

40008

COMBINED CRISPR/CAS9 AND AAV FOR THE GENERATION OF CONDITIONAL ISOGENIC GENE KNOCK-INS

Prajwal Boddu, Abhishek Gupta, Todd Waldman and Manoj Pillai
Translational Science, Policy, & Health Outcomes Science

ABSTRACT IMPACT: We describe a novel methodology that combines CRISPR/Cas9-induced double stranded DNA breaks with homology dependent repair from an adeno associated virus (AAV) encoded template to generate single-allele edited isogenic cell line models of cancer-associated mutations with high efficiency. **OBJECTIVES/GOALS:** Conventional approaches to creating isogenic knock-ins, to model disease-associated mutations, are limited by poor efficiency and loss of the mutant allele on extended culture. We present an optimized editing approach combining CRISPR/Cas9 with Adeno-associated virus, using mutant SF3B1 as a prototype. **METHODS/STUDY POPULATION:** Left and right homology arms for SF3B1 were PCR amplified and cloned into pAAV-SEPT-Acceptor plasmid (containing a chimeric intron, neomycin resistance cassette and polyA tail). The disease-associated K700E mutation was introduced by site-directed mutagenesis. Single guide RNA (sgRNA) complexed with recombinant Cas9 along with the AAV donor were delivered into K562 cells, G418 resistant clones selected, and screened for integration by PCR. Confirmed clones were then transduced with a doxycycline-inducible Cre-recombinase containing lentiviral vector. Inducible expression of Cre-recombinase and expression of the mutant allele were confirmed by Western blot and Sanger sequencing respectively. **RESULTS/ANTICIPATED RESULTS:** Targeted-integration efficiencies among the Neo-resistant clones, generated by AAV-alone and AAV+CRISPR/Cas9, were 16% and 94%, respectively. Single cell cloning after Cre-mediated excision of loxp was unsuccessful presumably due to toxicity of the K700E mutation. To overcome this limitation, clones were transduced with doxycycline-inducible Cre-recombinase lentiviral vector. Doxycycline induction of Cre-recombinase resulted in reliable excision of the loxp cassette and expression of mutant allele at about 50% variable allele frequency (as determined by Sanger sequencing). The approach was validated in additional cell lines and for introduction of N-terminal FLAG tag for SF3B1. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** Combining AAV and CRISPR/Cas9 can generate scalable single-dominant allele mutants with high precision and efficiency compared to AAV or CRISPR alone. Together with inducible Cre-recombinase, our approach can generate isogenic models where the mutation confers a growth disadvantage.

67553

Exoskeleton dynamics alter upper-limb coordination in a virtual reality reaching task

Alexander Brunfeldt¹, Alexander Dromerick¹, Barbara Bregman¹ and Peter Lum²

¹Georgetown University and ²The Catholic University of America

ABSTRACT IMPACT: This proof-of-concept study demonstrates that systematically altering limb dynamics with two exoskeleton devices alters ingrained, bilateral upper-limb coordination with the potential to rehabilitate functional reaching in chronic stroke survivors **OBJECTIVES/GOALS:** Advances in virtual reality and exoskeleton technologies have allowed researchers to alter upper limb coordination with more precision than ever before. The goal of this study was to

systematically enhance the use of the nondominant limb during a bimanual reaching task, with an eye towards improving rehabilitative strategies post stroke. **METHODS/STUDY POPULATION:** Healthy, right-handed volunteers performed a bimanual reaching task in virtual reality (VR) space while simultaneously moving under the influence of two exoskeleton devices. The VR task had participants move a shared cursor, displayed at the midpoint between the hands, to targets arranged at shoulder and eye levels and located at 70% of full arm extension. Two exoskeleton devices applied either resistive torque to the dominant limb or assistive torque to the non-dominant limb. Three-dimensional hand position data were recorded at 50 Hz and analyzed offline. The primary outcome measure was relative contribution, calculated as the ratio of dominant/non-dominant displacement. **RESULTS/ANTICIPATED RESULTS:** Preliminary results from 3 participants showed that during baseline trials, when no torque was applied by the exoskeletons, relative contribution was 50.6% in favor of the dominant hand, with the dominant hand reaching on average 1.1cm farther than the left. When the exoskeletons resisted movement in the dominant limb while simultaneously assisting movement in the non-dominant limb, relative contribution was 49.7% indicating an increase in non-dominant limb usage. Further analysis showed that this effect was driven by one participant who reached 3.7cm farther with her non-dominant hand compared to baseline. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** These pilot data suggest our testing platform is capable of altering normal coordination patterns and is likely the result of participants adopting an optimal control strategy imposed by the shared cursor. These findings will form the basis for a rehabilitation intervention to promote the use of the paretic limb in chronic stroke survivors.

68415

Neural Impact of Neighborhood Disadvantage in Traumatically-Injured Adults: a Multi-Modal Investigation

E. Kate Webb¹, Carissa Weis¹, Ken Bennett², Ashley Huggins¹, Elizabeth Parisi¹, Jessica Krukowski³, Terri deRoos-Cassini⁴ and Christine Larson¹

¹University of Wisconsin-Milwaukee, ²Montana VA Healthcare System, ³Marquette University and ⁴Medical College of Wisconsin

ABSTRACT IMPACT: Neighborhood disadvantage was significantly associated with brain structure and function in trauma-exposed adults, providing evidence that contextual factors should be assessed in mental health research, particularly in high-risk populations. **OBJECTIVES/GOALS:** Over 13 percent of Americans live in a socioeconomically disadvantaged neighborhood. Previous work has linked lower individual socioeconomic position to alterations in brain structure and function. However, the neural effects of area-level socioeconomic factors, such as neighborhood disadvantage, are unclear. **METHODS/STUDY POPULATION:** We recruited two-hundred and fifteen traumatically-injured participants from an Emergency Department in southeastern Wisconsin. An Area Deprivation Index (ADI) score, a national measure of neighborhood socioeconomic disadvantage, was derived from each participant's home address. Two-weeks post-trauma, participants underwent a battery of self-report measures and functional magnetic resonance imaging (fMRI) scans. Using a multi-modal approach, we investigated the impact of ADI on brain structure as well as neural activation during rest and during an emotional uncertainty task. We sought to disentangle the relationship between neighborhood and individual socioeconomic position and neural activity in the context