ABSTRACTS

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LJA5: A Novel Population of Neurons in the Brainstem

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Abstract

Background: Dynorphin is an inhibitory neuropeptide that is expressed in well-characterized nuclei throughout the brain. However, we have discovered a novel population of dynorphin expressing neurons in the caudal ventrolateral pons which we have named LJA5. We aimed to map the afferent and efferent connections of LJA5, and to test the function of this novel population.

Hypothesis: Based on its location in the pons (lateral, juxta the catecholamine group A5), we hypothesized that LJA5 would have long, interesting projections to the spinal cord, and would suppress interoceptive functions including temperature, pain, or itch.

Methods: To selectively map neuronal pathways from the LJA5 dynorphin neurons, we used adult mice with Cre recombinase inserted after the dynorphin gene (Pdyn-IRES-Cre mice), and made microinjections of Cre-dependent adeno-associated viral (AAV) vectors into the brainstem. Mice were deeply anesthetized and stereotaxically injected (10-50 nl) in the LJA5 region with an AAV coding for channelrhodopsin and cholera toxin subunit B (CTB), an anterograde and retrograde tracer, respectively. The AAV vector carries a gene for red fluorescent protein (mCherry) that is in reverse orientation (DIO) that can be inverted only by Cre recombinase. Thus, mCherry is produced only in cells expressing Cre, which in this mouse strain is limited to those that express dynorphin. Four weeks after the injections, we transcardially perfused the mice and removed their brains. Anterograde axonal labeling with mCherry and retrograde labeling with CTB was revealed by immunostaining. We then repeated the injections with an AAV coding for hM3, an excitatory DREADD (designed drug exclusively activated by designer receptor). Four weeks later we made an intraperitoneal injection of a designed drug, clozapine-n-oxide (CNO) to selectively activate the LJA5 dynorphin neurons.

Results: The anterograde and retrograde tracers revealed numerous and robust afferent and efferent projections to areas known to be involved in pain and temperature regulation such as the periaqueductal grey, and parabrachial nucleus, and the most striking finding, lamina I of the spinal cord. Additionally, the DREADD technique produced robust c-fos expression (a marker of activation) specifically in the LJA5 neurons as compared to saline controls.

Conclusions: The dorsal horn of the spinal cord receives sensory information regarding itch, pain, and temperature. Many labs study the peripheral pathways for these sensations, but how the brain modulates these sensory inputs remains relatively unknown. We have discovered a novel population of dynorphin expressing neurons in the caudal ventrolateral pons that sends very dense and specific projections to lamina I of the dorsal horn, which contains all the neurons transmitting itch, pain, and temperature sensations to the brain. We believe this new, top-down pathway has the potential to suppress itch, pain, or temperature sensations, a hypothesis which we are currently testing with the DREADD technique.

The Usage of Telemedicine for the Evaluation of Pharyngitis Compared to In-Person Physical Exams

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Study Objectives: Telemedicine is an interface that has been used to facilitate direct-to-patient medical care, especially for urgent care complaints. One complaint for which telemedicine has been specifically marketed is for patients with sore throat (pharyngitis). For these patients, the physical examination is an important aspect of directing clinical management, but the ability to accurately evaluate patients with pharyngitis using a telemedicine interface is unclear. The objective of this study is to compare the accuracy of the physical examination using telemedicine vs. face-to-face examination.

Methods: Cross-sectional diagnostic study comparing face-to-face and telemedicine for patients presenting with sore throat to a 60,000-vist academic emergency department between March and August 2016. An advanced practice provider scored each physical examination in a face-to-face encounter, then a second provider (blinded to the first examination) scored the same examination using a telemedicine connection (VidyoMobile^{TM}, Vidyo, Inc, Hackensack, NJ). Patients were allowed to use a flashlight and tongue depressor to optimize the view for the telemedicine provider. The study was powered for non-inferiority to detect a 20% difference in the rate of pharyngeal redness, 62 patients were estimated to be required. Secondary outcomes included agreement on other aspects of the physical examination.

Results: We enrolled 46 patients. The agreement between the two examiners on identification of posterior pharyngeal redness was only 65%, which was below the predetermined threshold we identified for non-inferiority. For secondary outcomes, agreements were: 95% asymmetry of posterior pharynx, 53% tonsillar size, 95% uvular deviation, 86% soft palate color, 70% uvula color, 74% tonsillar color, 65% posterior oropharynx color, 98% exudate, 86% swelling, 100% ulceration, 83% - 96% for lymph node tenderness, and 87% - 98% for lymph node swelling.

Conclusion: The pharyngeal examination performed by telemedicine correlates poorly with the face-to-face examination. Future work should characterize the importance of the pharyngeal physical examination in influencing medical therapy, and the accuracy of other physical examination findings useful in direct-to-patient telemedicine applications.

Compensatory Effects of Carotid Body in Lmx1b Mice Suggests Central Chemoreceptor Role of Serotonergic Medullary Neurons

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Background: Sudden Unexpected Death in Epilepsy (SUDEP) is one of the most common causes of death in epileptic patients. While it is not as lethal as many other neurological disorders, the loss of quality years of life due to SUDEP is incredibly high, with the average SUDEP patient being 30 years old. Recent studies have suggested that SUDEP may be caused by post-ictal respiratory failure. The serotonergic neurons in the Raphe Nuclei of the Medulla have been suggested to have a role in respiration as central chemoreceptors, stimulating breathing in hypercapnic environments. Previous studies have used the Lmx1b mouse model to study these neurons. The Lmx1b mouse model uses Pet1-Cre to knockout the transcription factor Lmx1b specifically in serotonergic neurons. These mice have no serotonergic neurons and often have apnea and high neonatal mortality. Previous work to study the hypercapnic ventilatory response (HCVR) in this mouse model has shown a higher HCVR than was anticipated. It has been suggested that the carotid body, which acts as a peripheral chemoreceptor to stimulate breathing during hypoxia, may have some sort of compensatory effect in this mouse model. The purpose of this study was to study the HCVR in Lmx1b mice with inhibited carotid body function

Hypothesis: Lmx1b mice will have a decreased hypercapnic response after denervation of the carotid body.

Methods: Lmx1b mice were anesthetized with 2% isofluorane and were then operated on. The glossopharyngeal nerve and carotid body were resected bilaterally. The mice were given 3 days to recover after the surgery and were given Meloxicam to aid in their recovery. Before and after the surgery, Plethysmography was performed to measure the minute ventilation of the mice at different concentrations of oxygen and carbon dioxide. Breathing was measured at 50% oxygen (hyperoxia), 10% oxygen (hypoxia), and 50 oxygen with both 5% and 7% carbon dioxide (hypercapnia).

Results: Our Preliminary data shows that mice with a successful surgery had a reduced minute ventilation at both 50% oxygen and 10% oxygen. The mice also showed a blunted HCVR at both 5% and 7% oxygen respectively after carotid body denervation

Conclusion: This data suggests that the carotid body does have a compensatory effect in Lmx1b mice. This reinforces the concept of the serotonergic neurons in the Raphe Nuclei as central chemoreceptors that drive respiration.

Redefining the Target Area for Leprosy Elimination Programs Through Serological Evaluation of a Broader Definition of Leprosy Contacts

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Background: Brazil has the highest incidence of new cases of leprosy in the world with 33,000 new cases reported in 2014. Leprosy is caused by *Mycobacterium Leprae* infection and is spread from person to person mostly through the upper respiratory tract. Subclinical individuals are greatly involved in silent transmission. Therefore, to improve control and eventual elimination efforts it is pertinent to analyze the risk that exposure to a multibacillary leprosy patient poses in order to identify who may be at the greatest risk to develop disease or silently propagate infection. Currently, evaluation of only household contacts (HHC's) has been the cornerstone of case-finding campaigns, yet incidence stubbornly fails to decrease. In the Northeast of Brazil several studies have been conducted to better understand the risk of exposure in HHC's as well as in neighbors. Regarding multibacillary leprosy cases (the group considered to be the primary source of infection) no significant difference has been reported between seropositivity of their HHC's compared to their next door neighbors. These results hold extremely important implications for control program protocols since the foci of infection is larger than the focus on only HHC's allows for. The purpose of this study was to analyze the rate of seropositivity in individuals with close contact to a leprosy patient compared to those without close contact when extending the definition of close contact to include individuals living within three blocks of a leprosy case.

Hypothesis: Individuals who live with *or near* a person with clinical multibacillaryleprosy (defined as living less than 3 blocks away) will be more likely to be seropositive for *M. leprae* compared to individuals who do not live with or near an individual diagnosed with leprosy (defined as living farther than 3 blocks away).

Methods: A cross-sectional study design was used for this study which was conducted in five areas of the perimetropolitan areas of Natal in Northeast Brazil: the North district, São José de Mipibu, Várzea, Nova Cruz, and Arez. Index leprosy cases (9) who were under current treatment were identified at the referral center for leprosy at Giselda Trigueiro Hospital. Home visits were made to the index leprosy cases and surrounding households between June to August 2016. 59 participants with contact to a leprosy patient (within 3 blocks of the patient) and 80 controls (participants who did not have contact with a leprosy patient but lived in the same neighborhood more than 3 blocks away from the patient) were recruited. A consent form and a three-page questionnaire were administered verbally to all participants. Blood samples were collected during the home visits via venipuncture. All participants' sera were tested for *M. leprae* infection using via ML Flow and LID-NDO via standard enzyme-linked immunosorbent assay (ELISA). Data from the questionnaires and serological tests were analyzed statistically using logistic regression to determine potential risk factors for seropositivity.

Results: A total of148 individuals were recruited to participate in this study. The average age for research participants was 35 years of age with ages ranging from 4 to 89 years of age. Females made up the majority of the research participants with 62% of individuals being females. When considering both the ML Flow and ELISA LID-NDO results, the rate of seropositivity was slightly higher among women with 20% of women compared to 16% of men reporting seropositivity. Seropositivity among contacts was twice the seropositivity rate among controls (22% vs 13%). An 84% concordance was found between the ML Flow and ELISA methods.

Conclusion: The results of this study will inform control program design as surveillance of multibacillary leprosy patients should include neighboring residents and not only household contacts in order to obtain elimination. This study also demonstrates the utility and simplicity of PGL-I or NDO-LID assays during home visits as effective tools for new case diagnosis in endemic areas.

Developing an Organ Culture Model for the Autoimmune Blistering Disease, Bullous Pemphigoid

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Background: Bullous pemphigoid (BP) is an autoimmune disease targeting epidermal attachment proteins resulting in inflammation and blistering of the skin. Interestingly, BP manifests exclusively in the skin, despite the fact that the target antigen is expressed in many other tissues throughout the body. Immune responses to epidermal antigens are generated largely within the skin itself through antigen presenting cell (APC)-mediated activation of various T cell populations. Local activation and proliferation of regulatory T cells (Treg) is a critical component of peripheral tolerance via their ability to suppress autoreactive effector T cells. In BP, the origin of the autoimmune response is unknown; however, the advanced age of onset, localization of lesions to the skin, and the transient nature of the disease suggest a defect in tissue-specific immune regulation. Previous experiments have found differences in dendritic and T-cell subsets in flaring BP skin biopsies compared to BP remission and control skin. Staining for MHC class II and langerin revealed a decrease in the overall number of APC and Langerhans cells in flaring BP skin compared to control and BP remission skin. There was also a significant decrease in the number of Tregs (CD4+/FOXP3+ cells) observed in flaring BP skin when compared to control skin. In our effort to explore the process of when and why these immune populations change in BP vs. control skin, we have developed a human skin organ culture model. Using organ culture, we were able to study different environments that may affect immune populations in the skin.

Hypothesis: Using organ culture to mimic the BP environment will affect immune populations differently than skin cultured in a control media environment.

Methods: We obtained discarded skin from skin cancer surgeries at the UIHC Dermatology Clinic. We then cultured this skin 1-3 days using control media, 20% BP sera supplemented media, 20% BP blister fluid supplemented media, or 20% BP antibody supplemented media. After culturing, we froze skin using OCT and then cut sections onto a slide to stain.

Results: Staining with CD207, we found that there were significantly fewer Langerhans cells in skin that had been cultured in media supplemented with BP serum compared to both skin cultured in control media and skin not cultured at all. Staining with CD4 and FOXP3, we found that there were not significant differences in Treg numbers in skin cultured media supplemented with BP serum vs. skin cultured in control media or the same skin that was not cultured. Using annexin staining for apoptosis, we confirmed that the Langerhans cells were not dying in any of the skin, but instead potentially migrating away in the skin cultured in BP serum. When observing other conditions that skin sections were cultured in, we found no significant differences in any immune cell populations in skin cultured in media supplemented with blister fluid from a flaring BP patient, however, exposure of skin to BP antibodies results in a decrease in Langerhans cells.

Conclusion: We were able to develop an organ culture model for studying different environments affecting immune populations in the skin. Culturing skin with BP serum and BP antibodies shows a decrease in Langerhans cells compared to skin cultured in control media. We believe Langerhans cells may be migrating away in these skin sections, leading to eventual loss of Tregs.

Physics-Based Protein Optimizations Determine Missing Loops and Predict Structural Consequences of Missense Variants

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ABSTRACT

BACKGROUND: Many approaches are being explored to assist clinical decision-making in light decreasing costs associated with genetic sequencing. For missense variants that have not been characterized biochemically, computational approaches can provide tremendous insight on the impact on protein structure, function and human phenotype. Success for computational approaches relies heavily on accurate protein structures for both wildtype and variant sequences. However, x-ray crystallography, a common method for elucidating protein structures, often fails to pinpoint the atomic coordinates of highly flexibly and mobile loops. Thus, missing loops must be built correctly to permit downstream molecular simulations. For example, the phosphate binding loops and/or activation loops of kinases are notoriously difficult to study experimentally, but of particular importance to function.

HYPOTHESIS: Optimal protein structures can be generated for incomplete protein structures using simulations to provide a physics-based model of intermolecular interactions for missense variants.

METHODS: Optimization using biased molecular dynamics, a polarizable force field (AMOEBA), and an electron density target was used to predict loops in a number of x-ray crystallographic structures. The approach combined experimental data from X-ray crystallography with a physics-based model of intermolecular interactions into a hybrid target function. Conformational space was explored efficiently by adding a time-dependent bias to the aforementioned objective function. Furthermore, a state variable (λ) controlled the strength of non-bonded energy terms (i.e. softcore van der Waals, electrostatic interactions terms) with λ varying continuously between 0 and 1. When was $\lambda=0$, the loops were in an unphysical vapor-like phase while the non-bonded energy terms were scaled to zero eliminating energy barriers. Similarly, $\lambda=1$ was consistent with the experimental crystal, and local minimizations were performed to fit only the x-ray data exclusively.

RESULTS: The improved optimization method was assessed to be deterministic using a 4-residue loop. 300 ns aggregate sampling among 30 contributing simulations proved to deterministically find the global minimum using the polarizable hybrid target function. The low energy configuration was observed in 90% of the simulation windows. Experimental fit (using RFree metric) and physical accuracy (using Molprobity Score) were compared with the current standard method, fixed charged simulated annealing (SA). Average RFree was 23.94 with AMOEBA and 24.53 with SA, and average Molprobity Score was 1.56 with AMOEBA and 1.75 with SA. Both RFree and Molprobity use lower numbers to signify better structures. Modeling of PAX6 and TP63 using these methods demonstrated unfavorable electrostatic interactions when assessing R19W and R26W on PAX6 and R343W on TP63.

CONCLUSION: A Polarizable force field, represents crystalline environments more accurately, yields better loops than a fixed charge simulations, and the hybrid target makes optimal use of weak loop diffraction data.

Quality of Life and Burn Support Services Among Burn Survivors in Iowa

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BACKGROUND: Burns result in 500,000 hospital visits and 40,000 hospitalizations annually in the United States. In addition to the immediate physical concerns and the trauma of the burn itself, survivors face a variety of psychosocial challenges during recovery. Changed appearance, physical injuries, and limb deformities can make re-entry to family life, society, and work incredible difficult.

Support services are a key factor in recovering quality of life after a serious burn. Studies have shown that participation in support groups is linked to better outcomes in a variety of areas. However, the Midwest has one of the lowest participation support group participation rates for burn survivors. The goal of this research seeks to demonstrate what the barriers to participation are, and how Iowa burn survivors are faring in their psychosocial recovery.

HYPOTHESIS: The barriers to participation in support services and quality of life among burn survivors in Iowa can be identified and enumerated.

METHODS: Current adult burn survivors with a length of stay of 5 or more days in the Iowa Burn Unit within the last ten years (n=968) were mailed fliers with a description of the study and the website, www.iowaburnstudy.com, where they could access the survey in the beginning of June 2016. In order to maximize response rate, posters advertising the survey were placed in the outpatient burn clinic and on the St. Florian Fire and Burn Foundation Facebook page. All recruited participants were called to remind them of the survey, and to offer an email with a direct link to the survey, or a mailed paper survey.

The survey was offered through Qualtrics Insight Platform and was divided into 5 categories: demographics, burn support group participation, Burn Specific Health Questionnaire, Community Integration Questionnaire, and Perceived Stigmatization Questionnaire. STATA software was used to perform descriptive analyses of 118 subjects.

RESULTS: One hundred eighteen responses were received. Counterintuitively, results suggest that burn support participants experience lower quality of life scores across a variety of areas. Forty-three percent of respondent stated they did not need support groups, while 38% responded they were not aware of the support programs. Seventeen percent gave distance or lack of transportation one of the reasons that limited their access to support groups.

CONCLUSIONS: There is a significant proportion of burn survivors who might benefit from support programs but have not been able to due to lack of awareness, transportation or distance. It would be beneficial to expand the outreach efforts to include rehab and nursing facilities so survivors can be made aware of support programs before they return home.

A Comparative Study of Ponseti Method Pedagogy in Peru

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Background/rationale: Idiopathic clubfoot is one of the most common congenital deformities in the world, with an incidence of about 1:1000 live births (1-3). While in the past surgical options have been favored, the current gold standard for treatment is the Ponseti method (4-5). The preference of the Ponseti method is due in large part to its low costs (6) and high success rate (7), which makes it a particularly attractive option for low to middle income countries (LMIC). It is estimated that between 150,000-200,000 children are born with clubfoot every year, with 80% of those being born in LMIC (1, 4, 7-8). Left untreated, individuals suffering from clubfoot are unable to work, unable to ambulate, and they are unable to be contributing members of society (8). The Ponseti method provides a low-cost treatment option for a disease that negatively impacts thousands of lives every year. This adheres to the principles set forth by the United Nations, which comments, "States Parties shall take all necessary measures to ensure the full enjoyment by children with disabilities of all human rights and fundamental freedoms on an equal basis with other children" (9).

Purpose of this study: hypothesis/aims: The research project focused on how doctors are trained in the Ponseti method for treating clubfoot, and by extension, which method of teaching provides the greatest physician satisfaction and maintenance of those skills. The three primary methods of teaching the Ponseti method include: 1) continuing medical education, (2) physician-mentorship program, and (3) video conferencing. By establishing a clearly favored training method, future Ponseti Method training sessions can better cater to the needs expressed by its practitioners, with their patients being the greatest beneficiaries.

Method: The principal investigator designed a survey which was disseminated either in person or via email to the Ponseti practitioners in Peru. The inclusion criteria for this study included any healthcare professional (such as a doctor or physical therapist) who treated idiopathic clubfoot using the Ponseti method in Peru. Practitioners who treated idiopathic clubfoot by other methods were not included. The survey consisted of 25 questions, which were estimated to take between 15-20 minutes to complete. In order to contact participants, Ponseti International Association (PIA) maintains a registry of all Ponseti Method practitioners in Peru. To the existing registry of 32 physicians, 5 more individuals who completed Ponseti training this summer were added for a total of 37 participants. From that final list of 37 individuals, 5 physicians did not have an email address listed. In addition, the email addresses for 3 individuals were found to be no longer active. In the end, 29 participants were contacted via email for this study, with the goal of reaching at least 20 participants. The surveys were disseminated starting on June 27, 2016, with a final date of participation to be July 22, 2016.

Results: Of the 9 physicians who have responded so far, 7 had their Ponseti trainings in Peru. Eight of the 9 have at least an 8/10 confidence level in their ability to correctly use the Method. With respect to their training methods, people seemed to appreciate the ability to practice the method with actual patients at Ponseti workshops, while they expressed concern that not all their patients would arrive with adequate material (i.e., casting material) to be properly treated.

Conclusions: Peruvian physicians surveyed for this study are committed to using the Ponseti Method as the gold standard of club foot treatment. The majority expressed an interest in having future Ponseti workshops followed by a physician mentorship program where learners could perfect their technique under the guidance of an experienced Ponseti practitioner. Perhaps the individuals surveyed might serve such leadership roles.

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The Utility of Magnetic Resonance Imaging in the Clinical Evaluation of Infantile Nystagmus

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Abstract

Background: Infantile nystagmus is an involuntary eye movement disorder. It is often considered a diagnosis, however it is usually a sign of ocular, neurologic, or systemic disease requiring a complex workup. Despite the wide range of possible diagnoses and the many testing modalities available, there is no agreed upon protocol as to which tests should be used first and for which patients.

Hypotheses: (1) Most patients referred to the pediatric eye clinic for infantile nystagmus will have a vision-related cause, rather than a neurologic cause. **(2)** MRI is the most common first test performed for infantile nystagmus.

Methods: A retrospective chart review of all patients presenting to the pediatric genetic eye disease service from 2008-2016 was performed. Clinical data collected included results from complete pediatric eye examination and all ancillary testing completed both before and after referral, with documentation of the order in which testing was performed. Final diagnoses were tabulated. Final diagnosis was classified as "unknown" unless definitive electrophysiologic, anatomic or molecular diagnosis was obtained. Exclusion criteria were absent or acquired nystagmus, or no eye examination recorded.

Results: EPIC and the genetic eye database were searched for nystagmus or nystagmus-associated diagnoses. 284 charts were identified. 202 charts fit inclusion criteria. The 3 most common causes of infantile nystagmus were albinism (18%), non-Leber Congenital Amaurosis (LCA) retinal dystrophies (16%), and LCA (14%). 28 patients had magnetic resonance imaging (MRI) performed as their first test for nystagmus without other neurologic signs; 0/28 had a diagnostic MRI. 45 patients had an MRI performed as the first test due to neurologic signs and 32% were diagnostic. When electroretinogram was performed as the first test, a retinal disorder could be confirmed in 56% of cases, when OCT was the first test 55% were diagnosed, and when molecular genetic testing was ordered as the first test 47% were diagnosed. For all 202 patients, 50% had an MRI performed at some point in their work-up and 16% of these were positive, however only 2% of patients had a purely neurologic cause of nystagmus. All 202 patients had a complete pediatric eye examination which led to a clinical diagnosis in 67%.

Conclusions:

The most common causes of infantile nystagmus in this pediatric ophthalmology cohort are retinal disorders, totaling 55% of all cases. Conversely, the most common first test in the nystagmus work-up was brain MRI, which cannot diagnose these disorders. MRI is not the best first test for patients with infantile nystagmus in the absence of other neurologic stigmata. ERG, OCT and molecular genetic testing in an order determined by clinical findings should be performed.

Identification of Key High Efficiency Practices of Providers in a Community Emergency Department

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BACKGROUND: Increasing emergency department patient volume and acuity, declining numbers of EDs, and increased reporting of efficiency metrics prompt ED providers to strive for increased efficiency. Yet, much variability in provider-level efficiency remains unexplained (defined as Relative Value Units per hour, RVU/h).

OBJECTIVE: The objective of this study was to determine behaviors and practices associated with high provider efficiency in a community ED.

METHODS: A mixed methods study design was utilized to identify key behaviors associated with efficiency:

Stage 1. A convenience sample of sixteen participants (4 ED medical directors, 4 ED nurses, 4 ED advanced practice providers, and 4 ED physicians) listed provider efficiency behaviors during semi-structured interviews. Theme saturation was achieved. Ninety-two behaviors were identified and distilled by a group of three EM physicians into 32 themes.

Stage 2. An observational study of 13 consenting (consent t rate= 71.4%) providers was performed in a 55,000-visit community ED during two 4-hour periods and recorded in minute-by-minute observation logs.

Stage 3. Each behavior or practice from Stage 1 was assigned a score within each observation period. The efficiency outcome was defined as RVU/h.

Behavior scores from Stage 3 were used to predict RVU/h for each provider, using univariate linear regression.

RESULTS: Mean RVU/h data for observed providers was 7.20 ± 1.13 RVU/h. A strong positive linear relationship was observed between simultaneous patients and productivity (b1=0.701, 95%CI 0.525 – 0.876). Moderate correlations were observed for the behaviors of visits to patient rooms/h (b1=0.4585, 95%CI 0.283-0.634) and percent time spent running the board (b1=0.6642, 95%CI 0.489 – 0.839). As a robustness check, sensitivity analysis was conducted by repeating the analysis with patients/h as the outcome, and the conclusions were unchanged as the two outcomes were strongly correlated (R^2=0.958).

CONCLUSIONS: More patients seen simultaneously, total times visiting patient rooms per hour, and time spent running the board is associated with enhanced provider productivity (RVU/h). Identification of behaviors associated with efficiency can be utilized by clinicians, trainees, and learners to improve personal efficiency or counsel team members. In the future, data from additional sites could be incorporated to develop more robust models of efficiency behaviors.

The effect of preoperative status of patellofemoral joint surfaces on the outcome scores of patients who have undergone Fulkerson realignment surgeries for chronic patellofemoral instability.

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ABSTRACT

BACKGROUND: Patellofemoral dislocation remains a difficult clinical problem despite the existence of surgical procedures that can effectively prevent recurrent dislocation episodes. Realignment of the extensor mechanism of the knee through a Fulkerson osteotomy is generally considered to produce good stability, but variable functional outcomes. Chondromalacia (CM), of the PF joint appears to be a limiting factor despite reestablishing stability. There is little known about the relationship between the presence of preoperative CM and their expected short or long-term outcomes. We plan to investigate this short-term relationship through the review of preoperative clinical exam records, MRI, direct perioperative visualization of articular cartilage changes, and standardized patient-reported knee surveys (KOOS, WOMAC). The purpose of this study is to determine if the preoperative presence of CM is an important clinical predictor of the short-term functional outcome success of this procedure.

HYPOTHESIS: We expect that patients without CM prior to surgery will have better short-term functional outcomes than those who do have evidence of CM.

METHODS: The electronic medical records of 97 UI Sports Medicine patients (ages 18.0-39.8 years) who underwent Fulkerson osteotomy procedures with MPFL reconstruction or MPFL reconstruction alone were reviewed as part of this retrospective study. From this list, 24 patients were excluded due to an age >40 years, confounding conditions, or insufficient data. For each patient, the status of the articular cartilage of each knee was assigned a chondromalacia grade ranging from 0-4 based on the modified Outerbridge grading system. Patients were then grouped into two groups: those without pathologic changes (grade 0) and those with pathologic changes (grades 1-4). KOOS, WOMAC, and SF36 survey scores obtained preoperatively and 6-84 months postoperatively were then compared between the two groups. Other data was also obtained from medical records, such as patient medical history, operative outcomes and complications.

RESULTS: Patients without CM (n=48) had significantly better average postoperative survey scores in the KOOS pain (73.37 (95% CI [68.82, 77.92]) vs. 60.25 (95% CI [51.39, 69.11)) and KOOS ADL (73.53 (95% CI [69.95, 77.56]) vs. 60.25 (95% CI [50.63, 66.79)) categories compared to patients with CM (n=25). Preoperatively, WOMAC pain scores were significantly better in patients without CM.

CONCLUSIONS: Patients without CM at the time of reconstruction experienced better outcomes in the early postoperative time frame with KOOS measures of ADL's. Despite higher levels of activity, patients without CM experienced significantly less pain both pre- and postoperatively. The long-term outcomes of patients with a normal PF surface is yet to be determined.

Early lung disease exhibits bacterial-independent pathogenesis in cystic fibrosis pigs

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Background: Lung disease characterized by airway infection, inflammation, and mucus accumulation is the primary cause of morbidity and mortality in individuals with cystic fibrosis (CF). However, it is still unknown if lung disease develops as a primary effect of CFTR dysfunction or is secondary to bacterial infection. Ethical limitations in human studies make it difficult to address this question. Development of a CF porcine model that spontaneously acquires lung disease enabled us to investigate the role of infection in the pathogenesis of CF lung disease by simulating a bacterial-independent CF lung environment with intensive antibiotic therapy.

