targeting co-morbidities in people living with HIV to account for both inflammation and dysbiosis.

4472

Plasma Neurofilament Light as a Biomarker for Pediatric Patients with Huntington's Disease

Jordan L Schultz[1]
[1]University of Iowa Institute for Clinical and Translational Science

OBJECTIVES/GOALS: The goal of this study is to compare plasma neurofilament light (NfL) concentrations in asymptomatic children and young adults that carry the gene expansion (GE group) that causes Huntington's Disease to similar subjects that do not carry this genetic mutation (GNE group). METHODS/STUDY POPULATION: Subjects from the Kids-HD study in the GE group were divided into groups based on their estimated years to motor onset. Each subgroup was compared to the subjects from the GNE group. Additionally, a group of participants with juvenile HD were compared to the GNE group. These comparisons were made by utilizing linear mixed effects regression models that included a random effect per subject and family and also included the covariates of age and parental socioeconomic status. A post-hoc analysis of subjects in the GE group who were within 20 years from their predicted motor onset was conducted to assess the relationship between striatal volume and plasma NfL concentrations. RESULTS/ANTICIPATED RESULTS: GE participants more than 20 years from their predicted motor onset did not have elevated plasma NfL concentrations relative to the GNE group. However, participants who were 15-20 years from their predicted motor onset had a mean NfL concentration of 1.61 pg/uL compared to 1.31 pg/uL in the GNE group ($p = 0.036$). Participants who were within 15 years from their predicted motor onset had a mean NfL concentration of 2.08 pg/uL, which was also significantly elevated relative to the GNE group ($t = 3.03$, $p = 0.003$). Additionally, the participants with juvenile HD had a mean NfL level of 3.22 pg/uL, which was significantly elevated compared to the GNE group ($p<0.0001$). NfL concentrations were significantly correlated with striatal volume amongst participants who were within 20 years of onset ($p = 0.017$). DISCUSSION/SIGNIFICANCE OF IMPACT: The huntingtin protein is essential to neurodevelopment but current gene therapies for HD focus on blocking production of this gene. These results will provide guidance on the optimal timing of administration of gene therapies by identifying neurodegeneration decades prior to motor onset of HD.

4346

Potential Sudden Unexpected Death in Epilepsy (SUDEP) Biomarkers in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes with DEPDC5 Loss-of-Function

Yanting Zhao[1], Helen Zhang[2], Jack M. Parent[2], and Lori L. Isom[3]
[1]University of Michigan School of Medicine; [2]University of Michigan; [3]Department of Pharmacology, University of Michigan

OBJECTIVES/GOALS: Sudden Unexpected Death in Epilepsy (SUDEP) is a leading cause of death in epilepsy patients. This study aims to determine whether cardiac mechanisms contribute to SUDEP in epilepsy patients with variants in *DEPDC5*, a gene encoding a member of the mTOR GATOR complex, to identify SUDEP biomarkers. METHODS/STUDY POPULATION: SUDEP has been reported in 10% of epilepsy patients with *DEPDC5* loss-of-function variants. We used human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to measure changes in cellular excitability that are known to be substrates for cardiac arrhythmias. CRISPR-derived isogenic *DEPDC5* iPSC-CMs and *DEPDC5* patient-derived iPSC-CMs were used in this study. Whole-cell patch-clamp was used to measure voltage-gated sodium current (I_{Na}) and calcium current ($I>_{Ca}$) in single iPSC-CMs in voltage-clamp mode; and to measure action potentials (APs) in 3-dimentional iPSC-CM-derived micro-tissues in current-clamp mode. RESULTS/ANTICIPATED RESULTS: CRISPR generated heterozygous deletion of 1 base-pair in the first coding exon of *DEPDC5* gene, resulting in a premature stop codon, simulated the variants identified in *DEPDC5* epilepsy patients. In CRISPR generated heterozygous*DEPDC5* iPSC-CMs, whole-cell voltage-clamp recordings revealed that I_{Na} was increased and I_{Ca} was reduced compared with isogenic control iPSC-CMs. Whole-cell current-clamp recordings revealed that AP duration at 80% and 90% of repolarization, APD_{80} and APD_{90}, respectively, were prolonged compared to isogenic control iPSC-CMs. Similar measurements will be performed for iPSC-CMs derived from *DEPDC5* patients. DISCUSSION/SIGNIFICANCE OF IMPACT: This study shows that epilepsy patients with non-ion channel gene variants in *DEPDC5* have altered CM excitability, which may serve as a substrate for cardiac arrhythmias in *DEPDC5* patients. Importantly, this work may allow us to identify biomarkers for SUDEP risk in these patients in the future. CONFLICT OF INTEREST DESCRIPTION: L.L.I. is the recipient of a collaborative research grant from Stoke Therapeutics.

