Somatic correction of junctional epidermolysis bullosa by a highly recombinogenic AAV variant.

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Public Summary:
Definitive correction of disease causing mutations in somatic cells by homologous recombination (HR) is an attractive therapeutic approach for the treatment of genetic diseases. However, HR-based somatic gene therapy is limited by the low efficiency of gene targeting in mammalian cells and replicative senescence of primary cells ex vivo, forcing investigators to explore alternative strategies such as retro- and lentiviral gene transfer, or genome editing in induced pluripotent stem cells. Here, we report correction of mutations at the LAMA3 locus in primary keratinocytes derived from a patient affected by recessive inherited Herlitz junctional epidermolysis bullosa (H-JEB) disorder using recombinant adenoassociated virus (rAAV)-mediated HR. We identified a highly recombinogenic AAV serotype, AAV-DJ, that mediates efficient gene targeting in keratinocytes at clinically relevant frequencies with a low rate of random integration. Targeted H-JEB patient cells were selected based on restoration of adhesion phenotype, which eliminated the need for foreign sequences in repaired cells, enhancing the clinical use and safety profile of our approach. Corrected pools of primary cells assembled functional laminin-332 heterotrimer and fully reversed the blistering phenotype both in vitro and in skin grafts. The efficient targeting of the LAMA3 locus by AAV-DJ using phenotypic selection, together with the observed low frequency of off-target events, makes AAV-DJ based somatic cell targeting a promising strategy for ex vivo therapy for this severe and often lethal epithelial disorder.

Scientific Abstract:
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