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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the structures of distant bacterial homologs. DISCUSSION/SIGNIFICANCE OF IMPACT: We are using these experimentally verified structures and functional data to answer questions about the mechanism of ferroportin iron transport, structural dynamics and the significance of mutations in ferroportin seen in different populations, especially the Q248H mutation found in Africans and black Americans with moderate to high prevalence.

4369

Reprogramming of vascular smooth muscle cells to multipotent progenitor cells contributes to progression of atherosclerosis*

Allison Milfred Dubner[1], Sizhao Lu, Austin Jolly, Keith Strand, Marie Mutryn, Rebecca Tucker, Karen Moulton, Raphael A. Nemenoff, and Mary C.M. Weiser-Evans
[1]University of Colorado at Denver

OBJECTIVES/GOALS: Our lab previously identified a population of vascular smooth muscle (SMC)-derived progenitor cells (AdvSca1-SM) which expand robustly in response to disease and can differentiate into multiple cell types. We now aim to define the role of these AdvSca1-SM cells in atherosclerotic plaque progression. METHODS/STUDY POPULATION: Goal one uses SMC lineage tracing mice and a model of atherosclerosis to track reprogramming of SMCs to AdvSca1-SM cells in the setting of disease. Arteries are analyzed using flow cytometry and immunofluorescence to quantify changes in number of mature SMCs and AdvSca1-SM cells. Goal two uses AdvSca1-SM lineage tracing mice with high cholesterol-induced atherosclerosis and plaque neovascularization. Arteries are analyzed to quantify expansion of AdvSca1-SM cells, subsequent re-differentiation into mature SMC, endothelial cells, or macrophages, and contribution to plaque neovascularization. Mechanistic findings from both goals are being investigated in diseased human coronary arteries. RESULTS/ANTICIPATED RESULTS: Flow cytometry from SMC lineage tracing mice revealed a 7- to 13-fold expansion of AdvSca1-SM cells in carotid arteries ($p<0.001$) and aortas ($p = 0.03$) after 6 weeks of western diet; no differences in macrophage numbers were observed. Additional SMC and AdvSca1-SM cell lineage tracing mice are on atherogenic diets to assess early and advanced atherosclerosis. We predict that AdvSca1-SM cells will contribute to macrophage accumulation as well as plaque neovascularization in the setting of severe atherosclerosis. Translational relevance of mechanisms driving SMC reprogramming and AdvSca1-SM cell contribution to plaque progression are being applied to studies of diseased human coronary arteries. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data suggest a role for AdvSca1-SM cells in atherosclerosis. Ongoing work will clarify the mechanisms driving plaque-associated AdvSca1-SM expansion and define the ultimate fates of these cells. *In vivo* modulation of this process could provide the basis for future anti-atherosclerotic therapies. CONFLICT OF INTEREST DESCRIPTION: AD - CCTSI TOTTS TL1TR002533; SL - 18POST34030397 from the American Heart Association; AJ – no conflicts; KS - 1F31HL147393 from the National Heart, Lung, and Blood Institute, NIH; MM – no conflicts; RT – no conflicts; KSM – no conflicts; RAN - R01CA236222 from the National Cancer Institute, NIH, and 2018-03 from the Lungevity Foundation; and MCMW-E - R01 HL121877 from the National Heart, Lung, and Blood Institute, NIH, and 25A8679 from the Chernowitz Foundation.

4307

Role of Pre-pregnancy Uterine Natural Killer Cells in Human Embryo Implantation

Jessica Kanter[1], Sneha Mani, Scott Gordon, and Monica Mainigi
[1]University of Pennsylvania School of Medicine

OBJECTIVES/GOALS: Human placentation requires complex coordination between maternal and fetal cell types but remains incompletely understood. **We hypothesize that uterine natural killer (uNK) cells, an immune cell type that increases in abundance during the implantation window, is essential for appropriate implantation and placentation.** METHODS/STUDY POPULATION: We plan to examine stromal cell (SC) decidualization, spiral artery remodeling, and EVT invasion, processes vital for early pregnancy establishment, in the presence or absence of secretory phase uNK cells. Fetal extravillous trophoblasts (EVTs) will be isolated from first trimester pregnancy tissue; maternal SCs, endothelial cells (ECs) and uNK cells will be obtained from secretory phase uterine tissue. SCs will be placed in monoculture and coculture with uNK cells and prolactin will be measured to evaluate decidualization. To study EVT invasion, we will utilize our novel "implantation-on-a-chip" device to determine how addition of uNK cells affects EVT migration through a collagen-matrigel matrix. In this system, we will also examine spiral artery remodeling with or without uNK cells via TUNEL staining. RESULTS/ANTICIPATED RESULTS: We anticipate that uNK cell addition to SCs will lead to a significant increase in SC prolactin levels, suggesting a role of uNK cells in endometrial decidualization. In vitro, we expect the addition of uNK cells will increase EC apoptosis and promote EVT invasion. DISCUSSION/SIGNIFICANCE OF IMPACT: Although decidual NK cells are known to participate in placentation, the role of pre-pregnancy uNK cells is unknown. uNK cell involvement in processes important for the earliest stages of pregnancy would provide a potential marker for abnormal placentation and offer avenues for intervention to decrease placentation associated perinatal morbidity.

4121

The beneficial, anti-fibrotic effects of chemokine receptor 2 and 5 antagonists on fat-exposed mouse primary hepatic stellate cells (pHSCs)

Annie J. Kruger[1], Bergman[2], Martha Gay[1], Hong Cao[3], Robin Tucker[3], Narayan Shivapurkar[3], and Jill P. Smith[3]
[1]Georgetown - Howard Universities; [2]St. Louis University School of Medicine; [3]Georgetown University

OBJECTIVES/GOALS: Non-alcoholic steatohepatitis (NASH) is a leading cause of cirrhosis in the world for which no anti-fibrotic therapies exist. We hypothesized that BMS-22 and maraviroc (MVC), chemokine receptor 2 (CCR2) and 5 (CCR5) antagonists, respectively, would diminish the fibrogenic activity of "fat-exposed" murine pHSCs. METHODS/STUDY POPULATION: pHSCs were isolated from livers of 6 week old male mice following 4 weeks on a NASH-inducing choline-deficient high fat diet (CDAHFD, "fat-exposed") or standard diet (SD) and passaged *in vitro*. Early passage (6-12) pHSCs were plate-adhered and TGF-b-treated (10ng/mL) to maximally activate their pro-fibrogenic genes, *collagen 1α1*

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