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Quantifying the art of surgical decision-making in total knee arthroplasty

Shady Elmasry[1], Carl Imhauser[1], Timothy Wright[1], Peter Sculco[1], Cynthia Kahlenberg[1], Geoffrey Westrich[1], Michael Cross[1], David Mayman[1], and Andrew Pearle[1]
[1]Hospital for Special Surgery

OBJECTIVES/GOALS: To quantify clinical exam in total knee arthroplasty by answering the following questions: (1) What are the magnitudes of forces applied by surgeons during the varus-valgus exam? (2) Is the choice of tibial insert thickness related to the magnitude of the applied forces? (3) How accurately does a surgeon estimate the gaps in the varus-valgus exam? METHODS/STUDY POPULATION: Three cadaveric knees were implanted with standard TKA trial implants. Four pliable force sensors were wrapped around the foot and ankle of each cadaver to measure the push-pull forces applied during the varus-valgus exam. Six surgeons with varying experience independently conducted a varus-valgus exam in extension and flexion and reported the gaps that they observed. Motion capture was used to measure the gaps between femur and tibia by placing cluster of reflective markers on femur and tibia. Subsequently, each surgeon chose the tibial insert that they thought best fit each knee. The measured peak applied forces were related to the insert thickness and the measured gaps were compared to the observed gaps by surgeons. Since insert thickness was in 1 mm increments, 1 mm gap error was considered a meaningful difference. RESULTS/ANTICIPATED RESULTS: The peak forces varied among surgeons for each cadaver. In cadaver one, the peak forces in varus and valgus in extension were 48±20 and 20±12 N, and in flexion were 27±14 and 8±11 N. Peak forces in cadavers two and three were similar; in varus and valgus in extension, 24±14 and 35 ±10 N, and in flexion, 23±12 and 20±10 N, respectively. It was observed that the larger the valgus force in extension, the thinner

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was the inserts choice ($\beta = -0.08$ mm/N, $p = 0.012$). In extension, the difference between estimated gaps and measured gaps was > 1 mm for 36% of all assessments and 91% of gaps were underestimated. Only one measure, however, was underestimated by > 2 mm. In flexion, gap estimates were > 1 mm for 35% of all measurements and 59% of all measurements were overestimated. Four measures were overestimated, and one was underestimated by > 2 mm. DISCUSSION/SIGNIFICANCE OF IMPACT: We found that the applied forces varied among surgeons and a negative association between insert thickness and forces in extension valgus exam. We also found that error in gap estimates among surgeons was > 1 mm a third of the time and that underestimation is more common in full extension, which may lead to using smaller inserts that affect knee stability. CONFLICT OF INTEREST DESCRIPTION: The corresponding author has no COI but my coauthors had the following COI:

1. Royalties from a company or supplier: *Zimmer; Stryker; Exactech, Inc; Lima; Mathys Ltd.*
2. Speakers bureau/paid presentations for a company or supplier*Acelity; Flexion Therapeutics; Smith & Nephew; Exactech, Inc; Mallinckrodt Pharmaceuticals; Stryker.*

3B. Paid consultant for a company or supplier*Acelity; DePuy Synthes; Exactech, Inc; Flexion Therapeutics; Intellijoint; Smith & Nephew; Zimmer; Stryker*

4. Stock or stock options in a company or supplier*Imagen; Insight Medical; Intellijoint; Parvizi Surgical Innovation; OrthAlign; Orthobond.*
5. Research support from a company or supplier as a Principal Investigator*Acelity; Exactech, Inc; Intellijoint; Smith & Nephew; Mallinckrodt Pharmaceuticals; Stryker; Lima.*
6. Royalties, financial or material support from publishers (The following conflicts were disclosed) *Exactech, Inc.*
7. Medical/Orthopaedic publications editorial/governing board*Bone and Joint Journal 360; Journal of Orthopaedics and Traumatology; Techniques in Orthopaedics.*
8. Board member/committee appointments for a societyKnee Society; *Eastern Orthopedic Association.*