Hypothesis: We hypothesized that i) CF pigs develop lung disease characterized by infection, histological markers, and air trapping at 3 weeks, and ii) intensive antibiotic therapy to CF pigs from birth would prevent the development of lung disease during the first 3 weeks of life.

Methods: Pigs in each cohort (CF, CF + antibiotic therapy, non-CF) were assessed for lung disease at 3 weeks by characterizing bacterial colonization with standard microbiology techniques and quantitative PCR (qPCR), evaluating airway histopathology, and performing computed tomography (CT) imaging of lungs.

Results: CF pigs had more bacteria present in their lungs at 3 weeks ($\mu=6.7 \times 10^3$ CFU/sample, range=0-2.2×10^6 CFU/sample) than non-CF animals ($\mu=31.8$ CFU/sample, range=0-2.5×10^4 CFU/sample, $p<0.01$ non-CF v. CF), a finding similar to our earlier studies in newborn pigs. A similar number of bacterial species were recovered between genotypes. Similar to humans with CF, a dominant species emerged in CF pig lungs when analyzing bacterial species with a cutoff of 10^4 CFU/sample. Compared to non-CF animals, CF animals had significantly greater histological evidence of lung disease and air trapping at 3 weeks. CF pigs receiving intensive antibiotic therapy had lung bacteria abundance ($\mu=9.3$ CFU/sample, range=0-2.2×10^4 CFU/sample) comparable to non-CF animals, but significantly less than non-antibiotic treated CF animals ($p<0.05$). Surprisingly, CF pigs treated with antibiotics had histological lung disease and air trapping similar to untreated CF animals. Bacterial abundance was comparable among all three groups when quantified by qPCR likely due to the inability to differentiate between live and dead bacteria. Lung bacterial abundance was not related to airway inflammation, obstruction, mucus accumulation, or air trapping in any cohort.

Conclusions: CF pigs spontaneously developed lung infection with a dominant bacterial species, as well as histological lung disease and air trapping at 3 weeks. Intensive antibiotic therapy in CF pigs from birth reduced lung bacteria to non-CF levels, but some aspects of lung disease and air trapping developed that were comparable to untreated CF pigs. These findings suggest that both bacterial-independent and -dependent mechanisms underlie CF airway disease pathogenesis. Possible etiologies of bacterial-independent CF lung disease may include airway developmental structural changes, altered mucus properties, or an altered inflammatory response due to loss of CFTR function.

Regulatory consequences of mutation in a human mental illness risk gene

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Background/Rationale:

In the early 2000s a translocation event disrupting the Npas3 gene was observed in a mother and daughter who both had schizophrenia (SZ) and intellectual disability (ID). A mouse model was quickly made and found to have several behavioral abnormalities along with decreased neurogenesis in the hippocampus, which is a well-characterized phenotype of schizophrenic patients. Several years later it was observed that Npas1 repressed hippocampal neurogenesis, opposite of the Npas3 gene's effect in this region. Our study aims to explore the regulatory networks controlled by the Npas1 and Npas3 transcription factors (TFs) with the goal of relating these findings to neurogenesis and human mental illness.

Purpose of the study: Hypothesis/Aims:

Define genes regulated by Npas1 and Npas3, their regulatory context, and potential roles in human mental illness.

Methods:

Using Npas1 and Npas3 knock-out (KO) mice, we used RNA-Sequencing to define a list of genes which were differentially regulated compared to wild-type (WT) mice. We also performed ChIP-Seq to find areas in the genome which were bound by Npas1 and Npas3. These two overlapping sets of genes were used for subsequent bioinformatics analyses.

Results:

We found that 506 and 1,347 transcripts were differentially expressed in Npas1 and Npas3 KO mice, respectively. We also found 7,183 and 12,914 binding events for the Npas1 and Npas3 transcription factors (TF), respectively. Using ENCODE mouse forebrain data, we identified a recurrent trough pattern in many histone marks and DNAse-Seq data. Using Brainspan data we found that Npas3 differentially expressed genes (DEGs) shared a high level of correlation with Npas3 expression in human hippocampal data. Using clinically related data we show that Npas3 DEGs which had a binding event upstream of or within their gene structure had a higher mutational burden in schizophrenic patients compared to healthy volunteers. Lastly, we show significant overlap between gold standard intellectual disability genes and our Npas1 and Npas3 DEGs.

Conclusion/Discussion:

The primary findings of our study are the direct and indirect targets of the Npas1 and Npas3 transcription factors. These unbiased analyses provide an important foundation for future mental illness related studies. Using ENCODE we show that Npas1 and Npas3 binding events have an epigenetic profile typical of regulatory regions, helping verify our data. Further, we conclude that mouse-based Npas3 results are broadly generalizable to humans due to Npas3 DEGs showing significant correlation with Npas3 expression in human hippocampal data. Lastly we used SZ and ID human data to show that our findings are relevant to human disease. In conclusion, we have defined a regulatory network of genes shown to be relevant to human disease which can serve as a useful starting point for future SZ and psychiatric disease research.

Effects of trypan blue 0.06% on mitochondrial respiration in human corneal endothelial cells

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Abstract

BACKGROUND: Descemet membrane endothelial keratoplasty (DMEK) is an increasingly popular cornea transplant technique that involves one-to-one replacement of diseased patient corneal endothelium-Descemet membrane complex (EDM) with donor grafts that have been separated from the donor corneal stroma. DMEK preparation and transplantation has relied on off-label usage of trypan blue, a vital dye stain, for visualizing the EDM. The success of this technique speaks to the overall safety of trypan blue. However, one major limitation of studies to date is that indirect methodologies have been used to assess cellular damage and little attention has been given to the effects of trypan blue on corneal ATP production, which has direct effects on corneal pump function and visual clarity. In 2014, only 11% of all endothelial keratoplasties were DMEK surgeries. As this technique grows in popularity, it will be important to be able to determine what steps during preparation and transplantation may affect visual outcomes.

PURPOSE: To survey the metabolic effects of trypan blue 0.06% ophthalmic solution on human corneal endothelial cells.

METHODS: Corneoscleral tissues were obtained from cadaveric sources according to Iowa Lions Eye Bank (ILEB) and Eye Bank Association of America (EBAA) standards with research consent. Human corneal endothelial cells (HCEC) were isolated to produce a single-cell suspension amendable to growth in culture. Confluent cultures were assayed for mitochondrial activity following treatment with trypan blue 0.06% for various durations (1, 5, and 30 minute exposures) using extracellular flux analysis of oxygen and compared to untreated control wells using HCECs from the same donor.

RESULTS: Following trypan blue exposure using X samples per group, no statistically different responses were detected in any metabolic parameters measured (basal respiration, ATP production, proton leak, maximal respiration, spare respiratory capacity, or non-mitochondrial respiration) for any group.

CONCLUSIONS: Exposure to trypan blue 0.06% for up to 30 minutes did not alter mitochondrial respiration parameters and is unlikely to have acute toxicity in this setting. Additional studies may be warranted to determine the effects of trypan blue exposure in native graft tissue where this dye may be present in Descemet membrane and stroma for more prolonged duration following surgery.

Medical Imaging for Lung Cancer Detection
Testing Statistical Models to Improve Screening

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BACKGROUND: Lung cancer is responsible for over 1 in 4 cancer deaths in the United States. The National Lung Screening Trial (NLST) demonstrated that CT screening detects more early-stage cancers than chest radiography and reduces mortality from lung cancer. As a result, the US Preventive Services Task Force has recommended annual CT screening for high risk individuals. However, 96.4% of the abnormal CT screens from the NLST were false positive results, requiring additional imaging and/or invasive procedures to resolve the diagnosis. In an effort to decrease false positive rates of these screens, we studied the relationships between 144 radiomic features of nodule and surrounding tissue – size, intensity, shape, texture – and clinical information from a cohort of 198 individuals screened for lung cancer.

HYPOTHESIS: Radiomic features of both nodule and parenchyma can be used to improve the specificity of lung cancer screening CTs.

METHODS: This was a retrospective study that evaluated the use of clinical and radiomic features to improve the classification of lung cancer status based on CT. We used clinical/ demographic data and CT scans from the medical records of 198 subjects that met the criteria of: a) positive for a solitary lung nodule (5-30 mm) detected by CT and b) benign tumor or noncancerous condition confirmed by pathology or additional imaging (control) or pathology-confirmed primary lung cancer (malignant). Diagnosis was confirmed via surgical resection (78 and 15.7%), bronchoscopic biopsy (16.5% and 23.6%), fine needle aspiration (5.5 and 56.2%), or additional imaging (0 and 4.5%) for malignant diagnosis group and control group, respectively. Radiomic data were collected by first segmenting the image into nodule and parenchyma and then using algorithms to extract statistics for intensity, shape, border, and texture of both the nodule and parenchyma. Next, data were analyzed using R statistical software. We used supervised statistical learning methods to develop Stochastic gradient boosting, neural network, partial least squares and penalized logistic regression models to predict cancer status based on age and smoking quantification of each subject and 144 radiomic variables of each subject's CT. We utilized cross validation while generating our models to establish predictive performance. The model that maximized the area under the ROC curve was selected as the best performing multivariate model to predict cancer status.

RESULTS: Our best performing model was the elastic net penalized logistic regression, with 0.84 area under the ROC curve. For this model, the clinical variables were the best predictors of cancer status but several of the nodule and parenchyma features also improved classification.

CONCLUSION: Our model suggests that we may decrease the false positive rate of lung cancer screening by using the radiomic data contained within those scans, thereby reducing the number of patients who unnecessarily undergo further imaging and invasive procedures. These are intermediary results, and we will need to test our models on data from population targeted by the screen.

Importance of physical exam in the diagnosis of symptomatic synovial plica syndrome in patients with anterior knee pain

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Abstract

Background: In the general practice of medicine, it is not uncommon for physicians to be perplexed by a patient presentation of knee pain and order an MRI to aid in making a diagnosis. In many cases anterior knee pain can result from the presentation of synovial hypertrophy or even the synovial plical complex syndrome. Dr. Albright and other co-authors have established the clinical and radiographic criteria for describing the existence of excessive synovial tissue in the knee on MRI's that has led to anterior knee pain syndrome. They have shown patients with MRI evidence for synovial plical syndrome, whom underwent surgical treatment, experienced successful alleviation of their anterior knee pain. It is the purpose of this study to establish the value of specific physical exam findings in patients with anterior knee pain.

Methods: All patients in this descriptive study were those of Dr. Albright. Thirty-nine patients who were shown by MRI to have synovial plica syndrome and a resulting synovium removal surgery were included in the study. Documentation of the presence or absences of physical exam findings consistent with "synovitis" were recorded. This included the presence and peripatellar location of areas of tenderness as well as palpably thickened joint lining tissue. The presence or absence of parapatellar "popping" under the examiner's finger with active motion from 90 degrees flexion to complete extension was also documented. Synovial crepitus was again described as a parapatellar soft tissue popping sensation identified by both the examiner and patient occurring with active range of motion. The tenderness, crepitus and/or popping were also identified by every study group patient as being consistent with the problems that brought them to seek help and led to surgical intervention as described by McCunniff et al.

Results: 30 of the 39 (76.9%) study group patients had a palpable band on physical exam that matched radiographic MRI findings. Arthroscopy in the surgery was also able to capture images of the synovial hypertrophy and plical band condition in the patients. These images help visualize the symptomatic synovial plica syndrome. Additional physical exam findings are as follows: soft tissue crepitus was found in 11 of the 39 patients. Popping with motion was found in 22 of the patients. A history of "locking" was positive in 12 of the patients.

Conclusions: The information presented above suggests a careful physical exam of patients presenting with anterior knee pain can play a major role in confirming the relevance of MRI findings of synovial hypertrophy. All thirty-nine patients were relieved of their symptoms from the synovial excision surgeries. This suggests a successful progression from physical exam to operative procedure. If an MRI is obtained it is important the radiologist is able to make a diagnosis of plica syndrome and note the excess synovium to initiate the proper solution for resolution of anterior knee pain.

JNK inhibitor AS602801 (bentamapimod) decreases schwannoma tumor volume and cell proliferation

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ABSTRACT

BACKGROUND: Vestibular schwannomas are a type of benign nerve sheath tumor that most commonly occurs around CN VIII and in the process can negatively affect hearing. The prevalence of vestibular schwannomas is reported to be between 6-10 people per million, but the incidence has increased over the past 30 years, which is likely to be due to better imaging modalities. Most vestibular schwannomas are sporadic, but bilateral vestibular schwannomas are seen in neurofibromatosis type 2. Studies have shown that a family of proteins called c-Jun N-terminal kinases (JNK) has an anti-apoptotic role in vestibular schwannomas. Furthermore, one of the proteins in this family, JNK2, is upregulated in many schwannomas. Currently, the main treatment option is surgical removal, which in many cases results in deafness on the patient's affected side. While some drugs have shown some efficacy in delaying tumor growth, few options for most patients currently exist.

HYPOTHESIS: We hypothesized that a drug that inhibits JNK could either slow tumor growth or decrease tumor volume.

METHODS: A tumor was removed from a patient and placed in approximately twenty nude mice. The tumor was allowed to grow for four weeks, after which initial MRI scans were taken. The mice were subsequently assigned into two random groups: 30 mg/kg of AS602801 or a control group (water). The mice were treated twice a day for approximately 65 days, and MRI scans were taken immediately after treatment ceased. The mice were injected three times with EdU within 24 hours of death. Once the mice were sacrificed, the tumor was collected along with two pieces of gut tissue (which served as the EdU positive control). The tissue was sectioned and subsequently stained for DAPI (cell nuclei), S100 (schwannoma tissue), and either EdU (cell proliferation) or TUNEL (cell death). Multiple pictures were taken, and nuclei were counted using ImageJ. Nuclei with either EdU or TUNEL staining were counted manually, and an EdU:DAPI ratio or TUNEL:DAPI ratio was taken by dividing the number of EdU or TUNEL nuclei by the total number of nuclei. Cell staining analysis was blinded, and a student's t-test was performed in both cases.
Collaborators at Indiana University used a periostin-Cre mouse model with the same treatment and length as described above. Samples were then sent to the University of Iowa where they were sectioned, stained, and analyzed in the same manner as described above.

RESULTS: Here we show that mice treated with the drug AS602801 (a small molecule JNK inhibitor) over a 65-day period displayed a significantly decreased tumor volume in a xenografted mouse model ($p<0.001$) and decreased cell proliferation in both a xenografted mouse model ($p=0.004$) and periostin-Cre recombinant mice ($p<0.001$). However, AS602801 does not increase rates of apoptosis in either mouse model ($p>0.05$).

CONCLUSION: Our study shows that treatment with AS602801 leads to a decreased tumor volume and decreased cell proliferation, suggesting that this drug could potentially be used to help treat patients with vestibular schwannomas in the future.

Evaluation of Temporal Control in Parkinson's Disease

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BACKGROUND: Parkinson's Disease—a neurodegenerative disorder of midbrain dopaminergic neurons—is often characterized by motor symptoms of tremor, rigidity, and bradykinesia. However, this disease is also accompanied by cognitive impairments. These include deficits in executive functions (e.g., attention, planning, working memory, etc.) and can predate the diagnosis of PD through motor symptoms. These impairments—hypothesized to involve prefrontal dopamine circuits—have significant impact on the morbidity and mortality of PD patients. Early identification of impairments in PD could potentially lead to earlier interventions and better prognosis. Interval timing, or the ability to guide movements in time to achieve behavioral goals, is a process that requires the prefrontal cortex and has previously been shown to be impaired in PD patients. Interval timing shares executive resources, and PD patients have previously demonstrated marked variability in their ability to time. In this study, we apply a timing task to patients diagnosed with PD and analyze the relationship between timing accuracy and cognitive impairment as a possible methodology to accurately assess PD cognitive impairment.

HYPOTHESIS: We hypothesized that temporal timing impairment in patients with PD will demonstrate correlation with cognitive impairment as measured through standard assessments.

METHODS: 12 patients diagnosed with Parkinson's Disease were recruited from the University of Iowa Hospital and Clinics. Patients were asked to estimate intervals of time using a computer task. The computer task consisted of training—in which patients were trained to estimate either "short" (3 sec) or "long" (7 sec) intervals of time following a visual stimulus—followed by 80 trials divided over 4 blocks. In each trial, patients were asked to estimate either short or long intervals by pressing down on the space bar right before they believed the time interval was approaching and releasing the space bar after they believed the interval had elapsed. Performance feedback was given following $1/3^{rd}$ of test trials. Patients were instructed not to count during this task. Montreal Cognitive Assessments (MOCA) were administered to patients as a measure of cognitive impairment. Data collected from the experiment were analyzed via bivariate linear regression to determine correlation. Variability in patients' start, middle, release times as well as average absolute error were analyzed against MOCA scores for correlation and significance.

RESULTS: Correlation of variation in patients' average start times for both the short and long intervals was weakly correlated to MOCA scores ($R=-0.312$, $p=0.323$ for short, $R = -0.409$, $p = 0.187$ for long). Correlations of MOCA with variability in average release times ($R = -0.373$, $p = 0.232$ in short, $R = -0.409$, $p = 0.187$ in long) and variability in average middle times ($R = -0.352$, $p = 0.262$ in short, $R = -0.407$, $p = 0.189$ in long) were similarly correlated. Average absolute error—difference between patients' average times and interval length— were weakly correlated with MOCA scores in short intervals ($R = -0.268$, $p = 0.399$) with low correlation in long intervals ($R = -0.13$, $p = 0.967$).

CONCLUSION: Based on this data, we did not find strong relationships between MOCA scores and interval timing. Further testing with a larger subject group will delineate this relationship further.

An *in vitro/in vivo* genome-wide CRISPR screen identifies novel tumor suppressors controlling sensitivity to endocrine therapies and tumor metastasis

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Background: A majority of breast cancers are estrogen receptor positive (ER^+). The invention of endocrine therapies (such as tamoxifen) has revolutionized the treatment of ER+ breast cancer, but resistance to therapy eventually occurs in a large number of patients and represents a significant issue for optimal clinical management. The molecular mechanisms underlying endocrine therapy resistance are not fully understood, which has limited the development of effective approaches for preventing and overcoming resistance.

A genome-wide screen is a powerful unbiased approach for uncovering novel molecular alterations that contribute to drug resistance and disease progression. Previously, several RNAi-based screens were conducted to identify genes controlling tamoxifen sensitivity and breast cancer metastasis. However, the screens identified only a few genes involved in tamoxifen response and metastasis that could be validated in pre-clinical settings. The problem with RNAi-based screen is likely due in part to the inherited limitation of RNAi, which only partly inhibits gene activities and has extensive off-target effects.

The purpose of this study is to take advantage of the recent advance in the CRIPSR-Cas9-based genome-editing approach to develop a more powerful and specific genome-wide screening strategy for identifying novel tumor suppressors that regulate endocrine resistance and tumor metastasis.

Hypothesis: A combination of *in vitro*/*in vivo* genome-wide CRISPR-Cas9 screen will identify novel genes whose loss or downregulation drives ER+ breast cancer resistance to endocrine therapies and tumor metastases.

Methods: We transduced an ER^+ breast cancer cell line, MCF7 cells, with a genome-wide lenti CRISPR-Cas9 knockout library (the Gecko V2 1A library from Addgene) that encodes Cas9 and 67,405 guide RNAs (sgRNAs) targeting 20,611 human protein-coding genes (3 different sgRNAs per gene). Transduced cells were exposed to 4-hydroxytamoxifen (4OHT) to select for 4OHT-resistant cells. 4OHT-resistant cells were then implanted into the mammary gland of female nude mice, in the absence of implanted estrogen pellets, to screen for gene mutations that render cells capable of forming primary and metastatic tumors. Deep sequencing analysis were used to identify sgRNAs enriched in the genomic DNAs of the Gecko-transduced MCF7 cells and primary and metastatic tumors.

Results: We has identified a list of candidate genes whose loss may drive endocrine resistance and tumor development. Importantly, many of these genes have not been found in previous studies, but a handful of them have already been implicated as tumor suppressors. Moreover, compared to the normal tissues, most of the genes were expressed at lower levels at various molecular subtypes (including ER^+ luminal A and B) of breast cancer in TCGA database. Kaplan-Meier analysis of a large dataset of human breast cancer (2014 version, n=4142) indicated these genes were expressed at levels that correlated with disease-free survival of ER^+ breast cancer patients (n=3554; Fig. 3B), supporting their role as tumor suppressors. Preliminary studies also showed that inhibition of some of these genes promoted MCF7 cell proliferation and migration *in vitro*.

Conclusion: Our unique combination of *in vitro* and *in vivo* screens likely has identified novel tumor suppressors that control tumor development and sensitivity to endocrine therapies.

Is Multifocality an Indicator of Aggressive Behavior in Small Bowel Neuroendocrine Tumors?

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ABSTRACT

BACKGROUND: Small bowel neuroendocrine tumors (SBNETs) are indolent tumors that originate from enterochromaffin cells and frequently metastasize to the liver. Currently, surgery to resect the primary is the first treatment for SBNETs, with cytoreduction of liver metastases when possible. It is not unusual to find multiple primary SBNET tumors intraoperatively when carefully assessing the bowel. The reported incidence of multifocality ranges from 26-33%, and the mechanisms by which multifocal SBNETs arise are unknown. One theory is the true or initial primary spreads submucosally to other regions of the small bowel. Another is field cancerization, where genetic and epigenetic changes accumulate over time resulting in multiple independent malignant tumors. Multifocality is common in other cancers, and large retrospective studies have associated this with tumor aggressiveness in lung and breast cancer. There are few reports of this phenomenon in SBNETs, and given their relative rarity, large retrospective studies are difficult to perform. The purpose of this study was to investigate the incidence of multifocality in SBNETs, and to determine if there is any association between multifocality and SBNET aggressiveness.

HYPOTHESIS: SBNET patients with multifocal disease will have more aggressive disease, resulting in lower progression free and overall survival. Clinicopathologic factors will differ significantly between multifocal and unifocal SBNET patients.

METHODS: This was a retrospective, single center case control study that evaluated the frequency of multifocality in SBNET patients, and compared clinicopathologic factors, progression-free survival, and overall survival to patients with unifocal disease. The Howe lab maintains a large database of NET patients whom were operated on between 1999-2016 at the University of Iowa Hospitals and Clinics (UIHC) that includes clinicopathologic factors, immunohistochemistry, and demographic details. The database was updated with new information from EPIC electronic medical records system and from queries to the UIHC Holden Comprehensive Cancer Center Oncology Registry. Statistical comparisons between unifocal and multifocal SBNET patients for clinicopathologic factors, progression free, and overall survival were calculated using the Kaplan-Meier method, Welch's t test, and Fisher's exact test with the statistical package R.

RESULTS: In total, there were 184 SBNET patients in the database, with 87 (47.3%) having multifocal tumors and 97 (52.7%) unifocal tumors. There was a slight male predominance in both the multifocal and unifocal group. Additionally, over half of the patients in both groups were diagnosed before the age of 60. Statistically, multifocal patients were no more likely to be diagnosed at a younger age (<60 years, p=0.883) or be female (p=0.30). Median progression-free survival for multifocal disease was 2.5 years and 2.8 years for unifocal disease. Median overall survival was 10.5 years for unifocal disease and was not reached for multifocal disease. There were no significant differences in overall (p=.61) or progression-free survival (p=0.66). The size of the largest primary tumor was smaller for multifocal tumors, which approached significance (p=0.06). However, there was no significant difference in the primary tumor being found by imaging with CT (p=0.72), OctreoScan (p=1.0), or endoscopy (p=0.22). The lab values chromogranin A (p=0.24), serotonin (p=0.47), and pancreastatin (p=0.66) were just as likely to be elevated in multifocal as unifocal disease. There was no significant difference in the frequency of low grade tumors (p=1.0), T3/T4 disease (p=0.855), N1 disease (p=1.0), or M1 disease (p=1.0).

CONCLUSIONS: SBNET multifocality is not associated with more aggressive behavior, and multifocal SBNETs tend to behave similarly to unifocal SBNETs. As a result, multifocal and unifocal SBNETs can be treated effectively in the same manner. Multifocal disease is common in SBNET patients, and surgical treatment should include open surgical exploration to find and resect all tumors.

A Novel Method for Extracorporeal Life Support (ECLS) Oxygenator Monitoring

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ABSTRACT

BACKGROUND: Extracorporeal life support (ECLS) is a partial cardiopulmonary bypass strategy used in the intensive care setting to support patients with cardiac and/or pulmonary failure who do not respond to conventional therapies. The goals of ECLS are twofold: (1) to support O_2 delivery and CO_2 clearance and (2) to reduce the workload on diseased heart and lung tissue during convalescence. Clinical data collected from a consortium of health centers show that oxygenator failure is a leading cause of ECLS mechanical complications. Oxygenator-related complication rates are significant across all age-groups (18% of all cases) and are associated with high mortality rates (40-80%). Hollow membrane fiber oxygenators (HFMO) – the most common type of oxygenator in current use – often fail due to occlusion caused by irreversible fibrin and thrombin deposition on its membrane surfaces. Occlusion of the oxygenator fibers compromises its capacity to exchange gases and replacement becomes imperative. However, oxygenator replacement is a resource-intensive effort as well as a traumatic experience for the patient. Therefore, early detection of oxygenator obstruction is critical to our efforts to reduce mortality rates and improve patient outcomes.

Currently, clinicians monitor oxygenator status by tracking changes in pressure differential across the oxygenator over time. Pressure readings are recording hourly by nursing staff using in-line pressure monitors. This configuration poses a risk of air entrainment, which can be fatal.

HYPOTHESIS: Flow differential is a safer and more effective indicator of oxygenator obstruction than pressure differential.

METHODS: Through *in vitro* studies using adult and pediatric ECLS circuits, pressure and flow gradients (ΔP and ΔQ, respectively) were measured and evaluated across a range of flow rates and simulated obstruction levels. ECLS circuits were adapted from replica adult and pediatric ECLS circuits. The closed circuit loop comprised of a venous reservoir, pump, and oxygenator connected in series – with a shunt line branching distal to the pump and reconnecting proximal to the pump. Two modifications were made this standard circuit: (1) Transonic flow transducers were attached 5 cm proximal and distal to the post-pump shunt and (2) an adjustable steel hose clamp was attached immediately downstream of the oxygenator to simulate obstruction. 0.9% saline solution was used as the fluid media. Saline was pumped through the circuit at flow rates of 500, 1000, and 1500 mL/min for the pediatric circuit and 2000, 3000, 4000, and 5000 mL/min for the adult circuit. For each of these flow rates, pressure and flow differentials were recorded across four predetermined levels of simulated obstruction (none, mild, moderate, and extreme).

RESULTS: N=3 experiments were performed on the adult and pediatric circuits. ΔQ and ΔP increased predictably in response to increases in simulated oxygenator obstruction at each flow rate. The elevations in ΔQ above baseline were statistically significant under Student's t-test ($p<0.05$) in all but one condition – this exception being mild obstruction at the lowest flow rate for the adult circuit (2000 mL/min). Elevations in ΔP were statistically significant across all conditions.

CONCLUSION: Our study suggests that ΔQ is equally as effective as ΔP at indicating oxygenator obstruction at high flow rates. However, the results at low flow rates were inconclusive. Comparison of standard deviations within the data suggests the N=3 is an inadequate sample size for drawing conclusions about ΔQ at low flow rates.

The NLRP3 Inflammasome as a Candidate for Pro-Inflammatory Signaling in The Fluid Percussion Mouse Model of Traumatic Brain Injury

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ABSTRACT

BACKGROUND: Traumatic brain injury is a serious clinical concern, with more than 1.7 million Emergency Department visits every year in the United States alone. The initial injury sets off an inflammatory response which, over the following days, can cause additional damage and potentially threaten the life of the patient. Physicians currently have limited clinical options for treating patients with traumatic brain injury and associated inflammation. Advancing our understanding of the mechanism behind this inflammatory response is critical in developing and evaluating new approaches and treatment options in cases of traumatic brain injury.

The potent pro-inflammatory cytokine IL-1b is known to be present in the brain tissue of mice subjected to the fluid percussion injury model of traumatic brain injury. IL-1b is produced as a precursor form that must be activated through cleavage by Caspase-1, which is also present in these damaged tissues and can itself be activated through multiple pathways. The purpose of this project was to determine if the mechanism responsible for IL-1b activation proceeds specifically through the NLRP3 inflammasome, a pathway known to activate Caspase-1.

