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GENETIC ENGINEERING

Allele-specific genome editing of disease loci

A goal in the treatment of Huntington disease, an autosomal dominant disorder caused by a gain-of-function mutation in one allele of the huntingtin (*HTT*) gene, is to inactivate the harmful mutant allele without affecting the normal version that is required for cell integrity. Now, Shin *et al.* have achieved this objective in cells derived from a patient with Huntington disease through a novel personalized approach involving a dual guide RNA (gRNA) CRISPR–Cas9.

The Huntington disease mutation, which involves expansion of a CAG repeat that results in the expression of an additional, toxic version of the HTT protein, has arisen independently many times in humans. It is thus found on many different haplotypes, each of which is associated with a distinct set of polymorphic variants. Shin *et al.* reasoned that they could selectively inactivate the mutant *HTT* allele by targeting mutant-haplotype-specific variants that introduce PAM (protospacer adjacent motif) sequences, which are recognized by Cas9. The researchers compiled a map of PAM-altering *HTT* variants in data from the 1000 Genomes Project and selected PAM sites to target on the basis of their potential to discriminate between mutant and normal alleles.

The authors then transfected cells derived from a patient with Huntington disease with two gRNAS that were targeted at mutant allele-specific PAM sites upstream of the *HTT* promoter and within the coding sequence. Their aim was to remove the CAG expansion repeat by introducing a large deletion between the target PAM sites. Sequence analysis of PCR products amplified from the dual gRNA-transfected cells was consistent with the successful generation of 44 kb-sized deletions that, importantly, occurred exclusively in the mutant allele.

Further analyses to determine the functional consequences of this approach confirmed the loss of the CAG expansion repeat from both the *HTT* DNA and RNA and complete loss of the mutant HTT protein. Notably, the excised mutant DNA fragment had not become integrated elsewhere in the genome.

Finally, the researchers used different allele-specific gRNAs targeting other PAM-altering SNPs to successfully eradicate the CAG expansion from additional independent patient cell lines, suggesting a broad applicability of their approach.

With sufficient haplotype characterization, the authors suggest that this technique could be extended to a range of autosomal dominant gain-of-function diseases.

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ORIGINAL ARTICLE Shin, J. W. *et al.* Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. *Hum. Mol. Genet.* http://dx.doi.org/10.1093/hmg/ddw286 (2016)