4278

Recovery Time is Exaggerated in Individuals with Degenerative Cervical Myelopathy Following Standing Lateral Waist Pulls

Timothy Boerger[1], Learon McGinn[1], Marisa Clare[1], Marjorie Wang[2], Brian D Schmit[1], and Allison S Hyngstrom[1]
[1]Marquette University; [2]Medical College of Wisconsin

OBJECTIVES/GOALS: The aim of this study was to quantify balance impairments in stance in individuals with degenerative cervical myelopathy (IwDCM) in response to external perturbations. IwDCM have damage to their spinal cord due to degeneration of the cervical vertebral column, but little is known about balance. METHODS/STUDY POPULATION: Recovery time following a perturbation may be an important measure of balance. Changes in recovery time were measured in 7 IwDCM (2m, 58.59±15.00y) and 6 controls without DCM (2m, 56.91±11.04y) as they stood on an instrumented treadmill and received cued (predictable) and uncued (unpredictable) lateral pulls to the waist at 12% (high) and 6% (low) pull magnitudes. Individuals stood with feet together, shoulder width, and wide. Recovery time was defined as the time following pull onset when the absolute value of the center of pressure velocity returned to <1x baseline standard deviation. Repeated measures ANOVA was performed on recovery time. RESULTS/ANTICIPATED RESULTS: We anticipate that feet together standing, unpredictable, higher magnitude perturbations will be most challenging evidenced by longer recovery times. For waist pull recovery time, there was a trend for a Group x Predictability x Magnitude x Stance Width interaction ($p = 0.1$) which we anticipate being greater with additional participants. There were significant Group x Predictability x Stance Width ($p = 0.01$) and Group x Magnitude x Predictability ($p = 0.01$) interactions. IwDCM had exaggerated recovery times in narrow and wide stances with unpredictable pulls. IwDCM recovered more slowly in response to unpredictable higher magnitude pulls. DISCUSSION/SIGNIFICANCE OF IMPACT: Balance responses in IwDCM are most impaired in narrow stances and when perturbations are unpredictable. Rehabilitation should focus on shortening latency of response timing and increasing power utilization during balance response to promote quicker recovery.

4096

Refined structure of human ferroportin using restraints from mass spectrometry

Christian S. Parry[1], Andrey Ivanov[2], Guelaguetza Vazquez-meves[2], Fatemah A. Alhakami[2], Jessika Agyepong[2], Kyungreem Han[3], Bernard R. Brooks[4], and Sergei Nekhai[2]
[1]Georgetown - Howard Universities; [2]Howard University; [3]NHLBI Laboratory of Computational Biology, NHLBI/NIH; [4]Laboratory of Computational Biology, NHLBI/NIH

OBJECTIVES/GOALS: Mammals require iron for hemoglobin, respiration, immunity and as cofactor in enzymes. But free iron is toxic from the production of reactive oxygen species. Ferroportin is the sole exporter of cellular iron and it crucially determines cellular and systemic iron levels. Labile iron must be tightly regulated. This requires structural understanding. METHODS/STUDY POPULATION: We built structure of human ferroportin (FPN1) using the ab ignition prediction approaches of Rosetta/Robetta and by comparative modeling with distance restraints in MODELLER. Templates selected were from solute carrier protein families of distantly related orthologs and homologs including a proton coupled peptide transporter (PDB ID: 4IKV) and the bacterial iron transporter in outward-open and inward-open states, (PDB ID: 5AYM, 5AYO). Each model was validated by experimental mass spectrometry data. The energy minimized structural model was inserted into a lipid bilayer, placed in a rectangular simulation box, covered with TIP3P water solvent balanced with counterions and conditioned. Finally, we carried out 350 nanoseconds molecular dynamics simulations. RESULTS/ANTICIPATED RESULTS: Our first model of FPN1 (571aa), using Rosetta/Robetta *ab initio* approach, resembles the structure of the proton-dependent transporter, POT and consists of 12 transmembrane helices. The membrane spanning helices veer away from the orientation in the structure of 4IKV. The alternate model using MODELLER and the method of satisfaction of constraints, returned one template, the structure of *Bdellovibrio bacteriovorus* iron (Fe^{2+}) transporter homolog (5AYN, 440aa) with sequence identity of 19%. Aligning FPN1 on the template sequence incorporating structural information revealed better conservation (29%). This model also comprises 12 transmembrane helices in two bundles separated by a large intracellular loop. The iron binding site predicted in both models match

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