HYPOTHESIS: The mechanism of IL-1b maturation in the fluid percussion mouse model of traumatic brain injury proceeds through the NLRP3 inflammasome.

METHODS: The NLRP3 inflammasome is composed of the Nod Like Receptor Family Pyrrhin Domain Containing Protein 3 (NLRP3), Apoptosis-associated Speck-like Protein Containing CARD (ASC), and Pro-Caspase-1 proteins. In order to assemble, the NLRP3 protein component must be de-ubiquitinated. The ASC protein component must be linearly ubiquitinated to likewise become capable of inflammasome assembly. By evaluating the size of these proteins in the context of this model via western blot, it can be determined if they are activated and capable of performing their pro-inflammatory function. In order to firmly state that the NLRP3 inflammasome is responsible for Caspase-1 and thus IL-1b maturation, it is additionally necessary to demonstrate its assembly *in vivo*. This can be determined through co-immunoprecipitation of the NLRP3 and ASC protein components.

RESULTS: Through western blot it was demonstrated that both NLRP3 and ASC are present in damaged tissue samples, and additionally are in their activated forms. NLRP3 appears at 105 kDa, consistent with its de-ubiquitinated state. ASC appears ~37 kDa, consistent with expectations of its linearly ubiquitinated form. Pilot immunoprecipitation experiments were conducted to determine the viability of NLRP3 and ASC antibodies for co-immunoprecipitation. The NLRP3 antibody used here was unsuccessful in precipitating NLRP3 protein from damaged tissue lysates, despite NLRP3 being detectable in those lysates. Fortunately, the ASC antibody was able to precipitate activated ASC from the sample lysates.

CONCLUSION: The presence of NLRP3 and ASC proteins in their activated forms indicates that the mechanism of IL-1b activation and its resultant inflammation may indeed proceed through the NLRP3 inflammasome. While it has yet to be demonstrated that this inflammasome is assembled and active in the fluid percussion mouse model of traumatic brain injury, an antibody capable of immunoprecipitation has been shown to successfully pull down the ASC protein component of the inflammasome. These results are promising for future experiments and indicate that the hypothesis remains valid.

Cardiomyopathy in the Dystroglycanopathies

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ABSTRACT

BACKGROUND: Hypoglycosylation of α-dystroglycan, a protein that is part of the dystrophin-glycoprotein complex, is the hallmark of a subset of muscular dystrophies known as the dystroglycanopathies (DGs), caused by mutations in one of 18 known genes. The glycosylation defect results in a spectrum of phenotypes, from severe variants of congenital muscular dystrophy to milder limb-girdle muscular dystrophy (LGMD). The largest subgroup of the DGs results from mutations in *FKRP*, which most typically causes LGMD2I. A recurring *FKRP* mutation is c.826C>A (p.L276I), which is found in most LGMD2I patients (homozygous or compound heterozygous). Glycosylated α-dystroglycan is found in cardiac as well as skeletal muscle. Cardiac abnormalities have been reported in DGs, with most series focused on subjects with LGMD2I. Reported frequencies of cardiac abnormalities in LGMD2I subjects have varied widely, and published information is inconsistent regarding the relationship between cardiomyopathy and LGMD2I genotype. Fewer studies have examined the frequency of cardiac abnormalities in other DGs. Finally, little is known about the relationship of cardiomyopathy to age, sex, and muscle disease severity.

HYPOTHESIS: The frequency of cardiomyopathy in patients with DGs increases with increasing age. Among individuals with LGMD2I, those heterozygous for the c.826C>A mutation in *FKRP* (who have more rapidly progressive skeletal muscle disease) will have earlier onset of cardiomyopathy.

METHODS: Echocardiograms from consented participants in a DG natural history study (NIH 2 U54 NS053672-11, IRB 200510743) were retrospectively reviewed, measures of ventricular function were collected, and echocardiograms were classified as normal or abnormal. Standard functional data (forced vital capacity and 10-meter walk test times) that had been collected annually were associated in time to echocardiograms. The probability of developing cardiomyopathy was assessed by Kaplan-Meier analysis for the whole cohort and different genotypes. Measures of clinical function were associated with cardiac function for the subgroup with *FKRP* mutations.

RESULTS: We reviewed 197 echocardiograms from 58 subjects with 7 different genes causing DG. Those with *FKRP* mutations constituted the largest subset (n = 39). At least one abnormal echocardiogram occurred in 31% of subjects, with a median age at first abnormal echocardiogram of 36 years (IQR 16-48) by Kaplan-Meier analysis, but was seen as early as age three years. There was no significant difference in age at abnormal echocardiogram between gene groups. However, in the subgroup with *FKRP* mutations, abnormal echocardiograms occurred significantly later for those homozygous for the c.826C>A variant compared to all other *FKRP* genotypes (median ages by Kaplan-Meier analysis: 48 years and 29 years, p = 0.0001). In the entire cohort, we found no effect of sex on probability of cardiomyopathy. Ejection fraction was positively correlated with 10-meter walk speed, but not with forced vital capacity.

CONCLUSION: Cardiac dysfunction is prevalent in the DGs. While the mean onset is in mid-adulthood, it can present in childhood. Among those with *FKRP* mutations, genotype can help to guide surveillance frequency.

Quantitative Assessment of Activity of Statistical Editor for Reviews of Anesthesia Patient Safety Research

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ABSTRACT

BACKGROUND: Recently, a narrative review summarized prior knowledge in statistical editing (Dexter and Shafer, 2016). A consistent finding among studies was that, generally, non-statistical reviewers (e.g., "scientific reviewers") and journal editors poorly assessed the quality of the statistical analyses. A statistical editor should be responsible for all manuscripts in general medical journals that involve data analysis. For 8.5 years, one author (FD) performed a statistical review of each manuscript accepted for publication and handled by the section editor (SJB) for anesthesia patient safety of the journal Anesthesia & Analgesia.

HYPOTHESES: Hypothesis #1: Most comments (> 50%) written by the statistical editor were about statistical methods. Hypothesis #2: Many comments (> 30%) about statistical methods included a theme that was a contemporary statistical topic. Hypothesis #3: Greater numbers of comments written by the statistical editor about statistical methods resulted in greater number of weeks until a revision was submitted by the authors.

METHODS: The statistical editor saved all his reviews. Before the start of this study, all statements identifying authors were reviewed and removed from the text to ensure anonymity. An initial set of statistical themes was created by the statistical editor using his notes. Using these statistical themes, words and phrases were identified inductively from the manuscript paragraphs to match the themes. The words and phrases for each of the 34 themes identified (Table 2) are shown in the Microsoft Excel 2010 file in the supplemental material. Statistical methods have precise names (e.g., "Clopper Pearson") and thus the only ambiguity present was the issue of punctuation. Although the possibility of a spelling mistake was checked for all themes of a statistical method, no misspellings were found that needed to be added to the list of themes in order to identify all relevant paragraphs. All calculations for the presence of the words or phrases in the paragraphs were performed using Microsoft Excel 2010 text processing without regard to text case. After themes of statistical methods were created, all remaining manuscript paragraphs were reviewed manually by the first 3 authors for any additional statistical method occurring at least 5 times (i.e., ≥ 1.0% of reviews) that had not been identified previously.

RESULTS: Among the 3274 paragraphs of comments, 72.2% did not include a theme of a statistical method (i.e., involved statistical writing); 95% CI 70.6% to 73.7%; $P < 0.0001$ vs. 50%. Among the 207 manuscripts with a review that included a statistical method, 47.3% included a contemporary topic such as generalized pivotal methods (95% CI 40.4% to 54.4%). However, among the 911 corresponding paragraphs, only 16.0% included a contemporary theme; 95% CI 13.7% to 18.6%. Among the 273 subsequent (i.e., secondary) reviews for manuscripts that were eventually accepted, the number of paragraphs including a theme of a statistical method was significantly but very weakly associated with longer revision times until the next review ($P = 0.0004$; Kendall $\tau_b = 0.139 \pm 0.039$).

CONCLUSIONS: These results show that most of a statistical editor's work is about editing, not statistical methods, and most of the work that does involve statistics involves classic methods such as Student's t-test. Editor requests for statistical changes during the review process seem to have negligible influence on the time to manuscript resubmission. Our case series of reviews was limited by being the result of a unique collaboration between two of the authors, working for one journal, and one discipline, anesthesia patient safety, for a well-defined period. The distribution of statistical methods surely may differ among anesthesia disciplines. Perhaps, if more of the journal's authors had Ph.D. training (versus medical backgrounds alone), the statistical editor's editing role would have been more about statistical methods and less about writing.

Retrospective Histopathologic Evaluation of Fibroblastic versus Myofibroblastic Entities of the Oral Mucosa

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ABSTRACT

BACKGROUND: Although soft tissue neoplasms are relatively uncommon in the head and neck region, fibrous proliferations of the oral cavity are fairly common and include a diverse group of reactive and neoplastic entities such as irritation fibromas, giant cell fibromas, inflammatory fibrous hyperplasias and myofibromas or myofibroblastic proliferations (MFPs). Immunohistochemical stains are usually not employed in order to diagnose these entities. MFPs, however, are proliferations usually arising in the head and neck area and are almost always confirmed with the use of immunohistochemistry due to their resemblance to other spindle cell entities. Although literature exists comparing the histologic features of MFPs to other spindle cell entities, to the authors' knowledge, there is no literature exploring the possibility that MFPs of the oral cavity are simply part of the spectrum of oral fibrous proliferations as the fibroblast and myofibroblast, components of both entities, are reliant upon each other in wound healing and repair and that fibrous proliferations of the oral cavity may be misclassified as MFPs.

HYPOTHESIS: MFPs of the oral cavity lie on the spectrum of oral fibrous proliferations and may be misclassified as vimentin, SMA and CD34 can be expressed by each of these entities.

METHODS: The Institutional Review Board approved this study**.** Two hundred and forty-nine tissue blocks were collected corresponding to the biopsy sites of 249 patients: 126 males and 123 females. The ages of the biopsied patients ranged from 2 to 92 years for females and 5 to 87 years for males, with a mean age of 50 years. Biopsy sites included mucosal surfaces (buccal, labial, and vestibular), tongue, gingiva, maxilla and sites not specified by the submitting clinician. Of the 249 blocks, 46 blocks were diagnosed as irritation fibromas; 44 blocks as giant cell fibromas; 44 blocks as inflammatory fibrous hyperplasias; 23 blocks as MFPs (11 myofibromas and 12 myofibroblastic proliferations); and 45 blocks as ulcerated irritation fibromas. As a control group, 47 blocks with diagnoses of focal keratosis and chronic mucositis were used. Immunohistochemical staining was performed according to a standard protocol. Staining patterns of vimentin, SMA and CD34 were graded as 0, 1 and 2 ($\leq$25%; $\leq$25-50%; and $\geq$50%, respectively). Cohen's kappa was used to calculate intra- and interobserver agreement of the investigators. The Kruskal-Wallis exact test was used to compare the percentages of the cases that reacted to vimentin, smooth muscle actin and CD34 as well as the percentages of cases that fell into each grade. Dunn's test was used to identify stochastic dominance between each possible group. Statistical significance was set at $P < .05$.

RESULTS: There were statistically significant differences in SMA staining of MFPs compared to the other categories although other entities expressed SMA to some degree. In addition, four of the other five categories demonstrated statistically significant differences in CD34 expression when compared to MFPs.

CONCLUSION: The results indicate that SMA immunohistochemistry is helpful but not definitive in delineating MFPs as a separate entity as it was expressed in other entities. The expression of SMA in other fibrous proliferations of the oral cavity may lead to misclassification of these lesions as MFPs. The results also indicate that although CD34 is deemed a specific marker for solitary fibrous tumor, this marker was not reliable in distinguishing the majority of the entities from one another and may not be as specific for solitary fibrous tumor as once thought. Further studies are needed to support these findings.

Evaluating the iNfluence of Diabetes STIGma on Medication Adherence: The ENDSTIGMA Study

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BACKGROUND: Disease-related stigma has been shown to negatively affect medication adherence in several chronic conditions, including HIV and schizophrenia. Although the International Diabetes Federation has identified decreasing diabetes stigma as a public health priority, the existence and importance of diabetes stigma has been debated in the literature. No scale comprehensively measuring diabetes stigma exists. The ENDSTIGMA study was created to address this research gap.

HYPOTHESES:

1. Diabetes stigma exists and can be measured quantitatively.
2. Higher BMI, insulin usage, type II (v. type I) diabetes, and lower educational attainment will be associated with higher diabetes stigma, while diabetes duration will not.
3. Increased stigma will be associated with poorer self-reported medication adherence and glycemic control.

METHODS: To develop the Comprehensive Diabetes Stigma Scale (CDSS), a literature review of existing chronic disease stigma scales and qualitative publications about diabetes patient experiences was conducted. An 81-item draft was distributed to diabetes and stigma experts for evaluation of content and face validity, then piloted with participants waiting at the Vanderbilt University Medical Center. Inclusion criteria for pilot recruitment were being 18 or older, having diabetes for at least 1 year, and taking at least 1 diabetes medication. Participants completed the CDSS, the Adherence to Refills and Medications Scale for Diabetes Medicines 11 (ARMS-D 11), and questions about demographic and clinical characteristics using the REDCap Mobile app on a Nexus7 (2013) tablet. The study was approved by the Vanderbilt IRB (#160986) and was registered on ClinicalTrials.gov (NCT02828995). Descriptive statistics were used to summarize continuous and categorical variables. Least squares linear regression was used to detect associations between continuous dependent variables and diabetes stigma. Ordinal logistic regression was used to analyze an exploratory association between hemoglobin A1c (HbA1c) and diabetes stigma. Cronbach's alpha and Pearson's correlations were used to analyze internal consistency and concurrent validity, respectively.

RESULTS: Preliminary results indicate the CDSS-81 had strong internal consistency (Cronbach's alpha = 0.95) and high concurrent validity with a measure of diabetes-related family stigma (Pearson's Correlation = 0.81). Of 599 potential participants approached, 181 were eligible, 44 were recruited, and 30 were included in the final data set. Participants were on average 61.3 ± 11.7 years old; 48.4% female, 26.7% Black; 3.3% Hispanic or Latino/a; and 33% had a high school education or less. In total, 30% of participants had suboptimal glycemic control (HbA1c >7.0%); an average BMI of 33.5 ± 9.1; and an average diabetes duration 17.1 ± 12.6 years. Additionally, 16.1% of participants had Type I diabetes and 58.1% used insulin. Diabetes stigma was found to be associated with education (F-statistic = 4.23, p = 0.05) and diabetes duration (F-statistic = 6.95, p = 0.01), but not with BMI (F-statistic = 0, p = 0.98), insulin usage (F-statistic = 0.52, p = 0.48), or diabetes type (F-statistic = 0.21, p = 0.65). Near-significant associations were found between stigma and medication adherence (F-statistic = 3.1, p = 0.09), and between stigma and HbA1c (z = 1.58, p = 0.11).

CONCLUSION: The END STIGMA study resulted in the development and piloting of a novel comprehensive diabetes stigma measure, the CDSS-81. Results indicate education may be associated with diabetes stigma. Interventions to increase knowledge may be effective in decreasing stigma. These preliminary results should be interpreted with caution pending finalized validation by expert input, cognitive interviewing, and verifying convergent and discriminant validity in a larger study.

The Effects of Risperidone on Human Gut Bacteria In Vitro

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ABSTRACT

BACKGROUND: Growing research suggests that the gut microbiome, the community of microorganisms residing in human and animal digestive tracts, can modulate metabolism and weight gain. Recent studies have shown a correlation between obesity and an altered gut microbiome in humans. The Kirby lab induces weight gain in mice by using risperidone, an antipsychotic that is used to treat schizophrenia and autism and is known to cause weight gain in humans. Previous studies have shown that treating C57BL/6J mice with risperidone (80 μg/day) results in significant weight gain and an altered gut microbiome. Transplanting fecal matter from risperidone-treated mice to healthy mice induces weight gain in the healthy mice, suggesting that the altered microbiome of the risperidone-treated mice is affecting metabolism and weight gain rather than risperidone itself. The purpose of this project was to investigate the effects of risperidone on human gut bacteria *in vitro* in order to understand how the microbiome is being altered.

HYPOTHESIS: Risperidone will inhibit the growth of gut bacteria *in vitro* and will more strongly inhibit growth in anaerobic conditions than in aerobic conditions.

METHODS: Strains of human gut bacteria were obtained from the National Institutes of Health (NIH). Five of the strains were facultative anaerobes, which could be grown in anaerobic or aerobic conditions, while the other strains were obligate anaerobes. Strains were incubated in lysogeny broth (LB) at 37° C in an anaerobic chamber and left to grow overnight. In the morning, optical density (OD) measurements were taken by a spectrophotometer at 600 nm, and the samples were diluted to an OD of 0.05-0.1. Varying risperidone concentrations (20-100 μg/ml) were then added to the samples. OD readings were taken hourly in order to compare growth among the samples. The experiment was repeated in aerobic conditions by growing the five facultative anaerobes at 37° C on a shaking platform.

RESULTS: Most strains showed inhibition of growth at 60-80 μg/ml of risperidone in comparison to the control, while some strains were inhibited at the lowest concentration of 20 μg/ml. All strains showed inhibited growth or no growth at 200 μg/ml. The growth of the facultative anaerobes was more strongly inhibited by risperidone in anaerobic conditions than in aerobic conditions. Facultative anaerobe growth was also inhibited at lower concentrations of risperidone in anaerobic conditions.

CONCLUSION: Risperidone inhibited the growth of most gut bacteria at 60-80 μg/ml *in vitro*, suggesting that risperidone might also have an antibiotic effect on gut bacteria *in vivo.* The facultative anaerobes were more sensitive to risperidone in anaerobic conditions than in aerobic conditions, suggesting that the effects of risperidone may be more pronounced in the anaerobic environment of the gut than in areas of the body exposed to oxygen.

Role of ULK1 and ULK2 Kinase Activity in Regulation of Skeletal Muscle Autophagy

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ABSTRACT

BACKGROUND: Autophagy is a catabolic process that cleans up damaged cellular components modulating cellular stress and ultimately aiding in the maintenance of homeostasis.[1] This process is pivotal for normal skeletal muscle function and is dysregulated in obesity, diabetes and with aging.[2,3] Conversely, previous studies have also demonstrated that autophagy is stimulated under nutrient deprivation conditions.[1] ULK 1, the mammalian serine/threonine protein kinase orthologue of the critical autophagy gene 1 (Atg1) in yeast, interacts with other Atg proteins in the ULK complex and has been shown to regulate autophagy in different cell systems.[1] However, autophagy regulation is tissue specific and very little is known about the function of its homologue ULK 2. Our laboratory has recently observed that ULK 2 is the predominant ULK in skeletal muscle, and that ULK 1 and ULK 2 regulate autophagy differently in this tissue. Essentially, our data demonstrates that muscle ULK1 is required for bulk autophagy activation, whereas ULK 2 seems to regulate degradation of damaged proteins and organelles through selective autophagy. ULK 2 and ULK 1 share 53% AA homology, but whether their kinase functions are required for their different roles in regulation of autophagy in skeletal muscle remains to be determined. Understanding the specific mechanisms that regulate the functional roles of ULK2 and ULK1 may be of therapeutic value under conditions of dysregulated autophagy.

HYPOTHESIS: We hypothesized that the kinase activity of ULK2 and ULK1 are required for muscle autophagy regulation.

METHODS: One tibialis anterior (TA) muscle of the mice was electroporated with different plasmids depending on the assigned condition (ULK2 or ULK1 kinase inactive, KI). The contralateral leg of each animal was electroporated with an empty vector plasmid acting as a control. We compared results of each mutated ULK2 or ULK1 with its respective control leg (N = 6/mutated ULK) under both non-fasted and fasted (24h) conditions. These conditions were selected to compare and contrast the impact of the loss of these kinases on basal and stimulated autophagy, respectively. Therefore, half the animals were fasted for 24 hours (food removed from cage 24 hours before tissue harvest), the remaining were not. The TA muscle was harvested, snap frozen and later homogenized. Muscles were sectioned and histologically examined for successful electroporation. Muscle protein extracts were analyzed for common autophagy markers (LC3, p62, GABAL1, NBR1, Ubiquitin) via western blots.

RESULTS: Our findings revealed a significant overexpression (≥ 3-fold) of the ULK1 KI and ULK2 KI in skeletal muscle for both fasted and non-fasted conditions. As expected, fasting stimulated autophagy as indicated by an approximate 4-fold increase in LC3 II/I ratio. However, neither the overexpression of ULK1 KI, nor the ULK2 KI, affected autophagy in skeletal muscle. This was evident by unaltered LC3 II/I ratio and p62 levels in both the fasted and non-fasted states.

CONCLUSION: Although these preliminary findings suggest that the kinase activities of ULK1 and ULK2 may not be required for autophagy regulation, further experiments still need to be performed to precisely test this hypothesis. Both ULKs are known to perform auto-phosphorylation. Therefore, an alternative explanation is that kinase activity is required for the formation of the ULK complex due to conformational changes resulting from auto-phosphorylation. Without these changes in conformation, the kinase inactive mutants may have not been able to interact with other Atgs in the ULK complex and, therefore, could not compete with the endogenous ULKs in signaling that modulates autophagy. Experiments using ULK1/2 KIs with AA substitutions that mimic auto-phosphorylation will provide important information in this regard.

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Proteolytic Regulation of Retinal Neurotransmission

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ABSTRACT

BACKGROUND: Families with inherited, hyper-activating mutations of calpain-5 (CAPN5) exhibit loss of photoreceptor neurotransmission, and thus the loss of vision. We identified Syntaxin Binding Protein 1 (STXBP1) as a novel target of CAPN5, a calcium-regulated protease. STXBP1 is expressed in photoreceptor synapses and is necessary for neurotransmitter exocytosis. STXBP1 ensures proper assembly of the exocytosis machinery by assisting in the priming and release of synaptic vesicles filled with neurotransmitters. Without STXBP1, there is complete loss of neurotransmission.

HYPOTHESIS: CAPN5 proteolysis of STXBP1 alters the interactions of STXBP1 with exocytosis proteins that would lead to loss of retinal neurotransmission.

METHODS: Proteins were generated by *in vitro* transcription-translation. CAPN5 cleavage of STXBP1 was verified using an *in vitro* proteolysis assay. Cleavage products were analyzed by SDS-PAGE, and the cleavage region was determined by molecular weight changes. To determine the functional effect of CAPN5 cleavage, an interaction between the full-length and cleaved form of STXBP1 to its well-known target, Syntaxin1a, was tested in pull-down assays. Finally, we generated a three-dimensional model of STXBP1 to evaluate how CAPN5 cleavage alters its tertiary structure.

RESULTS: CAPN5 cleaves an estimated 11-kDa portion of the C-terminal region of STXBP1. The CAPN5-cleaved STXBP1 retains the binding site for its target, Syntaxin1a. Nevertheless, cleaved STXBP1 fails to interact with Syntaxin1a, an interaction necessary for exocytosis and neurotransmission. Three-dimensional modeling revealed that removal of the 11-kDa C-terminus of STXBP1 resulted in the exposure of a hydrophobic hole with allosteric effects and potential destabilization.

CONCLUSION: CAPN5 cleavage of STXBP1 alters its ability to interact with Syntaxin1a, which is required for neurotransmitter exocytosis. This proteolytic mechanism could explain the loss of retinal neurotransmission observed in patients with hyper-activating CAPN5 mutations.

CGRP Influences periocular muscle activity and facial cues of discomfort in mice

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Background: Migraine is a chronic and debilitating disorder that affects over 10% of the population, however the etiology is not well understood and only marginal advancements have been made toward the treatment of migraines. While the presentation of migraine headaches can be heterogeneous, a common symptom and trigger of the disease is photosensitivity. Several recent studies have suggested the central role of calcitonin gene-related peptide (CGRP) in the pathophysiology of trigeminal hypersensitivity, photosensitivity, and migraine. Previous studies in the Russo lab have demonstrated that CGRP administration in a migraine mouse model enhances light aversive behavior. In this study, we attempt to gain a better understanding of the relationship between CGRP-mediated photosensitivity and migraines through the use of periocular electromyography (EMG) and quantitation of the palpebral fissure height and facial grimace in CGRP sensitive mice.

Aims and Hypothesis: The aims of this project were to study the use of periocular-EMG, palpebral fissure heights, and facial grimace as objective measurements of photosensitivity in a transgenic nestin/hRAMP1 mice and to evaluate the effect of exogenous CGRP administration on these measures. We hypothesized that exogenous CGRP administration would cause a significantly increased EMG response in the transgenic mice compared to controls and that these mice would also have decreased palpebral fissure heights and increased scores on the mouse grimace scale.

Methods: All animals were treated in accordance with procedures approved by the University of Iowa Institutional Animal Care and Use Committee (IACUC). The effect of light and CGRP administration on the blink reflex EMG activity, palpebral fissure height, and the mouse grimace scale was measured in adult male and female CGRP-sensitive mice (nestin/hRAMP1) and control littermates. The EMG mice were surgically implanted with a wireless telemeter inserted into the right dorsal flank, with electrode leads implanted over the orbicularis oculi muscle of either eye.

During the testing protocol, the mice were gently restrained using a custom made acrylic box that allowed video recording of each assay. A blue-red light protocol was created for the periocular EMG measurements and involved increasing light intensities from 0.76 cd/m^2 to 745 cd/m^2 for 5 second exposures. A focused air puff was directed at the corneal surface to reliably elicit blinks at the onset of light stimuli. A 2 minute dark – 2 minute light protocol was utilized for the palpebral fissure height and grimace scale experiments. All experiments involved injection of intraperitoneal CGRP (0.5mg/kg) or vehicle. All testing scenarios were recorded on video to assist in quantitation and data analysis. EMG data and video were exported for visualization and interpretation into Mathworks MATLAB. Palpebral fissure height and mouse grimace scoring were performed by two independent blinded observers on images pulled at 0, 30, 60, 90, and 120 seconds for each lighting condition.

Results: Our data indicate an unexpected decrease in the periocular-EMG of air puff induced blink responses across all light intensities in the transgenic mice and one control mouse after CGRP administration. We also found an overall reduction of periocular-EMG during the entire experiment in these mice. Video recordings of the mice allowed us to observe the stark effect CGRP had on the transgenic mice, who held persistent squints after injection whereas controls did not. Palpebral fissure heights were significantly decreased in both light and dark for C57 background mice ($p<0.05$, $p<0.01$). Scores on the grimace scale were also higher (more discomfort) for the C57 background mice ($p<0.05$). These results indicate that CGRP induces migraine-like discomfort through mechanisms both involving and independent of light.

Discussion: After administration of CGRP the C57 background mice hold persistent squints in the dark and light with a decrease in EMG signal. This suggests that CGRP may be causing discomfort through a light independent pathway in addition to causing established light aversive behavior. In the future, these objective measurements may be used in translational studies with automated facial recognition software that would allow rapid screening of mouse and human responses to migraine drugs, including the CGRP antibodies now in Phase III clinical trials.

Bone Contusion Patterns Used to Predict Certain Meniscal Tears on MRI when an ACL Tear is Present

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ABSTRACT

BACKGROUND: There is a somewhat high MRI error rate for diagnosing certain meniscal tears when an anterior cruciate ligament (ACL) tear is present. Occult meniscal tears that are not visualized by MRI can lead to their unexpected discovery during arthroscopy or other surgeries. Developing a method to more accurately diagnose the most common types of occult tears, such as peripheral vertical tears of the posterior horn of lateral meniscus, can improve patient outcomes by ensuring that all meniscal pathology is repaired at surgery.

In particular, bone contusion patterns can be representative of the mechanism of injury and the specific type of the knee injury that follows. The purpose of this study was to search for a bone contusion pattern that can tip physicians off to the presence of an occult meniscal tear.

HYPOTHESIS: The increased MRI error rate for peripheral vertical tears of the posterior horn of the lateral meniscus combined with ACL tears will be associated with a mechanism of injury, with relevant bone contusion patterns, that cause a concurrent ACL tear and peripheral vertical tear of the posterior horn of the meniscus.

