Therapeutic Editing of the *HBB* Locus Using the Endogenous *HBD* Locus as a Donor Template

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Introduction

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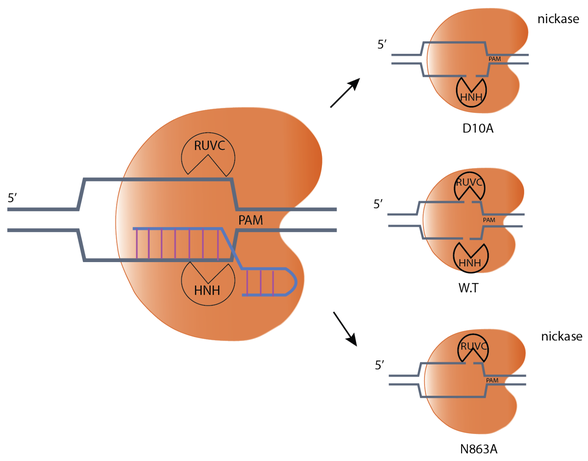
Sickle Cell Anemia is a recessive disorder caused by a single point mutation in the human beta globin (*HBB*) gene. Affecting nearly 1 million people worldwide, this disease is severely lacking in long-term treatment options and is a prime candidate for a gene editing therapeutic approach. Here we report the use of the CRISPR/Cas system to target the human *HBB* gene in the region of the sickle cell anemia-causing mutation.
Utilizing two different Cas9 nickases as well as the wild type nuclease, we are able to introduce blunt double-strand breaks, single strand nicks, and dual-nicks in which the nicks are placed on opposite strands and leave either 3' or 5' overhangs of varying lengths. Using either single strand oligonucleotide (ssODN) or plasmid DNA donors, we characterize several different DNA repair outcomes including indel mutations resulting from non-homologous end-joining, homology-dependent repair (HDR) using the donor as a template, and finally HDR using the closely related *HBD* gene as an endogenous template. Repair using homologous sequences from the *HBD* gene results in partial gene-conversion yielding a chimeric *HBB-HBD* gene. The region of gene conversion includes the sequence most commonly mutated in sickle cell anemia. The frequency of this event depends on the nature of the break. The data support a therapeutic approach in which correction of the sickle-cell mutation is efficiently mediated through HDR using a donor template or by gene-conversion using the endogenous *HBD* gene.

Fig 1: Schematic of Cas9 variants used in this study



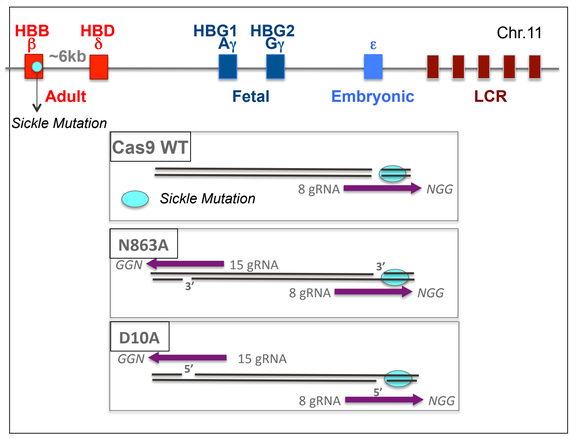


Fig.2
Schematic of the HBB locus indicating the position of the different gRNAs used in the study.

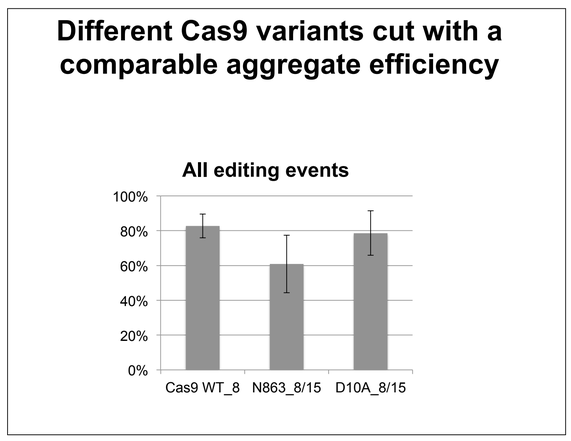


Fig.3
Total editing observed with three Cas9 variants. Results are compiled from at least 3 independent experiments for each condition.

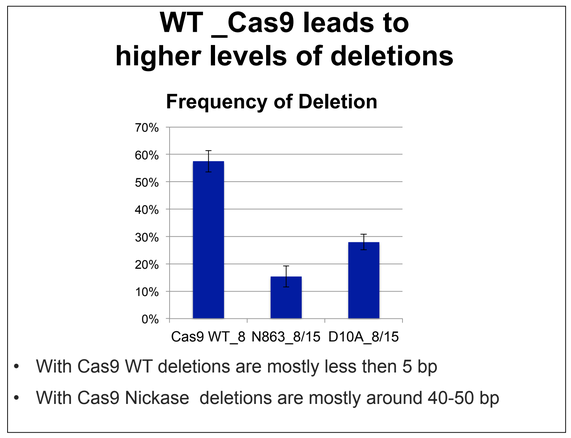


Fig.4
Frequency of deletion observed with three Cas9 variants. Results are compiled from 3 independent experiments for each condition.

