CRISPR–Cas9 to edit the genome of human embryos is the risk of mosaicism, whereby a developing embryo has a mixture of both edited and unedited cells, which would be unacceptable for clinical applications. The investigators demonstrated that co-injection of CRISPR–Cas9 and sperm into the human oocyte during metaphase II of the cell cycle was more efficient than injection into zygotes and, importantly, eliminated the occurrence of mosaicism.

Another risk of CRISPR–Cas9 is the introduction of off-target mutations in the genome. However, comprehensive whole-genome and whole-exome sequencing did not detect off-target effects. Importantly, CRISPR–Cas9-treated human embryos developed normally into blastocysts and embryonic stem cells, with no cytogenetic abnormalities.

Human genome editing raises many ethical and safety concerns. "There is now no question that human germline genome editing can be performed," says Kiran Musunuru (University of Pennsylvania, USA), who was not involved in the study. "Further improvements can and will be made to the genome-editing technique, so we now need to start having those serious conversations about which circumstances, if any, would permit the clinical use of germline genome editing." Eric Olson is also cautious: "aside from the many ethical issues, this method is impractical for human application any time soon or ever".

To guide future research, the American Society of Human Genetics has published a Position Statement in which they conclude "at this time ... it is inappropriate to perform germline gene editing that culminates in human pregnancy", but "there is no reason to prohibit *in vitro* germline genome editing on human embryos and gametes ... to facilitate research on the possible future clinical applications of gene editing".

Gregory B. Lim, Chief Editor,
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ORIGINAL ARTICLES Ma, H. *et al.* Correction of a pathogenic gene mutation in human embryos. *Nature* http://dx.doi.org/10.1038/nature23305 (2017) | Ormond, K. E. *et al.* Human germline genome editing. *Am. J. Hum. Genet.* **101**, 167–176 (2017)

FURTHER READING Strong, A. & Musunuru, K. *et al.* Genome editing in cardiovascular diseases. *Nat. Rev. Cardiol.* **14**, 11–20 (2017)