METHODS: This was a retrospective study that evaluated the bone contusion patterns of knee MRI's that had known occult meniscal tears compared to controls. We reviewed the database at the University of Iowa Hospitals and Clinics for all knee MRI's from June 1, 2009 to December 31, 2015, totaling 6392 knee MRI's. Occult meniscal tear cases were collected from these 6392 using the surgical report as a standard of reference for the presence of a meniscal tear. Two board certified musculoskeletal fellowship trained radiologists reviewed the collected occult meniscal tear cases to determine their validity. The 35 remaining occult meniscal tear cases' bone contusions were then compared to the bone contusions of the control group, which had 122 cases. Fisher's Exact Test was conducted to determine statistical significance between the control group and occult meniscal tear group.

RESULTS: The study revealed that 61% of the control group had a pivot-shift mechanism of injury compared to 83% of the occult tear group ($p = 0.06$). 19% of the control group had an unknown mechanism of injury compared to 17% of the occult tear group ($p = 0.19$). 14% of the control group had a bone contusion of the rim of the medial femoral condyle compared to 40% of the occult tear group ($p = 0.007$).

CONCLUSION: If a patient has an ACL tear and a bone contusion of the rim of the medial femoral condyle, then there is a much higher likelihood of an occult meniscal tear being present. This is useful to radiologists and surgeons to gauge the probability of the most common types of occult meniscal tears, like peripheral vertical tears of the posterior horn of the lateral meniscus.

Investigating Low Dose Decitabine as a Strategy to Delay Acquired Resistance to Vemurafenib in Melanoma.

Noah Frydenlund MS, Mohammed Milhem MBBS, Michael Henry PhD.

BACKGROUND: BRAF is a kinase that plays a central role in the mitogen activated protein kinase (MAPK) oncogenic signaling cascade. Activating mutations in BRAF are present in up to 50% of melanomas. The development of targeted therapy (vemurafenib) against the most common BRAF mutation (*BRAF-V600E*) in the past decade has resulted in significant improvements in progression free survival and overall survival in patients with advanced melanoma. However, most patients develop acquired resistance to vemurafenib within one year resulting in disease progression. To date, the mechanisms underpinning acquired resistance to BRAF inhibition are incompletely understood and have been suggested to include copy number alterations in *BRAF*, alternate splicing, additional point mutations and epigenetic alterations. Decitabine is a cytidine analogue that is capable of depleting the DNA maintenance methyl transferase (DNMT1) in numerous cancer types. DNMT1 depletion by decitabine results in global DNA hypomethylation and alters patterns of epigenetic inheritance. At higher doses, decitabine exhibits a cytotoxic effect, and for this reason has been investigated as a monotherapy in solid tumors with little success. However, DNMT1 depletion and functional DNA hypomethylation have been observed at significantly lower concentrations than those used in decitabine monotherapy. Low dose decitabine could be administered for extended periods of time in combination with vemurafinib disrupting the epigenetic plasticity that may be required for melanoma to acquire resistance to vemurafinib.

AIMS: To demonstrate *in vitro* depletion of DNMT1 by decitabine in the A375 human melanoma cell line at sub-cytotoxic concentrations. To demonstrate that concurrent treatment with decitabine delays acquired resistance to vemurafenib *in vitro* in the A375 human melanoma cell line.

METHODS: To determine the sub-cytotoxic range of decitabine in A375 cells, a 72 hour viability assay was performed at a range of concentrations from 1 nM to 100 µM with each concentration tested in triplicate. After determination of the sub-cytotoxic range, four concentrations were chosen from this range to determine the lowest concentration at which DNMT1 depletion could be observed. After 72 hour incubation with decitabine DNMT1 depletion was measured by western blot on whole cell lysates. After determining a minimum effective decitabine concentration, A375 cells were cultured in the presence of 3 µM vemurafenib or 3 µM vemurafenib + decitabine. Cumulative population doubling was calculated every 4 days to monitor the emergence of acquired resistance.

Results: Decitabine treatment for 72 hours produced a bimodal pattern of growth inhibition in A375 melanoma cells with a calculated IC50 of 6 µM. The sub-toxic range was determined to be 1-50 nM. Western blot analysis showed >50% DNMT1 depletion at 5 nM, the lowest concentration at which DNMT1 depletion was observed after 72 hours of exposure. Given these results, we chose to use 5nM as the starting concentration in long term studies of acquired resistance to vemurafenib. Low trial number in this exploratory study precluded statistical analysis.

Conclusion: Substantial DNMT1 depletion occurs at sub-cytotoxic concentrations of decitabine in A375 melanoma cells. Currently, experiments are underway to determine if combination therapy with low dose decitabine will delay the development of acquired resistance to vemurafenib in melanoma *in vitro.*

Factors affecting outcomes following the operative treatment for patellofemoral instability

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Background:. The stability of the patellofemoral joint is multifactorial and relies on interplay of limb alignment, osseous architecture of the patella and trochlea, soft- tissue constraints, and the dynamics of surrounding muscles. Stability is achieved when these factors can balance the laterally directed pull of the quadriceps mechanism. The osseous constraints consist of the bony articulation of the patella and femoral trochlea. When engaged in the trochlear groove at 10 to 30 degrees flexion, the patella becomes more deeply engaged within the trochlea as flexion continues. The primary soft tissue restraint to lateral patellar displacement is the medial patellofemoral ligament, which provides 50% to 60% of lateral restraint from 0 to 30 degrees flexion. The core, hip, and quadriceps musculature play an important in dynamic patellar stability, while the vastus medialis obliquus (VMO) plays a particularly important role in restraining lateral displacement of the patella.
Predisposing factors for patellofemoral instability are likewise multifactorial. These include: trochlear dysplasia, medial restraint deficiencies (MPFL), malalignment, hyperlaxity, and patella alta. Patella alta results in delayed engagement with the trochlea as flexion begins, which decreases the osseous stability. Patella alta can be ascertained by the Caton and Deschamps index and corrected by the distal transfer of the tibia tubercle. Trochlear dysplasia is represented by the crossing-sign on a lateral radiograph. A tibial tubercle-trochlear groove distance of more than 20 mm represents malalignment and can be corrected by medialization of the tibial tubercle. MPFL deficiencies are detected by MRI and can be corrected by MPFL reconstruction.
Deciding whether proximal or distal realignment is appropriate depends on the pathoanatomy of the patellofemoral joint. In general, deficient medial restraints, trochlear dysplasia, or VMO deficiency should undergo proximal correction, while patella alta, increased Q angle or TT-TG should undergo distal correction. However, as previously mentioned, patellar instability is multifactorial so a combination of both distal (TTT) and proximal correction (MPFL reconstruction) are often needed.

Methods:
A total of 76 patients were prospectively enrolled and IRB approval was attained.

a. Electronic medical records were used to gather demographic, preoperative, operative and postoperative outcome data for a cohort of TTT and MPFL reconstruction patients followed by UI Sports Medicine.
    i. Preoperative data points included the patient's age, BMI, presence of Ehlers-Danlos Syndrome, smoking status, Caton-Deschamps ratio, grade of trochlea dysplasia (A,B,C,D), crossing sign (yes or no), patella height, patella tilt, presence of axial x-ray subluxation, the presence of an MPFL tear (grade and location), TT-TG ratio, presence and grade of cartilage tear, apprehension, and previous surgeries.
    ii. Operative data points included type of surgery performed, fixation device, cartilage status, associated injuries or damage.
    iii. Postoperative data points included patient reported outcomes (SF-36, KOOS, Marx, Kujala), MPFL femoral tunnel on lateral x-ray, Caton-Deschamps ratio, physical exam finding, knee range of motion and strength.
b. Patients were categorized for analysis based on the procedure(s) performed including: Isolated MPFL reconstruction, isolated TTT, TTT and MPFLreconstruction, and if cartilage resurfacing was done.
c. Univariate and multivariate analysis was performed to determine associations between patient factors and outcomes.

Results: 76 patients with patellofemoral instability were enrolled. 51 (67.1%) had 6 month follow up. The average patient age was 23.8 years; 47 (61.8%) being female. 43 (56.6%) patients underwent TTT, 39 (51.3%) patients underwent MPFL reconstruction, and 32 (42.7%) patients had combined (soft tissue and osteotomy) procedures. 31 (43.7%) had grade C or D trochlear dysplasia, 24 (33.8%) had grade A or B, while only 16 (22.5 %) had no evidence of trochlear dysplasia (Dejour Classification). 45 (60%) patients had cartilage lesions with 34 (44.7%) having grade $\geq$ 3 or 4 lesions. Patients with grade 3 or 4 cartilage lesions were more likely to be obese (65.2% vs 35.8%) and older (28.1 vs 20.3 years). Additionally, at 6 months, patients with grade 3 or 4 cartilage lesions had lower Kujala scores (69.7 vs 62.2). However, regardless of procedure, patients got better when comparing pre- and postoperative subjective outcome scores. (56.7 vs 75.4 (KOOS symptoms)).

Conclusions: Uniformly, patients do well following operative treatment of patellofemoral instability. There was no difference when comparing tibial tubercle transfer, MPFL reconstruction, or combined procedure. Patients with high grade cartilage lesions had worse outcomes scores at 6 mos and more likely to be obese and older. Further studies are needed to determine optimal treatment approach for patients with patellofemoral instability and high grade cartilage lesion.

Small bowel obstruction, a recurrence of metastatic melanoma during the second trimester of pregnancy

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Introduction: The incidence of melanoma is on the rise. It Is particularly prevalent among women of childbearing age and is the most common malignancy diagnosed in pregnancy. The majority of cases are diagnosed as a clinically localized skin lesion, much fewer cases present as advanced stage disease.

Case: A 35-year-old female with a remote history of treated cutaneous melanoma presented to a local hospital with intractable nausea, vomiting and diffuse abdominal pain in her 23rd week of gestation. Imaging was concerning for small bowel obstruction and the patient underwent an exploratory laparotomy. A mass within the small bowel was excised and was consistent with recurrent metastatic melanoma. She presented to our institution for a multidisciplinary treatment plan. A healthy infant was delivered prematurely in the 27th week of gestation and the patient was started on an immunotherapy regimen of nivolumab and ipilimumab. PET CT scan shortly after delivery showed significant lesions in the liver, left lung and gastric outlet. PET CT scan after four and eight cycles of therapy revealed very good response with no new lesions and a significant improvement in metastatic sites.

Conclusion: Few cases exist in the literature in which patients present with metastatic melanoma, especially during pregnancy. There are no guidelines on how or when to proceed with treatment of disease or delivery of the fetus. Immunotherapy is changing the paradigm of melanoma response rates and is extending life expectancy. To our knowledge, this is the first reported case that resulted in preterm delivery and a positive disease response with immunotherapy.

Analyzing compound heterozygotes to identify novel mutations in epilepsy

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Background:

Epilepsy is a debilitating disease that causes seizures that affects roughly 1% of adults[1]. The causes of epilepsy are poorly understood, but genetics is suspected to play a major role[2,3]. Large genetic databases have been compiled[4], and have been analyzed using Genome Wide Analysis Studies, looking for homozygous mutations which have knocked out both copies of a gene[5].

Many disease-causing mutations inhibit a protein. However, because humans have both a maternal and paternal chromosome, the affected individual must have received two copies of a mutated gene. Often, these two genes are mutated in the same location, and so researchers have often searched for homozygous genetic mutations. Contrastingly, two different knockout mutations in the same gene are much rarer, and termed “compound heterozygote.” We have developed an automated pipeline to test patients for compound heterozygote mutations. This pipeline will allow us to find novel disease-implicated genes that would have been missed by a conventional genetic association study.

Methods:

Our pipeline only searches for rare mutations. The probability of a mutation being homozygous is $p_{homozygous} = p_{mutation}{}^2$. Therefore, common mutations will often be homozygous, and therefore caught by other, simpler genetic screens.

Results:

Our pipeline was designed to be used by other research groups, and so was implemented in the Python programming language. The pipeline consists of several filtering steps; removing mutations that are more common than a user defined threshold, synonymous mutations, and mutations in non-coding regions of the genome. Afterwards, a user-selected parameter controls if the program will identify compound heterozygous or homozygous mutations. The program takes in an annotated vcf file as input, and returns a list of patients and which genes were affected by mutations. This pipeline is available on the GitHub public repository.

Conclusions:

This pipeline represents a novel, out-of-the-box method for labs to screen for compound heterozygous mutations, which may contribute to many rare diseases.

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[5] Do, Chuong B., et al. "Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease."*PLoS Genet* 7.6 (2011): e1002141.

The Role of $Ca_v1.4$ $\alpha_2\delta_4$ Subunit in Vision Impairment

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BACKGROUND: The L-type voltage-gated Ca^{2+} channel $Ca_v1.4$ is a critical component of the vision pathway. Located on the presynaptic terminals of photoreceptors (PRs), Cav1.4 mediates Ca^{2+} influx into the PR which results in glutamate release and transmission of visual information. Spontaneous mutation of the gene coding for the $Ca_v1.4$ extracellular $\alpha_2\delta_4$ subunit (*CACNA2D4*) in mice results in disrupted PR synapses. In humans, a mutation in *CACNA2D4* has been linked to night blindness and progressive cone dystrophy. However, the role of $\alpha_2\delta_4$ in regulating PR synapse structure and vision remains unknown. mGluR6, a glutamate receptor located on post-synaptic bipolar cells, has been shown to interact transynaptically with adhesion proteins. These interactions are critical for proper synapse formation between rods and adjacent ON-bipolar cells. The purpose of this study was to define the role of $\alpha_2\delta_4$ in the retina and to investigate its potential interaction with mGluR6.

HYPOTHESIS: $\alpha_2\delta_4$ interacts transynaptically with mGluR6 to stabilize retinal synapses.

METHODS: $\alpha_2\delta_4$ KO mice were generated using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9n to introduce a stop codon in exon 2 of the mouse *CACNA2D4* gene encoding $\alpha_2\delta_4$. Retinas from WT C57BL/6 mice and $\alpha_2\delta_4$ KO mice were isolated at post-natal day 52 (p52) and either fixed for 1 hour in a 30% sucrose solution in 1x PBS or fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer for 24 hours. Sucrose-fixed samples were embedded in OCT compound and sectioned using a cryostat. Sections were stained with Hoechst stain. Samples fixed in glutaraldehyde-cacodylate buffer were embedded in resin and sectioned. Sections were stained with toluidine blue. OCT and resin-embedded samples were visualized using light microscopy. Measurements of the outer nuclear layer (ONL), outer plexiform layer (OPL), and inner nuclear layer (INL) were taken and averaged for WT and KO mice. To study the interaction between $\alpha_2\delta_4$ and mGluR6 in vitro, HEK293T cells were transfected to overexpress $\alpha_2\delta_4$ and mGluR6. Following transfection, cells were lysed and a co-immunoprecipiation (Co-IP) assay was performed using either $\alpha_2\delta_4$ or mGluR6 Abs. Cells singly transfected with either $\alpha_2\delta_4$ or mGluR6 served as controls for the Co-IP. Results were visualized by Western blot. Following the Co-IP, a GST pull-down assay was performed. HEK293T cells were transfected to overexpress $\alpha_2\delta_4$ and lysed. A GST-tagged fusion protein containing the extracellular domain of mGluR6 was used to isolate $\alpha_2\delta_4$ and results were visualized by Western blot. GST protein alone served as a control for the pull-down.

RESULTS: In p52 $\alpha_2\delta_4$ KO mouse retinas, the ONL thickness decreased by 25.2%, OPL thickness decreased by 60.1%, and INL thickness decreased by 13.5%. For the Co-IP, $\alpha_2\delta_4$ coimmunoprecipitated with mGluR6 antibodies and mGluR6 coimmunoprecipitated with $\alpha_2\delta_4$ antibodies. Finally, the GST-tagged mGluR6 fusion protein pulled down $\alpha_2\delta_4$.

CONCLUSIONS: In $\alpha_2\delta_4$ KO mice, the OPL, which contains the PR-bipolar cell synapses, decreased drastically more than the surrounding ONL and INL that contain PR and bipolar cell nuclei, respectively. This indicates that $\alpha_2\delta_4$ KO OPL thinning is caused primarily by disrupted synapses rather than by reduction in PR and bipolar cell number. The $\alpha_2\delta_4$/mGluR6 Co-IP and GST pull-down indicate that $\alpha_2\delta_4$ interacts with the extracellular domain of mGluR6 in vitro. Taken together, these results suggest that $\alpha_2\delta_4$ and mGluR6 may interact transynaptically to stabilize synapses in the retina.

Preliminary Comparison of Outcomes in Low-Risk Women in Centering Pregnancy® versus Traditional Certified Nursing Midwife Prenatal Care

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BACKGROUND

Centering Pregnancy® (CP) is an interdisciplinary approach to prenatal care characterized by group visits of women with similar estimated delivery dates. Group prenatal care such as Centering Pregnancy® has been associated with increased rates of breastfeeding and decreased cesarean deliveries in adolescent and low-income women, however these outcomes have not been studied in a generalizable population.

Understanding rates of breastfeeding and vaginal delivery for mothers participating in Centering Pregnancy® can offer additional metrics of outcome-related evidence associated with group prenatal care. As participation in group prenatal care has been associated with better overall pregnancy outcomes and lower costs, more specific knowledge of these rates may facilitate health providers applying group prenatal care models to their practice.

HYPOTHESIS

Women who participated in Centering Pregnancy® group prenatal care will report more breastfeeding six weeks postpartum and have higher rates of vaginal delivery than those who participated in individual provider midwifery care.

METHODS

IRB approved, retrospective chart analysis was conducted for women that participated in (1) CP care at UIHC between October 2012 and May 2016 and (2) certified nurse midwifery (CNM) prenatal care during the same time period. This is a preliminary analysis of the ongoing review. Breastfeeding status was recorded on all discharging women and at a scheduled 6-8 week postpartum visit. Delivery type was recorded at the time of delivery. Descriptive statistics were performed with $P<0.05$ considered significant.

RESULTS

115 women who participated in CP care and 469 who were involved in CNM individual prenatal care were reviewed allowing for a 3:1 control to intervention ratio. Rates of vaginal delivery (including operative) were not statistically different between CP vs CNM groups (77.4% vs 63.7%, p=0.062). Breast feeding rates at discharge following delivery were likewise not dissimilar (96.4% vs 92.7%, p=0.157). More women attended their postpartum visit in the CP group (93.9% vs 84.2%, p=0.007), and rates of breast feeding reported at the postpartum visit were higher in this group, though not significantly (89.8% vs 83.3% p=0.095).

CONCLUSION

At our institution, incorporating Centering Pregnancy® group prenatal care improved the rates of women attending their postpartum visit and led to greater likelihood of breastfeeding at the postpartum visit.

Determining Return of Scapulohumeral Rhythm in Patients After Reverse Total Shoulder Arthroplasty

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BACKGROUND: Cuff tear arthropathy (CTA) is a shoulder pathology defined by a full-thickness tear in two or more rotator cuff tendons, superior migration of the proximal humerus, collapse of the proximal aspect of the humeral articular surface and erosion of the acromion. This pathology is often the result of atraumatic cuff tears and predominantly affects an elderly patient population. Patients with CTA often present to the clinic with complaints of shoulder pain and an inability to flex or abduct the arm overhead. Their pain is the result of arthritis and is made worse by the migration of the humeral head and collapse of the articular surface. Loss of abduction to 90° – pseudoparalysis – results from the defect in the rotator cuff. Overall arm abduction is a result of combined glenohumeral (GH) and scapulothoracic (ST) motion. The ratio of GH to ST motion is referred to as scapulohumeral rhythm (SHR). CTA patients lose the ability to initiate GH motion and compensate with increased ST motion.

Reverse total shoulder arthroplasty (RSA) is a surgical option for CTA patients that both alleviates arthritic pain and restores motion. RSA reverses the native ball-and-socket anatomy of the shoulder, which allows the deltoid muscle to initiate overhead motion in lieu of the damaged rotator cuff muscles. While this surgery is highly successful in reducing pain and restoring arm abduction to CTA patients, RSA also suffers from high complication rates. The most common complication is scapular notching, which is damage to the implant and/or scapular bone resulting from undesired contact between the implant hardware and the bone immediately inferior to the glenoid. Studies of scapular notching have thus far presumed SHR returns to that seen in healthy shoulders after RSA. However, residual abnormalities in in ST motion could greatly influence the risk or severity of scapular notching.

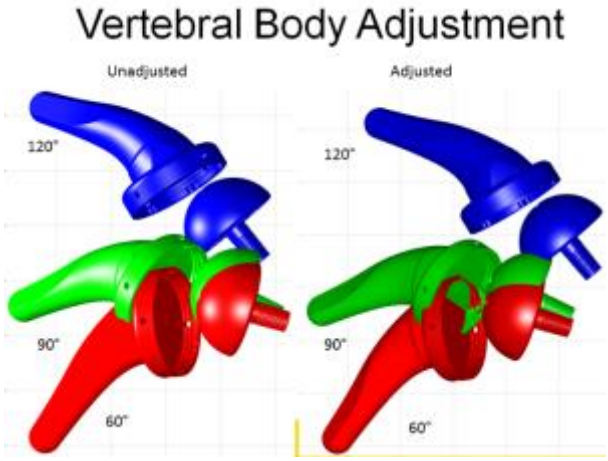
HYPOTHESIS: Patients will have persistent increased scapulothoracic motion after RSA.

METHODS: EOS radiographic images were obtained of patient's shoulders 6 months after RSA with a Tornier Aequalis Ascend Flex . EOS imaging is a technology that uses low dose x-ray to acquire simultaneous orthogonal biplanar radiographs. Images were acquired with the arm at 60°, 90° and 120° of abduction. EOS images and pre-operative CT scans were securely transferred to the Orthopaedic Biomechanics Laboratory at the University of Iowa. Two vertebral bodies were segmented from the CT scans using Osirix software. 3D surfaces of the vertebral bodies and the implant geometry – provided by Tonier, Inc. – were loaded into MATLAB. The 3D implant geometry was projected into 2D and aligned to the radiographic images collected using EOS. This 3D-to-2D model image registration method was previously reported by our group (Anderson DD, et al. 2012. *Comput Math Methods Med*). Humeral and scapular angles were subsequently calculated between 60° - 90° and between 90° - 120° arm abduction positions. A cylinder with known axis was fit in Geomagic Studio software to the distal aspect of the stem and glenosphere post representing the axis of the humerus and scapula respectively. The segmented vertebral bodies were then aligned to the EOS images using the previously described technique in to eliminate any motion of the thorax, allowing for a more accurate measurement of ST motion. GH and ST motion were then calculated to determine overall contributions to SHR.

RESULTS: Short-term follow-up results are based on the data for the first 2 patients enrolled in the study who have completed 6 months post-operative imaging analysis.

	SHR (GH: ST motion)	
Patient	**60-90**	**90-120**
1	0.586:1	0.655:1
2	2.943:1	0.271:1
Normal	2:1*	

* Inman, et al. Observations on the function of the shoulder join. J Bone Joint Surg, 26 (1944), pp. 1-26.



CONCLUSIONS: Patients having undergone RSA are able to regain the ability to abduct the affected arm, but they use increased ST motion to do so, particularly above 90 degrees. It is possible that this increased ST motion positions the scapula so that it is at a greater risk for impingement from the implant and may contribute to the explanation of why scapular notching is so common after RSA.

GWAS and System Biology Analysis of Depressive Symptoms among the COPDGene Cohort

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ABSTRACT

BACKGROUND: Depression is a very common disorder, having a prevalence of around 16.2% in the United States. This is around 52 million people—the most common psychiatric disorder in the United States. However, the etiology of depression is not yet completely understood. Although research on twin studies supports genetic factors influencing depression, few genetic factors have been found. Because a single genetic susceptibility factor has not been found, depression is likely a heterogenous disorder whereby synergetic effects from many mutations with small effect sizes cause depression. To identify such genetic factors, a hypothesis-free approach with a genome wide association study (GWAS) is needed. To detect such small effect sizes at the genome-wide significance level, a large sample size is vital. However, collecting both genotypic and phenotypic data on large sample sizes in a single study is usually quite difficult and expensive. Thus, to overcome these challenges, the current study uses the available genome-wide genetic data and phenotypic information from over 10,000 participants collected through the single largest genetic study among COPD patients—the COPDGene study—to find genetic factors associated with depression. The COPD patient population is useful because depression has both higher prevalence and mortality in COPD patients.

HYPOTHESIS: Certain genetic risk factors are associated with depression phenotypes among the COPDGene cohort.

METHODS: This study used partial data from the COPDGene study and created four subgroups based on ethnicity and depression phenotype. Depression phenotypes were defined using the Hospital Anxiety and Depression Scale (HADS), antidepressant use history, and the SF36 Quality of Life Mental Component Scale. An established pipeline of GWAS data was used to obtain a list of the top hit genes for each phenotype. The top hit genes were then analyzed in this study by being entered into different gene and pathway analysis programs such as GeneMANIA, DAVID, ConsensusPathDB, and GLITTER to determine if the top hit genes and their networks were relevant to depression. Specifically, DAVID and ConsensusPathDB were used to determine common pathways between the genes on the top hit list. GeneMANIA investigated the genetic interactions between the different genes, while GLITTER analyzed the expression of the top hit genes in tissues throughout the body.

RESULTS: Top hit genes were in the range of $p = \sim 10^{-7}$, not surviving at the genome-wide significance level. Among top hits, however, there were several genes associated with depression and other psychiatric conditions reported in the literature. Also, network analysis showed that top hit genes were forming networks involved in neurotransmitter and synaptic transmission functions. Further, top hit genes showed a trend of expression that was greater in brain tissues than other tissues in the body. These findings indicate that even from partial data with an imperfect phenotypic definition of depression, the COPDGene dataset can provide significant opportunity for genetic association studies of depression.

CONCLUSION: Future studies using data from the complete COPDGene study with improved psychiatric phenotype data would both greatly benefit and significantly contribute to the understanding of the genetic factors associated with depression.

Assessment of relationship between postoperative angles of alignment and functional outcomes following high tibial osteotomy

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Abstract

Background

In patients with medial unicompartmental osteoarthritis who undergo a medial open-wedge high tibial osteotomy (HTO), the accuracy of correction has been shown to play an important role in their long-term outcomes. Justin Chang found that computer navigation systems improve the accuracy of correction. The weight-bearing line should be between 50-75% of the medial to lateral edge of the tibial plateau, with optimal alignment at 62%, according to many authors. The purpose of our study was to determine if a relationship exists between post-operative alignment and the initial functional outcomes following high tibial osteotomy, primarily looking at post-operative KOOS scores and secondarily looking at the incidence of patients requiring a total knee arthroplasty (TKA) or long-term brace use.

Hypothesis

Our hypothesis was that in the short-term, under-correction would yield a higher incidence of TKAs and brace use as well as poor KOOS functional outcome scores in those patients who are living with the HTO results.

Methods

In this retrospective study, 38 patients with medial varus osteoarthritis who were managed by open-wedge HTO were included. Of the 38 patients, 31 were at least corrected into the target range or even over-corrected while 7 remained grossly under-corrected. Post-operative single-leg, long-leg standing alignment films were used to determine post-surgical correction by calculating the weight-bearing line from the hip to the ankle.

Results

There was found to be a higher incidence of TKA and long-term brace use in the grossly under-corrected group compared to the target range and over-corrected group. In the grossly under-corrected group, 100% of patients required a TKA or brace following surgery. In the target range and over-corrected group, only 13% of patients required a TKA or brace. On the other hand, for those with post-operative KOOS, there was not a significant difference when comparing the grossly under-corrected to the rest of the group, including target range and over-corrected.

Conclusion

In the short-term, there was a much greater incidence in TKAs and brace use in the grossly under-corrected, however when looking at functional outcome scores there was no statistically significant difference between the grossly under-corrected and the rest of the patients.

The Pathomechanical Basis of Post-Traumatic Osteoarthritis following Acetabular Fracture

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BACKGROUND: Posttraumatic osteoarthritis (PTOA) is a debilitating condition that presents following trauma to an articular joint. Development of PTOA is especially common in injuries involving fracture of the articular surface. Up to 25% of patients sustaining acetabular fractures develop PTOA despite surgical treatment.[1] Current PTOA prevention approaches are largely based on clinical intuition and accumulated experience. These approaches are subjective in nature and do not adequately improve patient outcomes or reduce PTOA risk. Recent research into the biomechanics of joint injuries has implicated mechanical factors in PTOA development. Acute fracture severity and chronic elevated contact stress are two mechanical variables that have previously been shown to influence PTOA progression.[2] Further research is needed to determine the role and relative contribution of these measures in PTOA development following fracture of the acetabulum. This research will provide objective measures to aid in predicting PTOA risk and improve how acetabular injuries are managed.