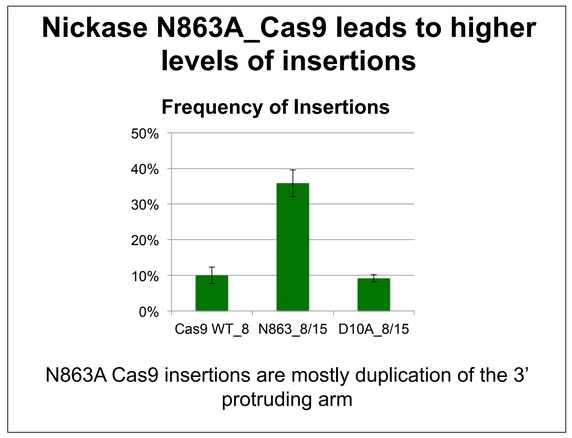


Fig.5
Frequency of insertion observed. Results are compiled from 3 independent experiments for each condition tested.

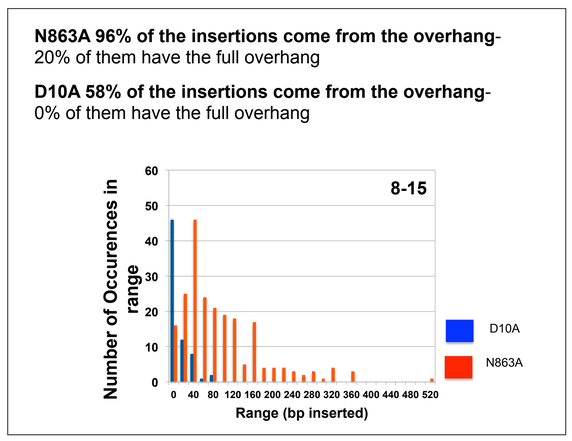


Fig.6
Histograms showing the length of the insertions for N863A and and D10A with gRNA 8/15.

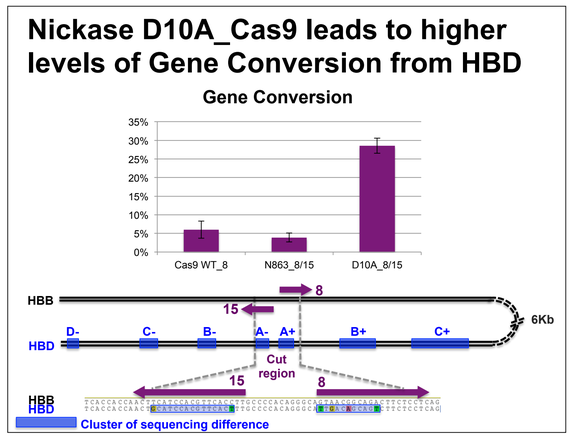


Fig.7
Frequency of gene conversion. A representation of at least 3 independent experiments for each condition is shown. Schematic representing the genomic organization and the region of similarity between HBB and HBD is shown on the bottom.

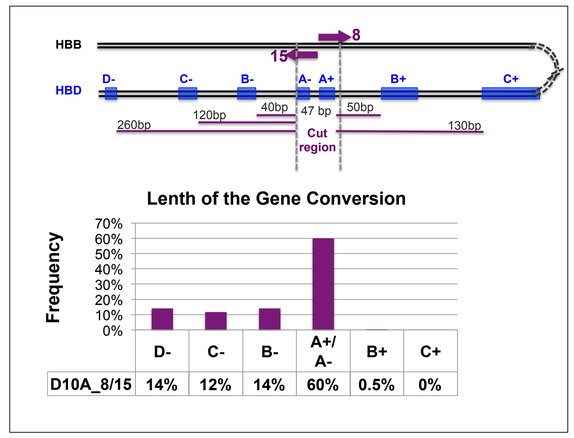


Fig.8
Frequency with which different lengths of HBD sequence are incorporated into HBB.

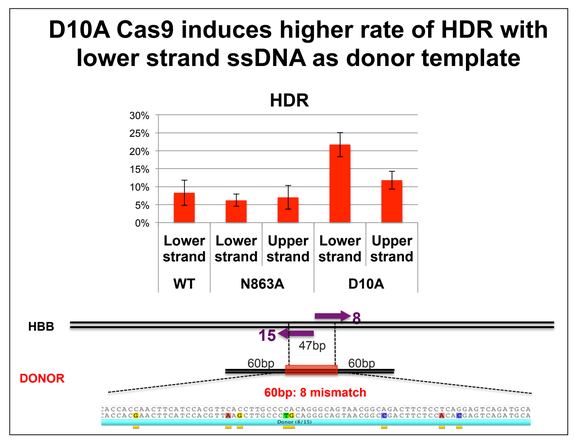


Fig.9
Frequency of HDR in response to different donors. Schematic illustrating the donor template is shown on the bottom.

Methods:

U20S cells were electroporated with gRNA and Cas Wt or mutant plasmid. Cells were collected 6 days after electroporation. gDNA was extracted, PCR amplification of HBB locus was performed and sub cloned into a Topo Blunt Vector. For each condition in each experiment 96 colonies were sequenced by Sanger sequencing.

Conclusion:

- Cas9 wt, N863A and D10A cut with a comparable efficiency
- Cas9 N863A Nickase induces a higher rate of insertions
- Cas9 D10A Nickase induces modification of the HBB locus using the HBD gene as a donor template
  - Predicted to repair the sickle mutation
- Cas9 D10A Nickase achieves a higher rate of HDR