HYPOTHESES:

1. Acute fracture severity is a significant predictor of PTOA in acetabular fractures
2. Chronic contact stress elevation following fracture reduction is a significant independent predictor of PTOA in acetabular fractures

METHODS:

Study population

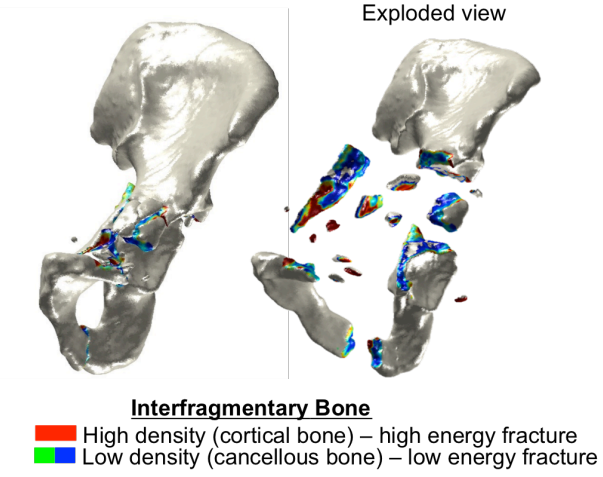
Seventeen patients with surgically reconstructed acetabular fractures were consented for this IRB approved study. Patients were selected from a larger series of 263 acetabular fractures for having pre-operative CT scans available with a minimum 24-month follow-up. Post-operative CT scans were also available for 7 of these cases.

Mechanical assessments

Fracture energy and articular fracture edge length (AFEL) were calculated from preoperative CT scans using previously established objective CT based methods.[3] Bone fragments created by fracture were segmented from preoperative scans to generate an accurate 3D model of the fractured pelvis. Fractured surface area was then identified on these fragments using a surface classification algorithm and used to calculate fracture energy and evaluate the AFEL. Post-operative CT scans were used to evaluate contact stresses within the reconstructed joints using previously validated methods.[4,5] Outcomes were evaluated using the Kellgren and Lawrence (KL) system to grade radiographs taken at least 24 months post-injury.

RESULTS

Fracture energies for the 17 acetabular fracture cases ranged from 4.6-32.8J with a mean of 17.6±8.6J. Corresponding maximum contact stress exposure for 7 cases with post-operative scans available ranged from 326-950MPa*s with a mean of 655±212MPa*s. When analyzed with KL grade, fracture energy did not initially appear to be implicated in PTOA risk. However, investigating contact stress measures for each case revealed that outliers within our dataset were heavily influenced by this metric. Excluding these points revealed a trend of increasing PTOA risk with increasing fracture energy. Plotting maximum contact stress alone against KL grade also revealed a positive trend, though neither relationship reached significance. A total of 3 of 7 patients developed PTOA as determined by a KL grade > 2 with a mean KL grade of 2±1.5.



CONCLUSION

This preliminary investigation into fracture severity and maximum contact stress within acetabular fractures has elucidated an interesting and complex relationship between these metrics and PTOA risk. Initial results suggest that maximum contact stress may play a larger role in PTOA progression than fracture severity within the acetabulum. It is possible that a dynamic relationship exists between the two measures. Increased fracture severity may be predictive of PTOA risk until a certain threshold of max contact stress is reached above which PTOA is unavoidable. These results encourage further investigation into pathomechanical variables and their ability to predict PTOA. An enhanced understanding and clinical application of these measures will provide an objective assessment of PTOA risk and may ultimately improve the standard of care of articular fractures.

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Recovery and Cycling Endurance Capacity Following Consumption of Chocolate Milk, Calorie Replacement Beverage or Beer

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Background: Previous research has demonstrated that chocolate milk is a more effective recovery beverage than a commercially available calorie replacement recovery beverage. However, this study suffered from potential funding conflicts of interest. In order to mitigate this conflict, our team replicated the study and added an additional study arm to evaluate another commonly beverage commonly consumed after exercise, beer. The purpose of this study is to determine which recovery beverage (Anderson Erickson Reduced Fat Chocolate Milk, Coors Banquet Beer, or Endurox R4) provides the most effective form of recovery following an exhaustive bout of exercise.

Hypothesis: We hypothesize that trained cyclists using beer as an exercise recovery beverage will perform the same during an exhaustive endurance task compared to cyclists using chocolate milk or Endurox R4 as an exercise recovery beverage.

Methods: This randomized crossover trial included 9 trained cyclists. Each cyclist performed 3 trials. Each trial included three stages: 1) an exhaustive exercise trial in which cyclists performed a linear ramp ergometer test of 1W/kg every ten minutes until failure, 2) a 4-hour recovery phase in which participants received an isocaloric, weight-based amount of the randomly-selected recovery beverage and 3) an endurance capacity trial, in which the linearly ramped endurance test was repeated to failure. Cycling power, heart rate, and blood lactate were measured throughout both the exhaustive and endurance capacity trials. Mood and hunger were surveyed during the recovery phase. The primary outcome was the difference in time to failure between the exhaustive trial and the endurance capacity trial.

Results: The mean difference in time to failure between the exhaustive trial and the endurance capacity trial was -13.1±102.0 seconds, -28.8±64.2 seconds, and -49.8±116.3 seconds for chocolate milk, Endurox R4, and Coors Banquet Beer respectively. Using ANOVA the difference was not found to be significant ($p>0.05$).

Conclusion: There was a trend, but no statistically significant difference, in exercise capacity following a recovery period using the three tested beverages.

Preparing Silastic Cochlear Implants with Surface Zwitterionic Polymer by Dip Coating and Photopolymerization

Ryan Horne, BS & C. Allan Guymon, PhD

Abstract

BACKGROUND: Cochlear implants have become a standard tool to restore auditory function. However, the foreign body response encapsulates cochlear implants soon after their implantation, causing the implant to transmit electric stimuli less precisely and requiring extra power output to transmit through the fibrosis. Coating cochlear implants with zwitterionic polymers has been proposed as a possible solution to the fibrosis issue. Zwitterionic polymers are anti-fouling *in vitro* and *in vivo*, durable, biomimetic mechanically, biocompatible, and straightforward to polymerize with UV light. Despite their desirable properties, zwitterionic polymers have proved difficult in practice to attach to cochlear implants. The monomer solution is aqueous, whereas the cochlear implant sheath is typically made of hydrophobic silastic (polydimethylsiloxane), making the wetting of the implant surface for polymerization impractical.

HYPOTHESIS: Introducing a silane surfactant will allow for consistent coverage between a solution of zwitterionic monomer sulfobetaine methacrylate (SBMA) and a silastic surface. This consistent coverage will allow a silastic object to be dipped into the monomer solution, be removed, and subsequently polymerized with UV light to form a consistent, thin zwitterionic polymer coating.

METHODS: Monomer solutions were made of SBMA in phosphate-buffered saline (PBS). Each monomer solution included 0.05% (w/w) of photoinitiator I-259. Silastic samples were soaked in a 50 g/L solution of benzophenone in acetone and vacuum dried to aid in surface attachment of SBMA monomer. Five silane-based surfactants were tested for contact angles, each at 0.8% (w/w) in monomer solution.

RESULTS: Contact angles were high (92° ± 4°) between 50% (w/w) SBMA solution and the silastic surface. Upon introduction of various types of surfactant at 0.8%, contact angles decreased, with the most pronounced reduction from Surfactant 1 giving a contact angle of 30° ± 2°. Further experiments established a relationship between concentration of Surfactant 1, concentration of SBMA solution, and the quality of coating produced from dip coating and photopolymerization. The best polymer coating on silastic slabs came from a monomer solution of 50% SBMA, and 0.8% Surfactant 1. These experiments also demonstrated a greater consistency of coating by introducing horizontally-oriented axial spinning under pure N_2 during the polymerization step. The optimized monomer solution yielded a consistent zwitterionic polymer coating when tested on an actual cochlear implant silastic with axial spinning under pure N_2. The control with no surfactant yielded only 4 beads of polymer under the same conditions.

CONCLUSION: Dip polymerization followed by photopolymerization yields consistent thin polymer coverage on silastic when surfactant is introduced into the zwitterionic monomer solution. The best coverage was achieved on silastic cochlear implants using the specific parameters of 50% SBMA, 0.8% Surfactant 1, polymerization under N_2, dip coating, and subsequent horizontally-oriented axial spinning. This technique could be used to coat silastic cochlear implants to prevent their fibrosis and infection.

Ponseti Method Treatment of Neglected Idiopathic Clubfoot: Preliminary Results of a Multi-center Study in Nigeria

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Abstract

BACKGROUND- Idiopathic clubfoot is the most common musculoskeletal birth defect and if left untreated can result in severe physical and social limitations. The Ponseti method has been established as the gold standard of treatment for idiopathic clubfoot in the newborn population. Untreated clubfoot in children above walking age has been classified as "neglected" clubfoot. Because of its scarcity in developed countries, there remains limited evidence on results of the Ponseti method in this population. The purpose of this study was to evaluate the effectiveness of the Ponseti method for initial correction of neglected clubfoot cases in multiple centers throughout Nigeria.

HYPOTHESIS- The Ponseti method is an effective treatment for clubfoot in children over one year of age.

METHODS- Patient charts were reviewed through the International Clubfoot Registry for 12 different Ponseti clubfoot treatment centers and 328 clubfeet (225 patients) met inclusion criteria. All patients were treated by the method described by Ponseti including manipulation and casting with percutaneous Achilles tenotomy as needed.

RESULTS- A painless plantigrade foot was obtained in 255 feet (78%) without the need for extensive soft tissue release and/or bony procedures.

CONCLUSIONS- We conclude that the Ponseti method is a safe, effective and low-cost treatment for initial correction of neglected idiopathic clubfoot presenting after walking age. Long-term follow-up will be required to assess outcomes.

Understanding of Cervical Cancer and Attitudes Towards Cervical Cancer Screening Following Loop Electrosurgical Excision Procedures in Haiti

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ABSTRACT

BACKGROUND: The incidence of cervical cancer in Haiti is estimated to be 94 per 100,000 women, and infection by carcinogenic human papillomavirus (HPV) is elevated in Haiti compared to other Latin American countries. This combined with lack of HPV vaccination, cervical cancer screening and effective intervention contribute to the extremely high death rate for Haitian women from cervical cancer. Efforts have begun to provide these services to women in the communities they serve as the resources needed to screen for and treat non-metastatic cervical cancer and pre-cancerous lesions have become increasingly available to both the government and non-governmental organizations throughout the country. Community Health Initiative Haiti (CHI) is a non-governmental organization that works in and around the community of Arcahaie. The CHI healthcare team has encountered many cases of cervical cancer during their mobile clinics which have advanced beyond domestic availability of treatment. To address this issue, CHI began offering cervical cancer screening during the June 2016 healthcare trip.

PURPOSE: As part of the organization's roll-out of their 'see and treat' cervical cancer screening and treatment program, an effort was made to assess the patient's understanding of cervical cancer and their satisfaction with the treatment. The overall purpose of the program was to best serve the women of the community and decrease the incidence of advanced stage cervical cancer.

METHOD: Over the course of 3 clinic days, approximately 60 women aged 30-49 were screened for cervical cancer using an acetic acid wash and colposcope. 14 women had a positive screen and were asked to return later in the week to be treated. Loop Electrosurgical Excision Procedures (LEEP) were used to remove the pre-cancerous tissue from the women's cervices. Following the LEEP, 9 women consented to participate in this study. Participants were immediately given a questionnaire consisting of 3 questions regarding their understanding of cervical cancer. Contact information was then collected and the participants were visited at their homes 3-4 weeks later, where they were given a second questionnaire. The second questionnaire assessed patient satisfaction with the procedure and their likelihood of recommending screening to friends and family. Participants were then asked open-ended questions regarding patient perspectives and quality improvement for future screening.

RESULTS: We found that while all of the participants (n=9) had heard of cervical cancer before, 56% were unsure of the cause. Of those who reported they knew the cause of cervical cancer, 22% reported it was due to a microbe from sex, 22% reported it was due to "bad water", and 11% reported it was due to voodoo. 56% of the participants reported that they currently know or had previously known someone with cervical cancer. Only 1 of the women surveyed reported that she had been previously screened for cervical cancer. At the follow-up visit, all of the women reported that they were happy with the procedure, would come back to the clinic to be screened again, and would recommend that their friends and family be screened for cervical cancer as well. Responses to open ended questions indicated that the majority of the women felt that their community could benefit from education regarding cervical cancer and that they had learned more about cervical cancer from their participation in the study.

CONCLUSION: While cervical cancer impacts the lives of many women in Haiti, this study confirms that a large majority of women have never been screened and a majority does not know what causes cervical cancer. After being screened for cervical cancer and treated for pre-cancerous cell growth, women from the community of Arcahaie reported positive attitudes towards the intervention and recommended that friends and family be screened in the future.

Iron Nanoparticle Contrast Enhanced Microwave Tomography for Emergent Stroke: A Pilot Study

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ABSTRACT

BACKGROUND: Stroke is a major cause of morbidity and mortality worldwide. Currently, emergent stroke is clinically evaluated utilizing existing hospital based imaging modalities (CT, MRI) capable of differentiating between ischemic and hemorrhagic cerebral circulatory changes. The AHA/ASA currently recommends that brain imaging be obtained before any specific therapy to treat acute ischemic stroke is initiated. Administration of rtPA within a 4.5-hour window following ischemic stroke onset has been associated with improved outcomes. Moreover, earlier treatment (i.e. within 90 minutes) is more likely to result in favorable outcomes. Unfortunately, only 1-8 percent of patients receive treatment within this window. Thus, an opportunity exists for the development of a device which can travel with first responders to the patient, and inform them whether intravenous thrombolysis is indicated, allowing for a more efficacious therapeutic response. Microwave imaging (MI) is an emerging alternative imaging modality which utilizes non-ionizing electromagnetic signals which operate over the frequency range of hundreds of megahertz to tens of gigahertz. Recent microelectronics and materials science advancements have allowed for the miniaturization of these devices. To produce signals or images, using tomographic techniques, MI has traditionally relied upon differences in tissue specific dielectric properties to create contrast. However, super paramagnetic iron oxide nanoparticles are known to interact with electromagnetic waves emitted during MI. Thus, these nanoparticles, when injected, could potentially be used to enhance contrast for MI when introduced into the cerebral circulation in the setting of emergent stroke. In this study we provide preliminary evidence that Ferumoxytol (iron oxide nanoparticles) causes attenuation of microwave signal amplitude in *in vitro* phantom simulations, New Zealand White Rabbits, and in Humans.

HYPOTHESIS/AIMS: 1) Construct and assess the functionality of our novel MI device *in vitro, in rabbit, and in humans* 2) Determine if Ferumoxytol iron oxide nanoparticles affect microwave signal amplitude in these respective imaging trials

METHODS: For all experiments a 4-channel Vector Network Analyzer (Anritsu) was connected to two 3D printed triangular copper patch antennas for transmission and reception respectively. The data was simultaneously analyzed via a custom Labview (National Instruments) program to achieve a measurement of signal attenuation (dB) as the microwave irradiation passed through the sample for each respective frequency transmitted. Microwaves were transmitted in a sweep pattern from 1-2 GHz for each individual measurement. To minimize multipath signal deflections resulting from the air/skull interface, a constant amount of commercial ultrasound jelly was applied to the patch antennas prior to each measurement antenna placement. For *in vitro* trials, a silicone brain phantom was created with either a pure water, single spherical iron nanoparticle/silicone inclusion, or a pure silicone inclusion placed in the midline a few centimeters below the surface of the simulated brain parenchyma. Additionally, a brain phantom was created with nanoparticles distributed in a crude approximation of a bilateral MCA ischemic infarct. Measurements were then obtained both on an anterior posterior axis and a lateral axis. Each frequency sweep was repeated 40 times and was reported as average attenuation at each respective frequency. Rabbit trials consisted of a baseline lateral measurement followed by lateral measurements 5 minutes post intravenous (IV) Ferumoxytol injection, and 30 minutes post injection. The latter occurred 3 minutes after the animal was declared deceased by veterinary staff. Each frequency sweep was repeated 20 times. For the human trials, a lateral baseline frequency sweep series was repeated 40 times, then Ferumoxytol was injected IV, and lateral frequency sweeps were conducted at 5 minutes post injection, 24 hours post injection, and 72 hours post injection.

RESULTS: Data for all three trials indicate that a measureable differential increase in signal attenuation exists across a frequency range of 1.3-2 GHz when Ferumoxytol iron oxide nanoparticles are introduced in phantom models, in New Zealand white rabbits, and in humans.

CONCLUSION: These results, to the best of our knowledge, provide the first preliminary evidence in humans that super paramagnetic iron oxide nanoparticles may be used as contrast in the setting of MI of the brain. These data will inform future efforts at image reconstruction and imaging device development.

Establishing reference intervals in laboratory testing for transgender patients. Robert M. Humble, Matthew D. Krasowski. Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242

BACKGROUND

Clinical laboratory testing is an integral part of modern medicine. In order to provide a context to results of laboratory testing, reference intervals are regularly included with laboratory test results. As access to transgender healthcare becomes more available in the United States, interpreting laboratory results is difficult within the male/female gender binary reference intervals. Previous research studied interpretation of laboratory results in transgender women (transfemales), identifying significant differences in transfemale hematocrit, hemoglobin, creatinine, ALP, LDL cholesterol and triglycerides compared to healthy cisgender controls. This study did not address the reference interval issue. There is little to no literature investigating the impact of hormone therapy (HT) on reference intervals in laboratory testing of transgender individuals.

HYPOTHESIS/AIMS

The objective of this study was to review and analyze laboratory test results for transgender patients in the UIHC pathology laboratories. In addition, we sought to investigate how HT impacts commonly ordered laboratory test values in order to determine whether new recommendations for laboratory test reference ranges are indicated to improve clinical care of transgender patients.

METHODS

In this retrospective study at an academic medical center, electronic health records were searched to identify transgender patients seen at UI Health Care in the time period of January 1, 2012 to May 1, 2016. Patients for analysis were verified as transgender by chart review, were at least 18 years old, had laboratory testing during the period of study, and were receiving either estrogen or testosterone as HT as a component of gender affirming treatment for at least six months.

RESULTS

Verified transgender patients were separated into a transmale cohort (n=119) and a transfemale cohort (n=119). For comparison, we identified patients that had lab testing done prior to initiation of HT for use as a baseline measurement, then had testing performed again after at least six months of HT. In the transfemale cohort, we identified individuals with lab results before and after initiating HT for creatinine (n=42), hemoglobin (n=11), hematocrit (n=11), ALP (n=8), LDL cholesterol (n=22), and triglycerides (n=11). There was a statistically significant difference ($p < 0.05$) in the hematocrit levels of the transfemale cohort after initiating HT. In the transmale cohort, we identified individuals with lab results before and after initiating HT for creatinine (n=10), hemoglobin (n=21), hematocrit (n=19), ALP (n=4), LDL cholesterol (n=10), and triglycerides (n=6). There was a statistically significant difference ($p < 0.05$) in the triglycerides levels of the transmale cohort after initiating HT, and a more powerful statistically significant difference ($p < 0.005$) in the creatinine, hemoglobin, and hematocrit levels of the transmale cohort after initiating HT. Data obtained during the study has shown to be complex to process and analysis is ongoing.

DISCUSSION/CONCLUSION

Our study is the largest effort to date to analyze the effect of hormone therapy on laboratory testing patterns in transgender individuals. Preliminary data suggest significant changes to laboratory test values in both the transmale and transfemale cohorts after initiation of HT. Further analysis is required to determine the full extent of the effects of HT on laboratory test values.

Examining the differences in characteristics and outcomes between married and unmarried radical cystectomy patients

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Objectives: Radical cystectomy (RC) for bladder cancer is associated with a significant risk of complications and mortality. Understanding the factors that contribute to cystectomy outcomes improves risk assessment for patient counseling and may provide insight into future care interventions. Being married has been reported to be a protective factor in cancer, but most reports are from cancer registry data which lacks information on factors such as medical comorbidity. Using an institutional cohort, we sought to expand on prior work to further evaluate the differences in patient disease-specific characteristics between married and unmarried cystectomy patients, and to report the association of marital status with postoperative complications and mortality.

Methods and Materials: 334 patients had radical cystectomy at our institution between 2000 and 2010. Marital status was identified prospectively by cancer registrars at the time of treatment. Marital status was defined as married versus unmarried (includes single, widowed, and divorced). Variables assessed were patient demographics, driving distance to our institution, comorbidity (Charlson Comorbidity index), body mass index, tumor staging, and treatment (neoadjuvant chemotherapy and type of urinary diversion). Outcomes measured were length of stay, discharge status (to home or to a skilled nursing facility/hospital), 30-day postoperative complications (Grade 2-5 Clavien-Dindo), and 4-year mortality. Statistical analysis was performed using univariate analysis, and then multivariate analysis was used to adjust for measured differences.

Results: On univariate analysis, unmarried patients (compared to married patients) were more likely to be female, to have more comorbidity, and to have undergone an ileal conduit urinary diversion ($p<.01$). Age, body mass index, clinical disease stage, and rate of neoadjuvant chemotherapy were not significantly different between the two groups ($p>0.05$). Unmarried patients were more commonly node positive (38% vs. 27%), but this difference was not statistically significant ($p=0.06$). With respect to outcomes, the rate of postoperative complications was not higher in unmarried patients (53% vs. 48%, $p=0.05$); however, unmarried patients had a longer length of stay (median 9 days vs. 8 days, $p=0.029$) and were more likely to discharge to a skilled nursing facility/hospital compared to married patients (30.4% versus 12.4%, $p<.001$). On multivariate analysis, marital status was not associated with complications, but continued to be associated with a longer length of stay (OR=1.18, $p=0.002$) even after adjusting for differences in age, gender, comorbidity, and diversion type. The 4-year mortality was higher in unmarried patients (65% vs. 46%, $p<0.001$), and the difference was most pronounced in the first 3 months after surgery. On multivariate analysis, not being married was associated with a 43% increased rate of mortality (hazard ratio=1.43, $p=0.047$).

Conclusions: In the radical cystectomy population, unmarried patients did not have a higher postoperative complication rate, but did have a longer length of stay and higher rate of discharge to a skilled care facility/hospital. Mortality was markedly increased in unmarried patients after cystectomy. This analysis suggests that further efforts should be directed at social support and surveillance/adjuvant treatment decisions in the unmarried patient population.

Comparison of Spinal and General Anesthesia in Unilateral Total Knee Arthroplasty

Lauren N. Javernick and Kyle R. Duchman

Background: Spinal anesthesia, compared with general anesthesia, has been associated with fewer complications postoperatively: deep vein thrombosis, blood transfusions, superficial infections, and overall mortality. However, this has not been studied in unicompartmental total knee arthroplasty where the patient population carries significantly increased proportions of comorbidities. Thus, the findings carry increased sensitivity to predisposing factors. The purpose of this study is to evaluate differences between spinal and general anesthesia in this higher risk population.

Methods: The American College of Surgeons National Surgical Quality Improvement Program (ACS NSQIP) database was searched to identify patients who underwent primary total knee arthroplasty between 2006 and 2011. The search criteria excluded non-elective and bilateral procedures, as well as patients with an INR greater than or equal to 1.4 who were not considered to be candidates for management with spinal anesthesia according to the anesthesia guidelines. Complications in those who had been managed with spinal or general anesthesia were identified. Propensity factor matching was utilized to adjust for selection bias. Multivariate logistic regression analysis was utilized to evaluate predisposing factors of 30 day mortality.

Results: The search of the ACS NSQIP database revealed 2141 patients available for analysis, with 56.3% receiving general anesthesia. Analysis of the spinal anesthesia group showed a lower risk of any complication (3.7% versus 6.5%; p=0.03), wound dehiscence (0.46 vs 0.00%;p=0.025), and length of stay (2.50 versus 2.01 days; p=<0.0001). No deaths were recorded for the first thirty days. Multivariate analysis identified spinal anesthesia as an independent protective factor for overall morbidity (1.820 Odds Ratio; 95% Confidence Interval 1.094-3.029) for those undergoing unicompartmental total knee arthroplasty.

Conclusion: Patients undergoing unicompartmental total knee arthroplasty are generally seen to have increased co-morbidities. General anesthesia was shown to be associated with a higher risk of overall morbidity as well as a longer hospital stay.

Impact of IL-1RI blockade on inflammatory response and functional outcome following murine traumatic brain injury

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Abstract

Background: Traumatic brain injury (TBI) is a leading cause of death and disability in the United States. TBI causes neurologic injury by two mechanisms: primary mechanical injury at time of impact, and secondary injury due to endogenous pathways activated by tissue trauma including inflammation. Neuroinflammation is mediated in part by the interleukin-1 family of cytokines. IL-1a and IL-1β are the best-characterized cytokines of this family, and both signal through a common receptor, IL-1RI. Following neurologic injury, activated microglia are an important source of pro-inflammatory cytokines IL-1β and TNFα. Because there are currently no targeted treatments for TBI, prevention of inflammatory cascades including the IL-1 pathway and microglial activation may improve outcome post TBI.

Hypothesis: Inhibition of the IL-1 pathway will decrease inflammation and secondary injury following TBI resulting in improved functional outcome.

Methods: IL-1RI knockout and wild type littermates (all on C57BL/6J background) underwent lateral fluid percussion injury. This is a well validated model of TBI that induces injury by a fluid pressure pulse delivered directly to exposed dura following parasagittal craniotomy. To evaluate motor function, Rotarod was used to compare injured mice to sham mice through time spent on the rod. To evaluate cognitive function, Barnes maze was utilized starting post injury day 13. Measurement of IL-1 and TNF was done through quantitative polymerase chain reaction from tissue isolated 6 hours post injury. To evaluate the impact of IL-1RI blockade on microglia function following TBI, initial trials were done using a Percoll density gradient to separate microglia from a whole adult mouse brain homogenate. Immunocytochemistry using Iba-1 and Lectin staining was used to determine cell type isolated.

Results: Following TBI, IL-1β expression was significantly decreased in left cerebellum and brain stem in IL-1RI knock out mice compared to wild type littermates ($p = 0.0413$, $p = 0.0295$). There was no difference in motor function between IL-1RI knockout and wild type littermates on days 1-7 following TBI, with a general trend of increasing recovery of motor function in both genotypes over time. In Barnes maze testing, IL-1RI KO littermates spent significantly more time in the escape quadrant following TBI compared to injured WT littermates. Immunocytochemistry using Iba-1 and Lectin labeling suggest this is a viable method to study an enriched microglial population following TBI; however, no quantitative measure has been completed.

Discussion: TBI studies done in IL-1RI knockout and wild type littermate mice demonstrate a mild decrease in secondary inflammation and a significant improvement in cognitive functioning with IL-1RI ablation. Therefore, blocking of IL-1RI using drugs such as Anakinra could be a beneficial treatment and studies using Anakinra treatment following fluid percussion injury in wild type mice are underway. Additionally, using Percoll density gradients for microglial isolation, measurement of microglia signaling post traumatic brain injury can be done. Through identification of signaling pathway and cell-type, treatments can be developed that are targeted towards halting secondary injury and preventing further cognitive or motor sequelae of traumatic brain injury.

The Impact of Legislation on Gas Can and Mattress Related Burn Injuries

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Background: Improvements in burn treatment have traditionally taken priority over improvements in burn prevention, even though prevention efforts are generally more cost-effective and impact larger portions of the population. Successful burn injury interventions focus on the three E's: education, engineering, and enforcement. These interventions can further be classified as either passive or active. Passive interventions are automatic and require little to no host action, while active prevention is voluntary, relying on host compliance. For these reasons, passive interventions are usually more effective injury prevention strategies.

Purpose: Burn prevention program success requires thorough evaluation and monitoring of intervention outcomes. The impact of two passive burn prevention regulations related to product engineering, the Children's Gasoline Burn Prevention Act (Public Law 110–278, effective 1/17/2009) and the Standard for the Flammability (Open Flame) of Mattress Sets (16 CFR 1633, effective 7/1/2007) will be assessed using nationally collected burn injury data.

For gas can burn injuries, we hypothesize that there will be a decrease in the risk of burn injuries in the years following passage of the law for children under the age of five, while the risk of burn injuries for those older than five will remain unchanged. For burn injuries involving mattresses, we hypothesize that the risk of burn injuries will decrease for all ages.

Methods: We conducted a retrospective review of records within the Consumer Product Safety Commission's (CPSC) National Electronic Injury Surveillance System (NEISS) from 1997-2015. Records were evaluated based on product codes, diagnosis code, and narrative fields to identify gas can and mattress burn injuries. Based on these records, national injury frequency was estimated by utilizing survey sampling weights associated with each record. Basic demographics were used to characterize the injuries. Logistic regression, incorporating estimated injury incidence and adjusting for gender (for gas can injuries) or gender and age (for mattress injuries), was performed to test for change in injury rates after the effective dates of these regulations. Statistical analysis was performed with SAS for Windows version 9.4 (SAS Institute, Cary, NC). This study received an exemption from institutional review board approval.

Results: Within NEISS, there were 493 burn injuries involving gas cans yielding an estimated 19,339 gas can related burn injuries (95% CI, 15,781-22,896) during the 19 year study period. After adjusting for gender, the odds of a gas can burn injury after legislation decreased by 67% for children younger than five (OR = 0.33, CI: 0.16-0.66, p-value = 0.0018). There was no significant change in the injury rate for persons five years of age and older (OR=1.07 CI: 0.8050-1.412, p-value = 0.66).

During the same time, there were 219 NEISS burn injuries involving mattresses yielding an estimated 6,864 mattress related burn injuries (95% CI, 5071-8658). After adjusting for gender and age, the odds of a mattress burn injury in the years following legislation decreased by 31% for all ages (OR = 0.69, 95% CI: 0.51-0.94, p-value = 0.02).

Conclusions: This study demonstrates that passive interventions involving the implementation of industry wide engineering standards remain a powerful tool for burn injury prevention and should be the focus of future efforts to improve burn care. Both the Children's Gasoline Burn Prevention Act and the Standard for the Flammability (Open Flame) of Mattress Sets decreased the risk of burn injuries in their target populations in the years following their enactment. The overwhelming success of these programs should allow them to serve as the basis for future burn prevention efforts.

What are the results of surgical treatment of postoperative wound complications in soft tissue sarcoma? A retrospective, multi-center case series

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Background: Non-oncologic complications, such as infection, wound dehiscence, and seroma formation, are common following resection of a soft tissue sarcoma. Although there are a number of studies illustrating the incidence of and risk factors for postoperative wound complications, we are unaware of any investigation that identifies the surgical treatment and eventual outcome of these complications.

Questions/Purposes: The purpose of this study was 1) to identify the time to recognition, treatment employed, and eventual outcome of wound complications and 2) to identify risk factors that may predispose patients to failure in complication management.

Patients and Methods: This was a multi-institutional, retrospective, consecutive case series of patient data from December 1, 2009 to November 30, 2014. We included all patients treated with a primary closure of a limb sparing resection of a soft tissue sarcoma of the pelvis or extremity who developed a non-oncologic wound complication requiring operative intervention. We excluded patients who were treated with a soft tissue reconstruction (free flap, fasciocutaneous rotational flap, or skin graft) at the time of tumor resection, infections present at time of resection, or use of a prosthesis or allograft. Participants were all fellowship-trained orthopaedic oncologists, and submitted de-identified patient data through the Research Electronic Data Capture (REDCap) into a central repository managed by the primary research team. We recorded patient (age, sex, body mass index, Age-Adjusted Charlson Comorbidity Index Score), tumor (histology, size, grade, location, depth, primary or recurrent), and treatment (chemotherapy, radiation) factors. The primary outcomes were a healed wound at the end of treatment and the total number of procedures required to address the complication. We performed a descriptive analysis to report the time to surgical treatment, modalities of surgical treatment, and final wound status. Bivariate methods (chi-square and Fisher's exact testing) were used to investigate clinical associations that resulted in failure of wound healing or requirement of multiple unplanned procedures.

Results: The median time from surgery to the initial recognition of a complication was 22 days (range 0-173 days), with 51 patients (84%) presenting in the first 6 weeks. The median time from the recognition of a complication to surgery was 5 days (range 0-219 days). The complications treated included infection (32), wound dehiscence/necrosis (23), and seroma/hematoma (6). The definitive procedures included primary closure (44), debridement with healing by secondary intention (9), muscle flap (6), and skin graft (2). No patient was treated with an amputation to manage the wound complication. Six patients (10%) had a wound requiring continued dressing changes after the treatment of dehiscence/necrosis (3) or infection (3). In these patients, the median time of followup from the time of the final procedure was 5.8 months (range 0.9-39.5 months). Four of these patients died prior to wound healing, 2 patients are currently alive 6.4 and 39.5 months after the last procedure. Twelve patients (20%) required at least one (median 2 [range 1-4]) additional unplanned procedure to address an infection (10) or hematoma/seroma (2). Eight patients had a planned two-stage procedure (six for infection and two for dehiscence/necrosis), all but one of whose wounds healed without further complication. In a bivariate analysis, we found patients with an infection were at increased risk of requiring multiple unplanned procedures ($p=0.024$). No other factors, including patient age, delays of treatment, type of complication, use of preoperative radiation, tumor location, or tumor size appeared to have any meaningful influence on wound healing or unplanned procedures.

Conclusions: Limb sparing resection of a soft tissue sarcoma is known to be at high risk of postoperative wound complications. We found that 84% of these complications were identified in the first 6 weeks after surgical resection and 72% were treated with a repeat primary closure. 31% of patients with a postoperative infection required additional unplanned procedures to adequately deal with the complication. In these patients, the treating surgeon may consider a planned two-stage procedure as part of the initial treatment for infection management. Patients may be counseled that a postoperative wound complication is rarely a devastating event, and resulted in a healed wound in 90% of cases and retention of the affected limb.

Vertebral Wedging in Adolescent Idiopathic Scoliosis: a Study Using 3-D Models Adapted from 2-D Radiographs

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BACKGROUND: Adolescent idiopathic scoliosis (AIS) is an abnormal curvature of the spine that presents in late childhood or early adolescence. In patients with AIS, the spine develops an abnormal lateral curvature with rotation in the axial plane making scoliosis a 3-dimensional deformity. Several authors argue that Hueter-Volkmann principle explains the asymmetric loading or compression of the growth plates on the concave side of the curve(s); this inhibits growth on that side, leading to wedging of the vertebral bodies. The principle states that the growth depends on the amount of compression of the growth plate – it is retarded by increased compression and accelerated by reduced tension. Due to axial vertebral rotation, the plane of maximal vertebral wedging may not be visible on a 2-D radiograph, making measurements from those images inaccurate. Therefore, in the absence of 3-D imaging, it is necessary to develop a model allowing a much more accurate depiction of the problem at hand. EOS imaging, a French company, has developed a system that uses high energy particle detectors to take simultaneous posterior-anterior (PA) and lateral x-rays. These x-rays can then be "united" via sterEOS® software to construct a 3-D model of the spine, similar to a CT scan. Furthermore, this 3-D model can be manipulated for various analyses. This has potential for a more accurate representation of the scoliotic deformity. These representations are the focus of multiple lines of research, but as yet, are not widely used for clinical purposes.

HYPOTHESIS: There is a reliable mathematical relationship between vertebral wedging measured on 2-D plain film radiographs and on 3-D models. Modeling this relationship will allow us to adjust the 2-D measurement so it more accurately reflects what would be seen if a 3-D model was available.

METHODS (PHASE 1): The Bracing in Adolescent Idiopathic Scoliosis Trial (BrAIST) conducted by Drs. Weinstein and Dolan in 2013 was stopped early by the NIH based on strong evidence that bracing was effective in reducing the risk of significant curve progression to the surgical threshold in patients with AIS. Samples for my research were taken from the subset of subjects in the BrAIST database who had both PA and lateral images taken with the EOS imaging system at three participating clinical sites. Both braced and observed subjects were included in this subset.

EOS® 3-D images were created via sterEOS® software developed by the parent company. The algorithms used to reconstruct the 3-D images are based on statistical modeling and bone shape recognition by the software. sterEOS® 3-D modeling allows the display of bone position, rotation and orientation. It also enables the display in different perspectives (e.g. plane of maximum deformity) which can further be manipulated to better understand the pathological anatomy. The software has built-in functions to measure both distance and angles on the reconstructions.

FUTURE WORK (PHASE 2): We will use multivariable regression to predict the 3-D wedging measurements as a function of the 2-D measurements and other candidate variables, such as the Nash Moe classification of vertebral rotation, location of the curve apex, and the Cobb angle. Variables that demonstrate a statistical relationship with the wedging ratio (p-values derived from t-tests or correlations) will be included as candidates. Full and reduced models will be compared using the r-squared statistic, root mean square error, and examination of the residuals (difference between the observed and predicted values). The model which minimizes residuals will be chosen. External validation (predictive capability) of the model will be evaluated by applying it to a set of films which were not used in the model derivation step. If an adequate fit is found, we will consider this model valid and reliable for use in converting 2-D to 3-D measures of wedging.

The role of BST-2 in anoikis resistance of breast cancer cells

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ABSTRACT

BACKGROUND: Several innate immunity genes are overexpressed in human cancers and their roles remain controversial. Bone marrow stromal antigen 2 (BST-2) is one such gene whose cancer-promoting functions are incompletely understood. BST-2 in breast tumors and mammary cancer cells is a strong predictor of tumor size, tumor aggressiveness, and host survival. BST-2 contributes to the malignant progression of breast cancer in part by promoting cancer cell migration, invasion, adhesion, and growth in suspension. Anoikis is a form of programmed cell death induced upon cell detachment from the extracellular matrix. This mechanism prevents adherent-independent cell growth and attachment to an inappropriate matrix, thus avoiding colonization of distant organs. Anoikis resistance and cell clustering are vital steps during cancer progression and metastatic colonization.

HYPOTHESIS: Since BST-2 is known to promote both cancer cell clustering and adhesion to other tumor microenvironment components and to promote cancer cell metastasis in vivo, we hypothesized that BST-2 plays a role in anoikis resistance of cancer cells.

METHODS: To test our hypothesis, we employed an in vitro model of anoikis. This model serves as an in vitro representation of circulating tumor cells that survive in the bloodstream before colonizing distant organs during metastasis. Using mouse BST-2 shRNAs to knockdown BST-2 in mammary tumor cells, we assessed the effect of downregulating BST-2 on anchorage-independent cancer cell growth.

RESULTS: Here we show that BST-2 is necessary for breast cancer cells to survive under anoikis conditions and that this occurs via downregulation of pro-apoptotic factors involved in anoikis.

CONCLUSION: These data suggest that 1) by enhancing cancer cell to cancer cell interactions, BST-2 initiates a signaling cascade that results in anoikis inhibition; and 2) BST-2 is a strong metastatic factor that could be targeted therapeutically to prevent cancer cell survival in circulation and seeding at secondary sites. Future studies will aim at deciphering the mechanism by which BST-2 inhibits anoikis and how that mechanism can be exploited therapeutically.

CORRELATION OF RADIOGRAPHIC AND BIOMECHANICAL DATA FOLLOWING PERIACETABULAR OSTEOTOMY

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PROBLEM

Developmental dysplasia of the hip is a condition described by a shallow acetabulum, thus creating an increased contact stress on the cartilage matrix of the hip joint. As the patient grows into adulthood, the severity of this condition can result in extreme pain during various physical activities. Currently, periacetabular osteotomy (PAO) is the procedure of choice in treatment of this condition, although still a rare procedure in and of itself. At the University of Iowa Department of Orthopaedics, one surgeon conducted these procedures and although there was great success, there is limited biomechanical evidence to document improvement.

HYPOTHESIS

Following the PAO, the lateral center edge angle will increase and the tonnis angle will decrease, resulting in a consequent reduction in pain experienced by the patient.

AIMS

Currently, PAO is offered as a procedure to patients with hip dysplasia without strong evidence to support or predict positive outcomes. Thus, there exists a wide range of success of the procedure. We intend to explore the correlation of both plain film and CT imaging with the severity of dysplasia and subsequent success of the procedure. The overall aim of the study is to perform the PAO with greater confidence and predictability.

METHODS

A retrospective analysis of 50 patients that underwent PAO by one operating physician at the University of Iowa hospital during the last 12 years was performed. For each patient, radiographic images were analyzed both before and after the surgery to measure lateral center-edge angle (LCEA), tonnis angle, posterior wall sign, and extrusion index on both the operative and non-operative hips.

DISCUSSION

As a result of the radiographic analysis, there is evidence of the success of the procedure with the LCEA increasing by an average of 12 degrees and the tonnis decreasing by an average of 8 degrees. There was one patient that showed a decrease in LCEA and a different patient that showed an increase in tonnis, though both are radiographically undesirable. This data is essential in establishing the correlation to mechanical data and patient-reported success, which is being conducted in future studies.

Safety and Efficacy of Atrial Anti-tachycardia Pacing in Congenital Heart Disease

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Background: Cardiac arrhythmias are a relatively common sequela following congenital heart disease (CHD) surgery resulting from cardiac anomalies, fibrosis and surgical scars. Atrial arrhythmias, including sinus node dysfunction, complete heart block and intra-atrial reentrant tachycardia (IART) are among the most prevalent. The presence of IART significantly increases morbidity and mortality. Atrial anti-tachycardia pacemakers (ATPM) have been utilized to prevent and treat IART in CHD but little is known about the safety and efficacy of newer generation ATPMs.

Methods: A single center, retrospective study was performed to determine if ATPMs are effective in the management of IART in children and adults with CHD. Demographic, CHD diagnosis, surgical, anti-arrhythmic, and atrial arrhythmia data were collected as well as therapy success and adverse events. Chi-square analyses and two sample t-tests were used for statistical evaluation of categorical and continuous data sets respectively.

Results: 91 CHD patients (median 27.3 yrs, range 6.9 – 59.8 yrs of age at time of first ATPM implant) underwent ATPM placement for the management of atrial arrhythmias at the University of Iowa Hospitals and Clinics and satellite centers between 2001 and 2016. Median number of annual follow up visits included in analysis was 4 (range 1 -15). Diagnoses included: D-TGA (atrial switch, n=26), Fontan (n=18), L-TGA (n=14), repaired TOF (n=13), and Ebstein's Anomaly (n=5) among others. Prior to ATPM therapy, 63 (69%) of patients were on anti-arrhythmic medication (digoxin, beta-blockers, calcium channel blockers, sotalol, amiodarone) and 22 (24%) were on multi-drug therapy. Due to physiologic limitations, 10 patients had only an atrial lead. 81% of CHD patients with a history of external DC cardioversion in the 5 years prior to ATPM placement (n=36) did not require subsequent DC cardioversion (median number of post op years =4). A total of 28 patients received ATP therapy. ATP therapy success, measured by average percent success by patient, was less successful in the L-TGA population (44.33%, n=4) when compared to the remaining CHD cohort (81.63%, n=24, p=0.03). One patient passed away as a result of respiratory failure and cardiac arrest secondary to IART which became intractable. No other patients died as a direct result of atrial arrhythmias, atrial antitachycardia pacing complications, or device failure.

Conclusion: ATPM therapy decreased the need for DC cardioversion in this CHD population. ATPM therapy was less successful in patients with L-TGA compared to other varieties CHD. No serious adverse events occurred as a result of ATPM therapy. These results support the safety and efficacy of ATPM use in CHD for the management of atrial arrhythmias. A large multi-center study is needed to determine the safety and efficacy of ATP among subgroups of CHD.

A MULTIPLEX APPROACH TO UNDERSTANDING THE PATHOGENESIS OF BULLOUS PEMPHIGOID

Leah Laageide; Mentor: Janet Fairley, M.D.; Collaborator: Kelly Messingham, PhD

Background: Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by IgG and IgE auto-antibodies targeting epidermal adhesion proteins. Clinical observations include urticarial plaques, elevated circulating IgE and blisters that correspond histologically to separation at the dermal-epidermal junction and an inflammatory infiltrate comprised primarily of eosinophils, mast cells and neutrophils. Since the mechanisms of BP are not well understood, systemic immune suppression remains the treatment of choice. It is not understood why BP patients develop IgE autoantibodies or if patients with high IgE represent a subtype of BP. Additionally, it is not known why BP patients typically exhibit localized areas of cutaneous disease, despite high levels of pathogenic antibodies in both the skin and circulation; however, these observations indicate a loss of peripheral tolerance in the skin, which typically is mediated by regulatory T cells (Tregs).

Purpose: The goal of this project was to utilize a bead-based multiplex assay to simultaneously evaluate human cytokines and chemokines classified as Th1, Th2, Th9, Th17, or Treg factors in a well characterized panel of sera BP patients and age- and sex-matched controls to determine the relationships between these factors or profiles and disease phenotype.

Methods:

Samples: BP patients (N=28; average age: 80.1) with a confirmed diagnosis of BP and age/gender matched controls (N=22, average age: 79.7) were recruited from the University of Iowa Hospital and Clinics (IRB#200712748) after giving written informed consent. Disease severity was measured using a scale of 1-6, from least to most severe, with all patients scoring 3 or greater (average grade 5). Patients' treatment status was also assessed with 71% taking no medications (none or PRN topical steroids) and 29% using systemic immunosuppressants.

Antibodies: BP IgE was determined by ELISA, BP180/230 IgG was evaluated with a commercially available ELISA, and total IgE was evaluated via electrochemiluminescence by the U of I Pathology Research Services.

Multiplex Array: A human cytokine (27-plex) and T-reg (12-plex) multiplex (BioRad) were used according to the manufacturer's instructions. Data was analyzed on the Luminex BioPlex at the University of Iowa Flow Cytometry Core Facility.

Results: Preliminary results, from the 12-plex, revealed elevated IL-19, 20, 35, and 10 in both patients and controls. The 27-plex also demonstrated elevation of cytokines in both patients and controls, including IL-1ra, 8, 9, 12, 17, in addition to elevated Eotaxin, FGF basic, G-CSF, IFN-g, IP-10, MCAF, PDGF-bb, MIP-1a, MIP-1b, RANTES. However, patients compared to controls had a greater elevation in the following factors: IL-9, IL-12, IL-17, FGF-basic, GM-CSF, IFN-y, IP-10, MIP-1a.

Conclusion: Overall, we observed an elevation in four main cytokines per bioplex, as described above. There was little obvious difference between cytokine elevation in patients versus controls. Therefore, further statistical analysis is to be performed to evaluate for statistical significance and ratios is cytokines between patients and controls. Ongoing analysis aim to determine any correlations between cytokine levels and disease severity, total IgE, IgG 180/230 values, eosinophil counts, and/or elevated T-regs in circulation.

The effect of 5-HT_{2A} and 5-HT_7 receptor antagonists on baseline breathing and the hypercapnic ventilatory response (HCVR) *in vivo*

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Background: Central chemoreceptors within the brainstem have the intrinsic ability to respond to changes in PCO_2 and transduce a signal to components of the respiratory network. A concept of multiple sites of chemoreception has arisen, but the focus for this project is serotonergic (5-HT) neurons in the medullary raphe. A subset of 5-HT neurons show chemosensitivity *in vitro* and *in situ* and have projections to various respiratory nuclei. *In vivo* studies in Lmx1b conditional knockout mice, which don't develop 5-HT neurons, show depressed baseline breathing and a 50% reduction in the HCVR. Despite this evidence, there maintains a theory that 5-HT neurons aren't central chemoreceptors and neurons of the retrotrapezoid nucleus (RTN) are the main central chemoreceptors. But recent unpublished results gathered in Dr. George Richerson's lab from co-culturing 5-HT and RTN neurons suggest RTN neurons are minimally intrinsically chemosensitive and most of their response to PCO_2 and pH is mediated by synaptic input from 5-HT neurons.

Aims and Hypothesis: Here we sought to further determine the role of 5-HT and RTN neurons in central chemoreception. In co-culture with 5-HT neurons, application of a 5-HT_7 antagonist nearly eliminated the response of RTN neurons to reduced pH. This suggests neurons of the RTN are just relays for chemoreceptor information from 5-HT neurons. A lesser effect is obtained through application of a 5-HT_{2A} receptor antagonist. Based on these results, it was hypothesized that *in vivo* treatment with a 5-HT_{2A} and/or a 5-HT_7 receptor antagonist would depress baseline breathing and blunt the HCVR.

Methods: Alzet mini osmotic pumps and brain infusion kits were used to constantly deliver a 5-HT_{2A} receptor antagonist (MDL 11,939) and/or a 5-HT_7 receptor antagonist (SB 258719) directly into the CSF through insertion into the lateral ventricle. Osmotic pumps with saline (for mice to be treated with SB 258719) or a combination of saline and ethanol (for mice to be treated with MDL 11,939) were placed initially for control. Mice were allowed to recover for four days following surgery before conducting plethysmography with the following gases: 0% CO_2, 5% CO_2 and 7% CO_2, all with 50% O_2 and balance N_2. Saline pumps were then switched to pumps allowing delivery of the SB 258719 at 0.75 or 7.5 ug/hr, and ethanol and saline combination pumps were switched to pumps allowing delivery of MDL 11,939 at 0.59 or 5.9 ug/hr. Following switching of the pump, plethysmography was performed as described previously 24 hours later. In most mice, the osmotic pump was switched a second time to the other concentration of the same drug and plethysmography performed 24 hours later. Additional experiments were conducted with intra-peritoneal (IP) injections of ketanserin (5-HT_2 receptor antagonist). Plethysmography was conducted before the injection as control and then started 15 minutes following injection of 3 or 10 mg/kg ketanserin, both at the gas concentrations described previously. A two-way ANOVA was used for statistical analysis.

Results: Intracerebroventricular (ICV) infusion of SB 258719 resulted in a dose-dependent decrease in minute ventilation (V_e) at 0% CO_2 (baseline breathing) and a dose-dependent reduction of the slope of the HCVR (n=6). With MDL 11,939 infusion there was a slight elevation in baseline breathing and a reduced slope of the HCVR at 0.59 ug/hr (n=6); there was depressed baseline breathing and a reduced HCVR at 5.9 ug/hr (n=2). Results with ketanserin were similar to those seen with MDL 11, 939; at 3 mg/kg there was an elevation in baseline breathing with a reduced slope of the HCVR (n=3), while at 10 mg/kg there was a large depression in baseline breathing and blunting of the HCVR (n=5).

Conclusions: SB 258719, MDL 11,939 and ketanserin all depressed baseline breathing at higher doses and reduced the slope of the HCVR. These results support the theory that 5-HT neurons are central chemoreceptors, and neurons of the RTN may function mainly as a relay for chemoreceptor information. Future directions will include gathering more data with higher doses of MDL 11,939 and determining the effect when SB 258719 and MDL 11,939 are combined at the highest doses.

Patterns of Vision Loss in Idiopathic Intracranial Hypertension: The Central vs. Peripheral Visual Field

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ABSTRACT

BACKGROUND: Idiopathic intracranial hypertension (IIH) is a syndrome of increased intracranial pressure (ICP) of unknown cause that occurs most often in obese women of childbearing age.[1] Papilledema, optic disc edema due to increased ICP, is the primary sign of IIH.[1] If untreated, papilledema can cause progressive visual field loss and optic atrophy. The visual field loss can go unnoticed by the patient until advanced, and IIH leads to blindness in about 5% of cases.[1] It is critical that visual field testing be performed early in the patient's course so that optic nerve damage can be found and blindness prevented. [2] Currently, standard automated perimetry (SAP) is the primary method used to detect visual field damage, and focuses on the central 24° of a patient's visual field.[3] The use of the standard small size III stimulus (0.43°) has been attempted with SAP in the far periphery, but with current methods the examination is a tedious, time-consuming task with poor retest variability. Consequently, the peripheral visual field remains unexplored with the current method of clinical testing. This seldom tested area represents over 3 times the territory currently evaluated. Efforts to efficiently test a patient's peripheral visual field is important because the peripheral field is critical for navigation of the environment, can be the earliest site of visual field defects, and may be the most appropriate territory to follow patients needing changes in intervention.

HYPOTHESIS: Idiopathic Intracranial Hypertension causes visual loss in the far peripheral visual field that is not present in the central 24° of the visual field. Peripheral visual field testing provides a more detailed and full evaluation of vision and will lead to improved clinical practice for those with IIH.

AIMS

1. Test the full visual field in patients with IIH to identify where visual field defects occur in the far periphery, especially early in the patient's course when optic nerve damage is subtle.
2. Compare results from the central visual field test to that of the far peripheral field to identify the relative merit of the two tests.

METHODS: 48 control subjects with normal vision were tested with a new perimetry test that covers the central and peripheral visual field. 15 patients seeking care for IIH at the University of Iowa Hospitals and Clinics were then recruited and underwent perimetry on the full visual field. All subjects were also tested on the Cirrus Optical Coherence Tomography (OCT) machine to determine retinal nerve fiber layer (RNFL) thickness. After creating a normative database for full field perimetry from the control subjects, the 95th, 98th and 99th percentiles for abnormality were determined for those with IIH. We then 1) characterized the visual field defects found testing the full visual field, and 2) analyzed the OCT structural data alongside vision function and determined the level of structure-function correlation.

RESULTS: The most common defects found in the central visual field was paracentral scotomas (27% of subjects), inferior arcuate defects (27%), and superior arcuate defects (20%). The most common defects found in the peripheral visual fields were supero-nasal loss (60%), infero-temporal wedge defect (60%), and infero-nasal loss (53%). 40% of peripheral tests also had generalized temporal visual field depression. 40% of subjects had normal central fields and 20% had normal peripheral fields. For those with infero-temporal visual field defects in the periphery (n=10), 80% had corresponding RNFL OCT thinning of the border of the superior and nasal optic disc that sends axons to this area. Only 1 subject had thinning of the supero-nasal disc sector without a corresponding infero-temporal visual field defect. Additionally, 27% of subjects had normal central visual field examinations with an abnormality in the peripheral visual field.

CONCLUSION: 60% of IIH subjects had temporal wedge defects, which is a finding that has only been rarely reported in IIH. Additionally, this particular defect showed excellent correlation with OCT structural data. Another important finding was that 4 of the 15 subjects (27%) had normal central visual fields with a visual field defect in the periphery. This finding supports our hypothesis that the peripheral visual field may show defects before the central visual field in patients with IIH. The current clinical standard for static automated perimetry, which tests only the central 24° or 30° of vision, would have missed these defects. Overall, testing the largely unexplored peripheral visual field may be important for clinically following patients with IIH.

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Observation of a Solvent Kinetic Isotope Effect (SKIE) in Flavin-Dependent Thymidylate Synthase Synthesis of dTMP

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Mentor: Amnon Kohen, PhD
Additional Collaborators: Kalani Karunaratne

Background/Rationale: Thymidylate synthase (TS) dihydrofolate reductase (DHFR), and serine hydroxyl methyl transferase (SHMT) are a group of enzymes responsible for converting uridylate to thymidylate. This used to be the only enzyme system known to synthesize thymidylate, a nucleotide essential for cell replication and survival. However, in 2002, a novel enzyme called flavin dependent thymidylate synthase (FDTS), notable for its dependence upon flavin, was discovered. The enzyme, which is present in certain bacterial pathogens such as *M. tuberculosis* but not in humans, uses a unique pathway to convert uridylate to thymidylate, which gets incorporated into DNA. Although there are several competing proposed reaction mechanisms, more work needs to be done to distinguish between them. Ultimately, understanding the structure and behavior of the intermediates formed in FDTS synthesis of thymidylate will allow the synthesis of highly specific molecular inhibitors, which could be used as antibiotics.

The solvent kinetic isotope effect (SKIE) arises in enzymatic reactions when rate constants are calculated in H_2O, D_2O, or mixtures of the two. Because there is more than one isotopically exchangeable site in a given enzymatic reaction, the causes of solvent isotope effects can be difficult to identify. Normally the presence of the heavier deuterium will cause the rate of the isotopic reaction to be lower than that of the normal reaction. However, sometimes the isotopic reaction occurs more quickly than the normal counterpart.

Hypothesis: A solvent kinetic isotope effect is present when FDTS-catalyzed dTMP synthesis is performed in D_2O.
Aims: Investigate whether D_2O exerts a solvent kinetic isotope effect (SKIE) on the reaction. Interpret these findings in the context of the current proposed reaction mechanism.
Method: Enzyme, substrates, and buffers were exchanged to D_2O. We performed quench flow experiments in both H_2O and in D_2O using both acid and base as quenchers to trap any intermediates along the reaction pathway of FDTS, and to follow the intermediate kinetics of the reaction catalyzed by FDTS. The reaction was quenched at different time points ranging from 0.002 s – 400 s. The collected time points containing dUMP, dTMP and intermediate were then analyzed and plotted. The amounts of dUMP, trapped intermediate, and dTMP at each time point were used to calculate the rate constant, k, for substrate consumption and product formation in H_2O as well as in D_2O with both acid and base. The ratio of the rate constants between H_2O and D_2O was calculated to get the value for the SKIE for both acid quenching and base quenching.
Results: We observed a SKIE when the reaction was carried out in D_2O. Specifically, the rate of substrate consumption was faster in D_2O whereas the product formation was slower compared to the rates in H_2O. This particular SKIE increased the speed of substrate consumption and decreased the speed of product formation.
Conclusion/Discussion: Quench flow analysis in H_2O and D_2O revealed a solvent kinetic isotope effect (SKIE). The faster rate of substrate consumption in D_2O may be due to the substrate binding being stronger in D_2O vs H_2O. The slower rate of product formation in D_2O can be explained by slower rate of deuteride (D^-) transfer from the reduced flavin because the N-D bond is stronger than its N-H counterpart.

CFTR Gene Editing by CRISPR/Cas9 to Repair Cystic Fibrosis Mutations by Non-Homologous End Joining or Homology-Directed Repair

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ABSTRACT

BACKGROUND: Cystic Fibrosis (CF) is an autosomal recessive disease caused by mutations in the Cystic Fibrosis Transmembrane Regulator (*CFTR*). This gene encodes a channel which transports ions across epithelial cell membranes, primarily chloride and bicarbonate. A mutation in this channel can lead to an excess of thick mucus which causes bacterial infections in the lungs, pancreas and other organs. While there has been some success in drug treatments of specific *CFTR* mutations, there is no curative treatment that addresses the needs of all people with CF. A successful new gene editing technique – CRISPR/Cas9 – offers the possibility of permanently modifying the CFTR locus by introducing DNA double-stranded breaks (DBSs) which are either repaired by non-homologous end joining (NHEJ) through insertions or deletions (indels) or by enhancing homology directed repair (HDR) to obtain precise gene correction through homologous recombination (HR). Here we evaluate repair by both strategies in two representative classes of CFTR mutations.

HYPOTHESIS: CRISPR/Cas9 can be used to correct specific *CFTR* mutations via non-homologous end joining or homology directed repair.

METHODS: We evaluated repair in two classes of CFTR mutations. For a mutation in intron 22-23, 3849 10Kb C>T splicing mutation, we attempted to remove the deleterious sequence by targeting DNA regions prior to and after the mutant site. By creating DSBs on either side of the mutation, the site would be removed and the DNA repaired by NHEJ. For the deletion mutation in exon 11, the commonly occurring ΔF508, we attempted to create a DSB near the mutation and proceeded to offer a single-stranded or double-stranded repair template to allow precise correction by HDR.
For both strategies, we utilized single or dual streptococcus pyogenes (SpCas9) or staphylococcus aureus (SaCas9) specific single-guide RNAs (sgRNA) introduced by non-viral or viral delivery. We screened 8 pairs of sgRNAS targeting intron 22-23 and 16 sgRNAs targeting exon 11 to identify those that most efficiently introduced DSBs at those two regions in the gene.

RESULTS: The 3849 10Kb C>T experiments showed significant editing with roughly 30% in HEK293T cells. 75% of these showed NHEJ repair via direct ligation of cut ends with 25% showing ligation with deletions. The ΔF508 experiments showed some correction via HDR when using a single-strand oligonucleotide – 5% in HEK293Ts and 1% in Calu3s. When dual plasmids (dF508 sgRNA-HDR template with spCas9) were co-transfected, HDR efficiency was 1% and indel efficiency was 12%.

CONCLUSIONS: Our results demonstrate CRISPR/Cas9 genome editing can disrupt mutations and/or induce correction by HDR at different regions of the *CFTR* locus. CRISPR/Cas9 offers a potential therapeutic strategy to treat cystic fibrosis.

Designing a Clinical Survey Study for Vulnerable Populations: Navigating Child Abuse Laws and Ethics

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ABSTRACT

RATIONALE: Adverse childhood events (ACEs) are traumatic events in a person's life before the age of 18. These events range in severity and quantity for each person and may be a predictive factor in the health outcome of individuals throughout their lifetime. It has been reported that ACEs are highly associated with negative health outcomes, such as health risk behaviors and acute and chronic diseases. There is also data to suggest a high rate of ACEs in caregivers of children presenting to acute care settings. The Central Iowa ACEs Steering Committee collected information on ACEs and their effects on Iowa residents. The results of this study found that 55% had experienced at least one ACE in their lifetime, with 15% experiencing four or more ACEs. This study also discovered a correlation between an increase in the number of ACEs experienced and health risk behaviors leading to acute and chronic diseases. This vulnerability can be mitigated through preventing children's exposure to ACEs, preventing health risk behaviors from developing as a result of ACEs, and changing the health risk behaviors that have already developed by building up resilience through Trauma Informed Care (TIC). Currently, the only departments implementing a form of TIC screening include the Child Protection Program Outpatient Clinic and a pilot study being implemented in the UIHC burn unit. Little work has been done investigating occurrence of ACEs among patients presenting to the Emergency Department (ED), and of the potential benefits and obstacles of screening for ACEs in the ED setting and referring families to an intervention clinic as a form of TIC.

PURPOSE OF THE STUDY: The original purpose of the study was to pilot a clinical survey and intervention to screen for ACES among parents and children presenting to the ED and to increase follow up by patients with four or more ACES to the University of Iowa Family Well-Being Clinic. In the course of designing this study a significant legal issue arose related to this sensitive topic. The results and discussion summarize this issue.

RESULTS AND DISCUSSION: During IRB discussion of the study, concerns arose related to potential legal requirements for researchers to report childhood physical and sexual abuse. Care providers are mandatory reporters. That means, if a parent or child verbally discloses abuse or if there are suspicions of abuse on exam, healthcare providers are legally required to report the case to the proper authorities for further evaluation and legal intervention. The IRB expressed concerns that if participants affirmed in the study that their child had a history of abuse, then the study enrollers and/or researchers would be required to report this—even if it had been previously reported to authorities or the abuse was in the distant past. In some states, laws related to such instances has been interpreted to mean that even if an adult reports a history of past sexual or physical abuse as a child, that it would need to be reported again upon disclosure. On the other hand, there was a grave concern among the research team that if families knew a report might be made to the authorities if they affirmed questions related to past abuse, then they would refuse to be in the study or would fail to answer study questions truthfully.

CONCLUSION: Legal roadblocks have caused some researchers to abandon research related to ACEs. In some cases, researchers have elected to gather data anonymously to get around possible reporting requirements. However, this prevents research on interventions that might protect patients from future harm. To address this issue and identify families and children at risk for health consequences associated with high ACEs, parents will read through the list of possible ACEs and record the number, but not specific experiences that apply to their child. We hypothesize that this will increase the likelihood of honest reporting due to removal of potential legal consequences. Of note, each person taking the survey will receive a message encouraging verbal disclosure of any concerns of current sexual or physical abuse, so that the proper authorities can be contacted in order to help protect the child and potentially other children as well. All cases where four or more ACEs are reported will be randomized to either receive a card that suggests follow-up at the Well-Being Clinic (control) or receive both a card and a phone call reminder for follow-up (intervention). The percent follow-up by control and intervention groups will then be determined.

Clinical Characteristics Associated with Allograft Performance and Future Biomarker Implications

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BACKGROUND: While it is well known that kidney transplantation is the most efficacious treatment for end stage renal disease (ESRD), there is a high demand for and relatively low supply of kidney donors. Deceased donor kidney selection is challenging due to variation of organ quality and lack of tools that adequately predict allograft function. These challenges have led to the possible use of suboptimal allografts for a given recipient and inappropriate discard of suitable organs. Delayed graft function (DGF) is a manifestation of acute kidney injury in the transplanted organ, which is associated with worse long-term allograft survival. Therefore, determining clinical risk factors and identification of donor biomarkers associated with development of DGF is vital to favorable kidney allocation.

METHODS: We performed a prospective cohort study with 50 kidney transplant recipients between 21 October 2010 and 28 January 2014 at the University of Iowa Hospitals and Clinics (UIHC). IRB approval was obtained and all participants were consented prior to transplantation. Donor kidney biopsies were obtained prior to implantation and stored for future transcriptome analysis while demographic and clinical data was collected for donors (age, race, gender, BMI, donor type), recipients (age, race, gender, previous renal replacement therapy, sensitization, previous transplant, CMV and EBV status, history of diabetes, serum creatinine levels at week 1, 1 year and 3 years post-transplant), along with HLA mismatches, cold and warm ischemia times, preservation method, and relevant procedure information (concurrent transplant, induction immunosuppression). Post-transplant outcomes were determined and include DGF status defined as need for dialysis or fall in serum creatinine less than 50% after 7 days of transplant, 1 and 3-year graft survival, 1 and 3-year eGFRs, length of initial hospital stay, rejection episodes, development of CMV, EBV and BK viremias, and post-transplant diabetes. All variables were analyzed by DGF status using the t-test and Mann Whitney u-test for continuous data and Fisher's exact test or chi-squared test for categorical data.

RESULTS: In our cohort 28% of patients developed the primary outcome of DGF. The clinical characteristics demonstrating statistical differences between DGF and no DGF groups were donor type, preservation method, recipient age, and donor BMI. eGFR means were numerically different between groups at 1 year (DGF 65.5; no DGF 54.6; p=0.1235) and 3 years (DGF 67.5; no DGF 56.3; p=0.2096) but did not meet statistical significance. All other variables were similar between groups.

CONCLUSIONS: Donor type, preservation method, recipient age, and donor BMI were associated with DGF in our cohort. Our results are consistent with other studies in this field. However, other groups have also identified variables that were not noted to be significant in our study highlighting the importance of identifying donor biomarkers that will better predict allograft function. Determining factors that predict DGF in all populations is important for kidney allocation given the growing need and paucity of this important resource. Future analysis of differential gene expression from our preimplantation biopsies will aid in the understanding of underlying mechanisms of graft function. This will inform biomarker identification thereby allowing us to predictably and consistently forecast DGF and long-term allograft outcomes by association.

Impact of Socioeconomic Factors on Racial and Ethnic Disparities in the Receipt of Mammography

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ABSTRACT

BACKGROUND: Breast cancer remains one of the leading causes of death among women in the United States. Over the past decade, technological advancements in mammography screening and treatment have substantially improved the early detection and prognosis of women with breast cancer. However, past studies have noted disparities between Hispanic (H) and non-Hispanic black (NHB) women in comparison to non-Hispanic white (NHW) women in regards to receipt of mammography. Health coverage, income, and education have also been implicated as strong predictors of women's use of mammography. The purpose of this study was to quantify the impact of race and ethnicity in H, NHB and NHW women on their utilization of mammograms, after accounting for socioeconomic differences.

HYPOTHESIS: Hispanic and NHB women are significantly less likely to receive a mammography than NHW women when controlling for health coverage, income and education.

METHODS: This was a retrospective, cross-sectional study that utilized the Center for Disease Control (CDC) data from the annual Behavioral Risk Factor Surveillance System (BRFSS) survey. The telephone survey collects data from U.S. residents in regards to their health-related risk behaviors, chronic health conditions, and use of preventative services, such as mammograms. The sample included 68,974 women, ages 50-54, who responded to the BRFSS survey in 2010, 2012, and 2014. Women were categorized into the following groups based on indicated race and ethnic preferences: NHW, HW, Other H, NHB, Asian, Hawaiian/Pacific Islander, Alaskan/American Indian, and Other. The relationship between receipt of mammography and racial, ethnic, and socioeconomic (SE) variables (income, educational level, and health plan coverage was examined using the chi-square statistic. Multivariable logistic regression was used to identify the independent association between race and ethnicity and receipt of mammography, while adjusting for SE factors and accounting for state-level variability using random effects. Results from the multivariable analyses were expressed as odds ratios for the receipt of mammography in different racial and ethnic groups, relative to NHW women.

RESULTS: The large majority of women reported having had a mammogram; of the 68,974 subjects only 4,104 (6.0%) did not receive a mammogram. Unadjusted rates of not receiving a mammogram were lowest in HW women (5.3%), followed by NHB (5.6%), NHW (5.9%), Other H (6.9%), Other women (7.5%), Asian (7.7%), Hawaiian/Pacific Islander (7.8%), and Alaskan/American Indian women (9.4%). However, in contrast to what we hypothesized, differences in rates between NHW, HW and NHB women were not significant (P=0.27). Socioeconomic factors were strongly associated (P<.001) with use of mammography. Non-receipt of mammography rates fell from 10.3% in women with incomes <$15000 to 2.9% in incomes >$75000. Non-receipt was also higher in women without health plans than in women with health plans (16.6% vs. 4.5%, P<.001) and in women with high school or less education than in women with college degrees (8.6% vs. 4.6%, P<0.001). Adjusting receipt of mammography for socioeconomic factors using logistic regression analysis had a large impact on the assessment of disparities. These analyses found that the adjusted odds of receiving mammography, relative to NHW women (OR=1.0) were substantially higher in HW (OR= 1.94 [95% CI, 1.63-2.31]), Other H women (OR= 1.48 [95% CI, 1.188-1.84]) and NHB women (OR= 1.51 [95%CI, 1.35-1.69]). However, odds remained lower in Asian women (OR=0.79 [95% CI, 0.631-0.985]), while there was no significant difference in receipt of mammography in Hawaiian/Pacific Islander, Alaskan/American Indian, and Other women.

CONCLUSIONS: Our results suggest that analyses of racial and ethnic disparities in the receipt of mammography must account for the lower SE statuses of black and Hispanic women. While the increased utilization of mammography services by HW and NHB women cannot be directly correlated to any one factor, the study suggests that other socioeconomic factors, such as income, education, and health care coverage, play a significant role in regards to predicting a woman's receipt of mammography.

Analysis of Compliance with Post-operative Follow-up Protocols after Anterior Urethroplasty

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BACKGROUND: Success rates after anterior urethroplasty are high (70-99%) and men report high satisfaction rates and quality of life after successful repairs. However, patient compliance with follow-up protocols after anterior urethroplasty is notoriously poor and poor follow-up has the potential to negatively (or positively) affect these reported success rates. While we often assume that men that do not follow-up are doing clinically well (and thus historically we tend to count them as "surgical successes" when reporting surgical success rates of our cohort in academic studies), we have shown that about 40% of men found to have recurrence of their stricture after anterior urethroplasty are relatively asymptomatic at the time the recurrence was found on cystoscopy. Thus, we believe that many of the men that do not follow-up are potentially putting their surgical repair, and secondarily the health of their bladder and kidney function, at risk by failing to return for routine follow-up. Our study aims to identify predictors of non-compliance with standard post-operative monitoring protocols after anterior urethroplasty.

HYPOTHESIS: Patient demographics, health insurance status, and distance of patient residence from UIHC will be independent predictors of follow-up compliance in urethroplasty patients.

METHODS: We identified patients that have undergone anterior urethroplasty by a single surgeon at UIHC (BAE) from 2010 to 2016 from the prospectively maintained Trauma and Urologic Reconstruction Network of Surgeons (TURNS) database. The medical records of these men were then retrospectively reviewed in EPIC for compliance with follow-up protocols. Standard TURNS follow-up protocol includes in-person visits at 3 and 12 months after surgery and then yearly thereafter. At these visits, patients will generally undergo a screening cystoscopy to evaluate the health of the urethra as well as complete a series of patient reported outcomes measures questionnaires. From the medical record, we obtained the following information: patient age, marital status, race, insurance status (categorized into commercial, Medicaid, Medicare and no insurance), distance of patient's primary residence from UIHC (calculated using respective zip codes and GoogleMaps route calculator, assuming shortest route taken). All data obtained that was unique to this study was stored in an IRB approved RedCAP database.

The outcome of interest was follow-up compliance at one year and two years. One-year compliance was determined by evidence of any follow-up clinic visit that was greater than 330 days after surgery and two-year compliance was greater than 660 days. Univariate predictors of one- and two-year compliance were analyzed with t-tests for continuous variables and Chi-squared analyses for categorical variables with a p-value of <0.05 being considered statistically significant. All analyses were performed using SAS 9.4 (Cary, NC).

RESULTS: There were 137 men eligible for 1-year follow-up and 97 men eligible for 2-year follow-up. Compliance with follow-up was 49% and 28% respectively. Predictors of compliance are shown in the Table. Compliance with 1-year follow-up was predicted only by insurance status ($p = 0.02$). Compliance with 2-year follow-up was greater in older patients (53.2 v 45.2, $p = 0.02$) and in those with commercial insurance. Travel distance, marital status and race were not found to be significant predictors of follow-up on univariate analysis.

CONCLUSION: While reported success rates for urethroplasty are high, routine follow-up compliance was found to be low. Patient age and health insurance status are statistically significant predictors of follow-up compliance. These predictors can be screened for in order to identify urethroplasty patients that are less likely to return for their routine in-patient visits. Lack of compliance puts these patients at risk for urethral stricture recurrence or other complications, especially those patients that are asymptomatic. Other methods of follow-up must be utilized in order to improve compliance in these patients. Potential options for alternative methods of follow-up may include after-hours clinics, remote follow-up via online questionnaire, and telecommunication. As smartphone access continues to grow among the patient population, applications may be developed to further promote follow-up compliance without returning in-person to clinic. However, this technology must effectively address asymptomatic recurrence of strictures without relying on cystoscopy.

Frequency and causes of lipemic interference on clinical chemistry laboratory assays

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Background: Lipemia is one of the most common pre-analytical interferences with laboratory testing and results from sample turbidity caused by accumulation of lipoprotein particles. Interferences can be a significant source of error with clinical laboratory testing. Lipemia can interfere with tests that uses spectrophotometric methods by increasing absorption of light. Other issues include volume displacement (impacting electrolytes) and aspiration/pipetting errors due to high turbidity. Severely lipemic specimens also have higher rate of hemolysis (lysed red blood cells) by as yet unknown mechanisms. The most common pre analytical cause of lipemia is not fasting prior to blood collection. The other main cause is hypertriglyceridemia, either resulting from a primary disorder (e.g., Fredrickson type I, IV, or V hyperlipidemia) or secondary cause. Common secondary causes of hypertriglyceridemia include metabolic syndrome, diabetes mellitus, alcoholism, renal disease, nonalcoholic fatty liver disorder, HIV infections, and medications. Some intravenous medications cause lipemia directly by containing lipid emulsions, either for parenteral nutrition or as the diluent for a non-polar medication (e.g., propofol). We utilized a large body of retrospective data from the core laboratory at University of Iowa Hospitals and Clinics (UIHC). We focused our chart review on specimens on the most severely elevated lipemic indices that are most likely to cause interference.

Hypotheses: We hypothesized that the most extreme lipemic specimens would occur in patients with medical conditions causing severe hypertriglyceridemia or in those infused with lipid infusions. In addition, we hypothesized that hemolysis rate would increase as the lipemic index increases.

Methods: Retrospective study was done on the data from the UIHC core clinical laboratory. Lipemic and hemolysis indices were available for all chemistry specimens analyzed over an 18-month period (n=552,029 specimens). Extensive chart review was done for all specimens with lipemic index greater than 500.

Results: There were 134 specimens from 68 patients that showed lipemic index >500. The most likely causes of high lipemic index (>500) were found to be recent infusion of lipid-containing intravenous medications which was 54.4% of total causes (fat emulsions – 47% for parenteral nutrition, propofol-7.4%) and diabetic mellitus which was 25% of total causes (mainly type 2-23.5%), according to our study which is in agreement with other previous studies. Over 70% of specimens with a lipemic index of greater than 300 are at least mildly hemolyzed. In the severely lipemic specimens, approximately 40% are markedly hemolyzed with a hemolysis index exceeding 300. The frequency of hemolysis increased with increasing lipemic index.

Conclusions: Diabetes and intravenous fat emulsion were the most common causes of extreme lipemia. While sample with extreme lipemia are relatively uncommon, some of these specimens can be nearly unanalyzable due to combination of severe lipemia and hemolysis. The high frequency of intravenous fat emulsion as a cause of severe lipemia suggest that education and interventions within the electronic medical receptor may be helpful in reducing extent of lipemia. For instance, warning alerts might prompt physicians and nurses about risk of lipemic interference, especially when samples are drawn close in time to lipid infusions.

Increasing Focus on Communication Skills in Clinical Teaching: A pilot study

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Background - Clinician-patient communication has been shown to significantly impact diagnostic accuracy, patient adherence, patient and clinician satisfaction as well as health outcomes and costs.[1-2] In medical education, the majority of formal communication skills (CS) teaching occurs preclinically[3-6] and a drop off in medical student's CS has been noted during clinical training years.[7] Researchers have posited that this decline in student CS may be due to lack of attention to CS during clinical teaching opportunities. The methods and frequency faculty teach medical students in clinic about CS has received limited research attention with a focus primarily on student and/or faculty self-report.

Objectives - The aims of the study were to identify current methods faculty utilize in teaching CS and the frequency of explicit emphasis of CS through faculty and student interviews as well as direct observation of teaching. These teaching methods were evaluated prior to a faculty development intervention informing faculty of key communication skills teaching methods and teaching opportunities.

Methods – All clinical teachers supervising medical students as part of the Outpatient Internal Medicine (OIM) clerkship and all M2 and M3 students rotating on the OIM clerkship during the study period (May 15 – Aug 15) were invited to participate in the study. Focus group interviews with medical students and one to one interviews with faculty focusing on if and how CS was being taught during clerkships were audiotaped. Clinical teachers were observed for 2-4 hours teaching medical students in the clinic. Interactions out of the room were audiotaped and transcribed. Detailed notes were taken during observations of in-room interactions to protect patient confidentiality. These observations and interviews took place before a faculty development workshop that focused on barriers, opportunities and specific strategies for explicitly addressing CS in the context of clinical teaching. Audiotapes and observation notes were transcribed verbatim and entered into the QSR NVivo 10 program for data coding and analyzed to identify common themes and methods related to CS teaching

Results- OIM faculty (N =29) and M2 and M3 students rotating on the OIM clerkship (N=45) took part in the study. 63 patient encounters (cases) that included both a faculty member and student and of these 63 cases, 37 cases of staffing (student presentations to faculty) following student interviews with patients were directly observed. Analysis of observation notes, interviews and focus groups identified four main approaches to clinical teaching: role modeling, faculty observing medical student- patient interactions, faculty teaching in patient's presence and feedback in response to staffing. The majority of faculty interviews and focus group participants described role modeling as the most commonly used clinical teaching technique. However, faculty and medical students described a lack of explicit discussion of CS immediately before and after role modeling. Research observation of role modeling supported these views, role modelling occurred in 57 out of 63 teaching cases observed. Discussion prior to role modeling occurred in 24 out of 63 cases with direct CS emphasis in 9 out of 63 cases. Observation of students with patients was described by students and faculty as the least commonly used teaching method. Faculty described that CS were emphasized as part of feedback after directly observing students, while students concluded they rarely received CS feedback. Research observation revealed that while observation of medical students occurred infrequently in 3 out of 63 cases, it was the teaching method with greatest CS feedback in all 3 cases. Most faculty and students described staffing outside the room and giving feedback about the information gathered, rather than CS used to gather the information. The faculty that described staffing inside the room described limited CS feedback after encounters. Direct observation discovered staffing occurred in 37 out of 63 cases. Despite the frequent cues medical students gave, staffing feedback occurred in 13 of the 37 cases with communication emphasis in only 3 cases.

Conclusion- The study identified the methods faculty use to teach medical students about communication skills as well as the frequency of use. Role modeling was the most frequently used technique followed by staffing feedback and observation of medical students. In addition, faculty and medical student perception of teaching CS in the clinics was also determined. In general, while both agreed the CS was important, the priority for teaching was low and the general consensus was that several barriers prevented CS teaching. The study highlights the limited CS teaching that occurs in clinics and the potential to emphasize methods for explicitly incorporating CS teaching into role modeling, staffing and observation of learners. The study also reveals perceived barriers as well as opportunities to teach about CS. Through these preliminary results feedback can also be given to faculty members that may enhance their CS teaching. Further research will be conducted to determine the impact of a faculty development workshop on the faculty CS teaching in the clinic.

Youth Football Injuries

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Abstract

Background: Football is among the most popular sports in the United States. Recently, there has been increased attention on the safety of football in both the popular and medical literature. Many leagues have made rule or equipment changes with the goal of improving player safety. Still, questions remain as to the safety of youth football. Past studies have yielded mixed results regarding injury rates, due largely to inconsistent reporting methods and small sample sizes. A review of past literature reveals injury rates ranging from 2.3% to 30.4% per year. Types of injury are also reported inconsistently. Although rates of objectively-reported injuries (such as fractures) are less variable, the rate of subjectively-reported injuries (such as sprains and contusions) vary from 9% to 55%. This study aims to describe the injury rates and types sustained by a large cohort of youth tackle and flag football players.

Hypotheses: 1. Youth flag football will have similar injury rates to youth tackle football. 2. Youth football has injury rates similar to previously reported injury rates in high school and college football.

Methods: We partnered with three football leagues in eastern Iowa. Two leagues were traditional tackle, and the third was a flag league. Using an online injury-reporting system, coaches from each of the three leagues took attendance at every practice and game to collect exposure data. Coaches also used the online reporting system to identify when an athlete was injured, when a player returned from a past injury, and what type of injury was suffered. We classified injuries as serious if a player was out for more than 7 days, fractured a bone, tore a ligament, or suffered from a concussion.

Results: So far, our group has collected 46,616 exposures from 3794 youth athletes, resulting in a total of 128 injuries. The injury rate was higher in the flag league (5.77 vs. 2.66 injuries per 1000 exposures). However, when comparing severe injuries, the ratio estimate of flag vs. tackle was 0.9489, indicating that the severe injury rate was similar between the two. Although it did not reach statistical significance, there is a trend towards a higher rate of concussion in the flag league compared to the tackle leagues (1.33 compared to 0.68 concussions per 1000 exposures). Tackle football athletes were slower to return from injuries. Given an injury, 50% of flag athletes had returned after 3 days, whereas 10 days passed before 50% of tackle athletes returned to practice. Further, the imminent probability of return ratio of flag vs. tackle football is 9.505, indicating a more rapid return to play among flag players.

Conclusions: Data collected from this study does not indicate that flag football is a safer alternative to tackle football. Injury rates and concussion rates were both higher in flag football. Further, the ratio estimate of severe injuries is similar between the two leagues. That said, the data shows that flag athletes return from injuries sooner. Data collection for a second year is ongoing.

Sex differences in the sleep state-dependence of seizure-induced respiratory dysfunction

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BACKGROUND: Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death amongst patients with refractory epilepsy and therefore a major public health problem (Thurman et al., 2014). The underlying mechanisms of SUDEP are not well understood, but seizure induced changes in respiratory and cardiac functions are thought to play a major role in causing death and SUDEP tends to occur at night (Massey et al., 2014; Ryvlin et al 2013). In previous experiments, it was found that vigilance state (Hajek & Buchanan, 2016) and serotonin (Buchanan et al., 2014) effects seizure propensity, seizure severity, and various breathing parameters. Within the human population and at least one known mouse model, there is a reduced incidence of seizure-associated death among females (Buchanan et al., 2014).

HYPOTHESIS: Seizures during NREM will induce greater respiratory dysfunction, this dysfunction can be rescued by increasing the amount of serotonin in the system, and the reduced mortality in female mice is due to decreased seizure-induced respiratory dysfunction in females.

METHODS: Epidural EEG and nuchal EMG electrodes were implanted in five male and three female C57BL6 mice. Mice were also stereotactically implanted with a bipolar stimulating/recording electrode targeting the right amygdala. After at least seven days of recovery, the threshold current for development of afterdischarges in the amygdala was determined and mice were kindled with two daily stimulations until three consecutive Racine grade 4 or 5 (Racine, 1972) seizures were observed. Once the animals were kindled, they underwent seizure inductions during wakefulness or non-rapid eye movement (NREM) sleep following intraperitoneal injection (100 ul) with saline (0.9% NaCl) or the selective serotonin reuptake inhibitor citalopram (20 mg/kg). EEG, EMG, and plethysmography were recorded throughout the experiments. Vigilance state was determined in real-time using established parameters and verified post-hoc (Buchanan & Richerson, 2010). Seizure and respiratory parameters were analyzed post-hoc. Amygdala electrode placements will be verified histologically at the completion of the experiments.

RESULTS: Compared to males, females demonstrated a higher threshold current for development of afterdischarges in the amygdala but required fewer repetitive stimulations to be fully kindled. Seizures induced during NREM were associated with increased respiratory rate variability. Gender analysis showed that females had lower variability in respiration in males. Citalopram had no effect on respiratory rate variability but did have a rescue effect on breath frequency.

CONCLUSIONS: Changes in variability corresponds to a dysregulation of respiration and increased vulnerability to seizure-induced morbidity. This data indicates that seizures occurring during NREM sleep can have detrimental effects on breathing which may contribute to increased risk of seizure related death. An increase in dysregulation of breathing in male mice may explain why there is a higher incidence of seizure related deaths in the male population.

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The Role of the Amygdala in Seizure Induced Apnea and Death

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ABSTRACT

BACKGROUND: Sudden unexpected death in epilepsy (SUDEP) is a devastating problem and the leading cause of death in patients with uncontrolled epilepsy, causing nearly 2,000 deaths per year in the US alone. Seizures cause defects in cardiovascular control and respiration, and ictal and post ictal respiratory depression can cause severe oxygen desaturation and death. Recent work suggests that SUDEP occurs first because of apnea, which is followed by bradycardia and eventual asystole. A study of intractable epilepsy patients conducted here at UIHC in the epilepsy monitoring unit has shown that central apnea and O_2 desaturation occurred if, and only if, seizure activity spread to the amygdala. This suggests that there is an inhibitory, neuroanatomical connection between the amygdala and brainstem respiratory networks which plays an important role in seizure-induced apnea. The purpose of this study was to determine if the amygdala is a necessary structure in the pathway leading to seizure-induced apnea and death and to see if lesioning the amygdala could prevent this effect.

HYPOTHESIS: The amygdala is a necessary neuroanatomical structure in the pathway resulting in seizure-induced apnea and death.

METHODS: For this study, DBA/1 mice were used as a seizure model because of their propensity to have respiratory depression during audiogenic seizures. DBA/1 mice were primed to have audiogenic seizures from postnatal ages 21-25 and experimental surgeries were performed at P65-P67. In the amygdala lesion group, a bilateral amygdala lesion was attempted using a stereotaxic apparatus, and an electrode. An electrical current of 10 mA was passed for 15 sec in order to make lesions at the coordinates of the amygdala, which were determined from previous trials and coordinates from Paxinos and Franklins "The mouse brain in stereotaxic coordinates" 2nd edition. An EKG was also implanted in order to measure cardiac function during the experiment. A control group was made by conducting sham surgery, in which the same procedure was used to introduce the electrode, but no electric current was passed; an EKG was implanted in this group as well. 4 days following surgery, audiogenic seizures were induced in a whole body plethysmography chamber which allowed real time recording of video, EKG and respiration. If respiratory arrest occurred during the seizure, recording was continued until the time of asystole. Following experiments, any surviving mice were euthanized, and all brains were sectioned and stained for microscopic examination with cresyl violet, and GAD67 antibody in order to evaluate the size and location of the lesions made, and to determine the extent to which the amygdala was lesioned.

RESULTS This study found that 100% (4/4) of mice in which the amygdala was successfully lesioned at least unilaterally, survived the audiogenic seizure induction trial. 3/4 of these mice showed seizure activity without prolonged respiratory arrest, and 1/4 showed no seizure activity or respiratory arrest during the trial. Only 20% (1/5) of mice in which lesions were made, but which did not accurately lesion either amygdala, survived the trial. The mouse that survived showed no seizure activity or respiratory arrest, while those that died showed seizure activity followed by respiratory arrest, asystole and death. In the control, sham surgery group, 50% (1/2) of the mice showed seizure activity, followed by respiratory arrest, apnea and death, while the other mouse showed no seizure activity and survived. Overall, unilateral or bilateral amygdala lesions significantly increased survival in an audiogenic seizure induction trial compared to mice without an amygdala lesion according to Chi-squared test, X^2 (1, N=11)= 9.923, p=0.0016.

CONCLUSIONS An amygdala lesion, at least unilaterally, significantly increased survival and decreased respiratory depression during audiogenic seizure induction in DBA/1 mice . This suggests that the amygdala may be a necessary intermediate in the pathway resulting in seizure induced apnea and death.

Developing Gene Therapy for Cystic Fibrosis

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Background/Rationale

Cystic fibrosis (CF) is an autosomal recessive genetic disease. It results from mutations in the *CFTR* gene which encodes an anion channel with important functions in multiple organs including the respiratory tract, pancreas, gastrointestinal tract, reproductive system, and sweat glands. CF results in a variety and range of symptoms but the major cause of morbidity and mortality is progressive respiratory disease. Delivery of a normal copy of the *CFTR* gene to airway epithelia is a strategy to treat or prevent CF lung disease.

In previous work in the CF pig model, a feline immunodeficiency virus (FIV) vector expressing wildtype CFTR was aerosolized in the airways of newborn CF pigs. Two weeks later tissues were harvested and it was found that there was partial rescue of the anion channel defect in the trachea, bronchus, and ethmoid sinus epithelia, as well as partial rescue of the air surface liquid pH and bacterial killing. All of these are promising signs of functional correction of some of the most important CF phenotypes.

Hypothesis/Aims

Although the CF pig results are promising and exciting, before this approach can be considered in people with CF, there are many questions that need to be answered. Given the complexity and heterogeneity of airway epithelium, we focused on two. The first is how many cells need to be successfully targeted to correct CF defects, and the second is which cell populations need to be targeted in order to achieve lasting functional correction of airway epithelia.

Methods

We plan multiple approaches to answer these questions. One is to generate a human immunodeficiency virus (HIV) vector to deliver *CFTR*, mCherry and puromycin resistance. Following transduction of CF airway epithelia and puromycin selection, we will obtain a population of *CFTR*-corrected, mCherry-labeled cells. We will then mix these cells at various ratios with un-corrected CF epithelial cells to determine the optimal proportion of corrected cells necessary to achieve functional correction with a lentivirus.

Since lentiviral vectors integrate into the host genome, we would like to transduce a population of airway cells with progenitor capacity. To answer whether such cells can be targeted using the HIV vector, we will use confocal imaging and flow cytometry to localize our vector, using mCherry, within airway epithelia using cell-type specific markers.

Results

We generated an HIV vector plasmid containing the *CFTR*, mCherry and puromycin resistance genes. When transfected into HeLa cells, we observed mCherry expression, and when placed under puromycin selection, the cells transfected with this vector survived drug selection.

We tested several antibodies recognizing cell specific epitopes in imaging and flow cytometry assays and we have identified potential combinations of antibodies to assess our cells on interest.

Conclusions/Discussion

The vector we generated along with the imaging and flow cytometry protocols we developed will help us address how many and which cell types we need to target for successful functional correction of CF phenotypes. Another question we may also be able to answer in future studies using these methods is how long the expression of these vectors persists in different cell types. Additionally, given that CF is a multi-organ disease, these techniques could be used to answer these same questions in different tissues. These are important questions that need to be addressed before this gene transfer strategy can be tested in clinical trials.

Evaluation of the Delirium Observation Screening Scale in the Diagnosis of Elderly ICU Patients

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BACKGROUND: Delirium is a fluctuating disturbance in consciousness with an acute onset of symptoms that can include confusion, disorientation, hallucinations, and agitation and is a result of an underlying medical condition. It has serious immediate and long-term consequences for patients and their families, especially when left untreated. Unfortunately, the oscillating, diverse, and ambiguous nature of its associated symptoms makes delirium especially challenging to diagnose. The Delirium Observation Screening Scale (DOS) only requires observation of patients, with no specific cognitive testing, making it ideal for routine use by busy nurses. However, it has yet to be validated in intensive care unit (ICU) patients over the age of 65 years.

PURPOSE: This study aims to determine the ability of the DOS to accurately screen ICU patients ages 65 years and older for delirium.

METHODS: The effectiveness of the DOS in positively diagnosing patients with delirium was assessed by comparing its results to those of a validated delirium diagnostic tool, the Delirium Rating Scale-Revised-98 (DRS-R-98). Participants were patients admitted to the University of Iowa Hospitals and Clinics' Surgical and Neuroscience Intensive Care Unit (SNICU), ages 65 and older. Patients who were intubated, who did not speak English, or who did not agree to participate were excluded. Nurses working in the SNICU recorded DOS scores in the electronic medical records of patients over the age of 65. A trained researcher administered the DRS-R-98 and collected data from assented patients within 24 hours of the nurse-recorded DOS score. The results of the DOS were then compared with those of the DRS-R-98 to determine the sensitivity and specificity of the DOS.

RESULTS: 71 assessments of 55 patients were conducted. 13 of the 71 interviews resulted in a DRS-R-98 score that was positive for delirium, demonstrating a delirium incidence of 18.3%. Comparing the results of the DOS to the results of the previously validated DRS-R-98, the sensitivity of the DOS was 84.6% and the specificity of the DOS was 94.8%.

CONCLUSIONS: The DOS appears to be a sensitive and specific test for the diagnosis of delirium in ICU patients over the age of 65 years. While further studies need to be completed for verification, the accuracy, coupled with the usability of the DOS, appear to make it an effective tool for busy ICU nurses to use to detect delirium while engaging in routine patient care.

Title: What is the clinical significance of incidental findings on staging CT scans in patients with sarcoma?

Authors: Zachary Mayo, Sean Kennedy, Yubo Gao, PhD, Benjamin Miller, MD

BACKGROUND

Upon the diagnosis of a sarcoma, a staging computed tomography (CT) scan of the chest alone or the chest, abdomen, and pelvis (CAP) will be performed to assess for distant sites of disease, most notably pulmonary metastases. Anecdotally, these staging studies often report additional findings that may or may not be related to the known malignancy; currently, there is a paucity of literature discussing the frequency and significance of incidental findings at the time of staging CT scan.

QUESTIONS/PURPOSES

The purposes of this study were to (1) record the frequency of all incidental findings in staging CT scans, (2) determine how many indeterminate nodules in the lung, liver, bone, and lymph nodes in staging CT scans became metastatic on follow up, (3) investigate the number of patients with metastatic disease who had an indeterminate nodule on a staging CT scan, and (4) determine which clinical risk factors are predictive of indeterminate nodules becoming clinically significant metastases.

METHODS

Patients diagnosed by a single surgeon (BJM) with a bone or soft tissue sarcoma from September 2010 to February 2016 were retrospectively studied. Inclusion criteria included a staging chest or chest, abdomen, and pelvis CT scan performed within two months of diagnosis with a formal report completed by a radiologist at our institution and available in the Electronic Medical Record. We were interested only in the initial presentation of primary sarcomas, so patients with recurrent or known metastatic disease were excluded. The radiology reports were analyzed to determine the presence of all metastatic lesions, indeterminate nodules, and incidental findings. Patients were required to have radiographic follow-up for a minimum of six months to observe for the development or progression of metastatic disease.

We recorded all mentions of lung, bone, liver, and lymph node nodules, which were classified as metastatic at staging or indeterminate depending on the number, size, and appearance of the nodules. All patients determined to have metastatic disease at the most recent follow-up either had a tissue diagnosis or progression of disease consistent with metastatic sarcoma

Patients with indeterminate nodules that progressed to metastatic disease were compared to patients with indeterminate nodules that did not progress to metastatic disease. Bivariate methods (chi square and Fisher's exact testing) were used to investigate an association between various clinical risk factors and metastatic progression.

RESULTS

We identified 147 patients that met our inclusion criteria. One hundred and thirty-two out of 147 (89.8%) patients had at least one incidental finding listed in the staging radiology report. Forty-six (31.3%) patients presented with indeterminate lung nodules, fifteen (10.2%) had indeterminate liver lesions, four (2.7%) presented with indeterminate bone lesions, and 55 (37.4%) had enlarged lymph nodes. Eleven of the 46 lung indeterminate nodules (23.9%), 1/15 liver nodules (6.7%), 1/4 bone lesions (25%), 3/12 lymph nodes ≥1.0cm (25%), and 2/49 subcentimeter lymph nodes (4.1%) were clearly metastatic on follow up.

Overall, fifty patients developed metastatic pulmonary disease (34.0%). Eleven of these patients (22%) had evidence at staging. Five patients (3.4%) developed liver metastases, fifteen (10.2%) developed bony metastasis, and twelve (8.2%) developed lymph node metastasis; one metastatic liver patient (20%), one metastatic bone patient (6.7%) and five metastatic lymph node patients (41.7%) had evidence at staging.

For indeterminate nodules progressing to pulmonary metastases, the only clinically significant risk factor was a primary tumor size ≥ 14cm ($p = 0.0003$). Primary tumor size ≥ 14cm was also a significant risk factor for patients with indeterminate lymph nodes becoming metastatic ($p=0.0044$). Lymph nodes ≥1.0 cm at staging also showed a statistical significance ($p=0.0477$). Analysis of the bone and liver yielded no statistically significant results.

CONCLUSION

It is extremely common for incidental findings to be present at the time of staging CT scan in patients with sarcoma. Additionally, enlarged lymph nodes and indeterminate nodules of the lung are very common, and the majority of those identified do not represent true metastatic disease. Our data shows that patients with indeterminate pulmonary nodules or enlarged lymph nodes are at greater likelihood of representing true metastasis if their primary tumor is ≥14 cm or if lymph nodes are ≥1.0 cm. This work will help assure patients that reports of indeterminate nodules do not indicate absolute presence of metastasis. Future work should focus on treatment and surveillance strategies in high- and low-risk patients.

Needs Assessment of Wellbeing and Unmet Health Needs in Homeless Persons

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Rationale: Homeless individuals are at increased risk for morbidity and mortality compared to the general population. They have unique needs, which Shelter House (SH)-- a local homeless shelter in Iowa City--attempts to meet.
Aims: A needs assessment was conducted at SH in order to characterize the wellbeing and unmet health needs of this unique population, as well as to understand how this organization was addressing those needs.
Method: A survey was created in collaboration with SH using questions regarding demographics, insurance status, and current health information and unmet needs. Following IRB approval, the study instrument was administered to 68 consenting adults (≥ 18 years old) who were seeking services at Shelter House during the spring of 2015. Results were analyzed for quantitative and qualitative trends.
Results: In terms of demographics, the average age was 42.4 years, with most (n=41, 60.3%) having been previously homeless. For insurance, 49 (72.1%) had some form of health coverage, with 15 (25.6%) rating their coverage as poor to fair. Only 29 (42.6%) had dental insurance. Regarding health, most individuals were satisfied with their overall health and mental health. The majority of those surveyed were smokers (n=48, 70.6%). There were 29 individuals (42.6%) with chronic healthcare conditions, 33 (48.5%) had primary care providers (PCP), and 32 (50.8%) had seen some type of health care provider in the past 30 days. There were 13 individuals that reported an unmet health care need, with dentistry (n=3, 30.8%) and mental health (n=2, 23.1%) being the most reported. Additionally, 28 (53.7%) felt that dentistry was important to overall wellbeing and 26 (48.1%) felt psychiatry was.
Conclusion: For this group of homeless persons surveyed at SH in Iowa City, many individuals had recent contact with health care providers and almost half had a PCP. For these individuals who had been seen recently, it is unclear whether this is due to poor health or indicative of good access to health care while at SH. Specifically, dental health and psychiatry were identified as important issues to subjects' wellbeing, as well as the most commonly reported unmet health needs. Other areas for intervention might include smoking cessation and enrollment in health insurance. Homeless individuals are a unique population with specific health needs that may require specialized interventions. With further research, this type of needs assessment made in collaboration with community organizations may be a useful tool for assessing wellness and unmet health needs in homeless individuals in order to better address them.

Identification and Characterization of Non-Coding DNA Regulatory Elements in the Zebrafish Periderm

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ABSTRACT

BACKGROUND: Cleft lip with or without cleft palate (CL/P) is the second most-common structural birth defect, affecting approximately 1 in 700 babies born worldwide. As with most congenital birth defects CL/P has a strong genetic component but the disease mechanisms underlying this heritability remain incompletely understood. Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) that are strongly associated with risk for CL/P. However, as is the trend with GWAS, approximately 90% of disease-associated SNPs are located in non-coding DNA. Most such SNPs are presumed to lie in *cis*-regulatory elements (i.e., promoters and enhancers) and to disrupt expression of neighboring protein-coding genes. Our understanding of how sequence variation alters the function of non-coding DNA regulatory elements remains in its infancy. Several genes implicated in risk for CL/P are expressed in periderm, the most superficial layer of embryonic epidermis and oral epithelium. The purpose of this study was to identify sequence features of periderm-specific enhancers in order to improve our understanding of how common sequence variation in these regions contribute to the heritability of CL/P. We used the zebrafish periderm as a model because it is accessible and amenable to transgenesis.

HYPOTHESIS: Regions of chromatin that are open (transposase accessible) specifically in cells of the developing zebrafish periderm possess sequence features of periderm-specific regulatory elements.

METHODS: Prior to my arrival in the lab, zebrafish embryos at 11 hours post fertilization (hpf) from a transgenic line expressing GFP driven by the periderm-specific *krt4* promoter were dissociated and GFP-positive and negative cells were sorted. Assay for Transposase Accessible Chromatin followed by high-throughput sequencing (ATAC-seq) was applied to both populations to identify regions of open chromatin. During my rotation, I identified genes neighboring periderm-specific ATAC-seq peaks using the Genomic Regions Enrichment of Annotations Tool (GREAT) and I cross-referenced the list with an experimentally-validated list of periderm-expressed genes from the Zebrafish Information Network (ZFIN). This analysis yielded 144 genes with at least one candidate cis-regulatory element located within 100kb. I selected and amplified specific genomic regions and cloned them into a reporter plasmid upstream of a minimal promoter and cDNA encoding a fluorescent protein (tdTomato). I co-injected these constructs with mRNA encoding Tol2 transposase into fertilized zebrafish embryos at the 1-2 cell stage and analyzed tissue-specific expression by fluorescence microscopy at 24hpf. I examined active elements for binding sites of transcription factors using JASPAR.

RESULTS: Three periderm-specific regions of open chromatin were identified on chromosome 15 located +6kb, +3kb, and -8kb relative to the transcription start site of the *cldne*, a gene that is specifically expressed in periderm and that encodes a protein involved in epiboly. Transient reporter assays demonstrated that the +3kb element most strongly drives periderm-specific expression while the others lacked activity. All elements possessed the binding site of Grhl3, a transcription factor essential for periderm differentiation.

CONCLUSION: The *cldne* +3kb element is a novel periderm-specific enhancer likely involved in the regulation of *cldne* and other periderm-expressed genes in this genomic neighborhood. Experimental validation of a greater number of enhancers will provide a more complete understanding of the sequence features that define a periderm-specific regulatory elements. This will facilitate a better understanding of how mutations in these regions cause CL/P.

Do High Healthcare Costs in Prior Years Reliably Identify High Healthcare Utilizers in Subsequent Years?

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Background: Health care spending in the United States consumes about 18% of our GDP. While this amount is more than two times higher than most other industrialized nations, the US lags behind in rankings on many quality measures. Much of these costs stem from acute care utilization, which could potentially be avoided with proper preventive management. Thus, populations that are at risk for incurring high costs may benefit from interventions to reduce unnecessary spending. Figuring out ways to target these populations effectively could greatly reduce health care costs and provide better care. Currently costs in prior years are often used to target patients, but little empirical data exists to show that this approach reliably identifies patients with high costs in subsequent years.

Hypotheses: This project tested two hypotheses. First, total healthcare costs in one year would be significantly correlated with costs in subsequent years and would be a reliable approach for identifying patients who would benefit from intensive care management interventions. Second, the top 10% to 20% of high cost patients account for a majority of total health expenditures.

Methods: Data for the study was derived from Medicare health care claims for inpatient, outpatient, skilled care, and pharmacy utilization for patients enrolled in the University of Iowa's Affordable Care Organization (ACO). The data on individual claims included demographics, comorbid conditions, and the dollar amount that was reimbursed for the claim by Medicare. Analysis was performed on the 8,547 patients in the ACO who were continuously enrolled during 2012-2015 or who died in this time interval. All of these patients' health care claims were aggregated to obtain total cost of care for each patient during each year. Patients were then categorized into deciles of increasing costs. All of the analyses were conducted using SAS. Correlations between total costs in prior and subsequent years were determined using the Spearman rank order and Pearson product moment coefficients. Comparisons of the costs in different demographics and clinical groups were determined using the t- or chi-square tests and ANOVA.

Results: Mean and median costs in 2012 were $8,860 and $2,002, respectively, and were higher (P<.001) in men than in women ($9,775 and $2,091 vs. $8,063 and $1,927) and in patients who died than in patients who did not die ($26,823 and $17,583 vs. $8,260 and $1,884). Pearson and Spearman correlations were highly significant (P<.001) between costs in 2012 and in 2013 (0.61 and 0.53, respectively), 2014 (0.59 and 0.42), and 2015 (0.52 and 0.35). In addition, of the patients in the highest cost decile in 2012, 45.3% were in the highest decile in 2013, while 64.1% and 75.6% were in the two and three highest deciles, respectively. Similarly, 38.9% and 36.1% were in the highest decile in 2014 and 2015, and 67.5% and 62.7% were in the three highest deciles, respectively. Furthermore, the top 10% of patients incurred 60.2%, 59.6%, 57.0%, and 58.3% of total expenditures in 2012, 2013, 2014, and 2015, respectively, while the top 20% of patients incurred 79.2%, 78.7%, 76.9%, and 77.6% of healthcare expenditures in those years.

Conclusion: Total healthcare costs in prior years can be a reliable predictor of costs in subsequent years and may be a reasonable approach for targeting interventions to reduce costs in subsequent years. Such high costs patients account for a majority of expenditures. Nonetheless, some patients had costs that varied from year to year and future studies should determine whether those patients have specific characteristics that allow them to be differentiated from those patients who continuously incur high costs.

Impact of a CMS Metric On Physicians' Brain CT Ordering Practices

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Abstract

Background:

Physicians are increasingly scrutinized with health care metrics. It is not known how such metrics impact patient care. After the Center for Medicare and Medicaid Services (CMS) announced CMS OP-15, Brain CT in the Emergency Department (ED) for Atraumatic Headache, we began a quality improvement intervention of reviewing headache charts and providing feedback to Emergency Physicians (EPs) on how their ordering practices accorded with CMS OP-15.

Hypothesis: In this study, we hypothesized that this intervention would decrease physician CT brain ordering. We also sought to measure whether any changes in ordering practices correlated with an increased or decreased rate of missed diagnoses.

Methods:

Our quality improvement intervention sampled approximately 10 headache visits per faculty physician per review period. In the pre-intervention period there were 198 visits. In the first and second post-intervention periods there were 242 and 256 visits, respectively. Between each period EPs reviewed the results of their chart reviews with our department chair. We investigated our study hypothesis by manually querying all reviewed charts to determine if a head CT was performed at the incident visit and whether any significant intracranial disease processes not known at the time of discharge from the initial visit were discovered within the next 24 months. We then compared epochs to determine if rates of CT ordering or missed diagnosis changed between pre and post-intervention periods.

Results:

We observed a decrease of CT ordering from 38.1% in the pre-intervention period, to 35.9% and 25.0% in post-intervention periods 1 and 2 respectively. Comparing all three cohorts there is an overall p value of .0041; pairwise comparisons demonstrated post intervention period 2 was significantly different from the pre-intervention with an odds ratio of 0.54 for likelihood of receiving a head CT (p=0.003). Sequentially, we observed a missed diagnosis rate of 1.52%, 2.94% and 0.38% in the three epochs. These changes were not statistically significant.

Conclusions:

We observed a decrease in the rate of CT ordering after Emergency Physicians began reviewing their head CT ordering practices that became more significant over time. Interestingly, this was not associated with an increased rate of missed intracranial diagnosis. This raises the possibility for improving CT ordering practices in regards to minimizing exposure to ionizing radiation.

Title: Intra-operative High Frequency Jet Ventilation Does Not Significantly Affect Outcomes During Patent Ductus Arteriosus Ligation

Authors: Mackenzie Noonan[1], Joseph W Turek MD PhD[2,3], John M Dagle MD PhD[2], Steven J McElroy MD[2]

Institution: [1]Carver College of Medicine, University of Iowa, [2] Stead Family Department of Pediatrics, University of Iowa, [3]Department of Surgery, University of Iowa

Introduction: Persistence of a patent ductus arteriosus (PDA) is a common occurrence in premature infants. Symptomatic PDA has been associated with intraventricular hemorrhage, pulmonary hemorrhage, necrotizing enterocolitis, and bronchopulmonary dysplasia. If initial pharmacologic treatment fails or is contraindicated, surgical ligation is commonly performed to close the PDA. Premature infants requiring ductal ligation are commonly on high frequency jet ventilation (HFJV) in an attempt to decrease lung damage from high tidal volume ventilation. The standard procedure for neonates undergoing PDA ligation is to temporarily transfer them from HFJV to conventional ventilation (CV) to create a more motionless field. However, there is currently no evidence that this step is necessary. The goal of our study was to determine whether or not maintaining neonates on the HFJV throughout PDA ligation is as safe and effective as conversion to CV prior to and during surgery.

Methods: This study was completed using retrospective data of neonates that underwent PDA ligation by a single surgeon at the University of Iowa Children's Hospital from July 2014 to July 2016. All infants in the study were on HFJV prior to surgery. Control infants (n=25) were transferred to CV prior to surgery and returned to HFJV post-procedure. Experimental infants (n=18) remained on HFJV. Demographic data obtained from patient records included birth weight, gestational age, sex, maternal race, day of life at time of surgery, weight on day of surgery, and size of PDA. Outcome data was obtained for the 24-hour period prior to surgery, the 12-hour period following surgery, one day post-operation, and one week post-operation. Data obtained included change in diastolic pressure during surgery, time in OR, surgical mortality, incidence of pneumothorax, chylothorax or laryngeal nerve damage, average FiO_2, mean airway pressure (MAP), serum pH, serum CO_2, and serum base deficits. In addition, we collected weights at one week after the surgery, and number of chest x-rays and blood gases obtained during the 48 hours around surgery. One- and Two-tailed T tests were used to compare values of patients in the control group to patients in the experimental group, and statistical significance was determined to be <0.05.

Results: There were no significant demographic differences between the groups (birth weight, gestational age, sex, maternal race, day of life at time of surgery, and weight on the day of surgery). The only significant difference in the 12-hour period following surgery was the serum CO_2. The experimental group had significantly lower CO_2 on the day of surgery ($p=0.0014$). There were no significant differences observed in any outcome measures examined.

Discussion: These results support maintenance of HFJV throughout PDA ligation as it does not pose a threat to the safety or efficacy of the operation. They also show us that eliminating the step of transferring the neonates from the HFJV to CV may result in improved ventilation immediately following surgical ligation.

Autophagy Induction by *Francisella tularensis*

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Background: *Francisella tularensis* is the causative agent of tularemia, with as few as 10 colony forming units sufficient to cause disease in humans. The virulence of *F. tularensis* is attributed to its ability to initially evade the innate immune response, escape phagosomal degradation, delay apoptosis, and replicate within the cytosol of phagocytes. Recently, several capsule and O-antigen mutants of *F. tularensis* have been isolated that exhibit significantly reduced virulence, and in contrast to wild-type bacteria may have an increased ability to induce autophagy, a cellular degradation process, within infected macrophages. The goal of this study was to explore the extent to which the *wbtA2* O-antigen mutant of *F. tularensis* induces autophagy.

Hypothesis: Transposon-based mutagenesis of the *wbt* gene cluster of *F. tularensis* resulted in the *wbtA2* mutant. Early biochemical characterization of this mutant revealed a lack of the O-antigen virulence factor at the cell surface. In view of these data we hypothesized that the *wbtA2* O-antigen mutant may stimulate initiation of autophagy.

Methods: *F. tularensis* strains were cultured on cysteine heart agar supplemented with sheep blood (CHAB) and brain heart infusion broth supplemented and antibiotics to maintain strain homogeneity. Human monocytes, isolated from the peripheral blood of healthy donors, were differentiated into macrophages for 5 days. Macrophages left uninfected, infected with the *wbtA2* mutant (MOI=5:1), or infected with wild-type *F. tularensis* (MOI=100:1) were characterized: 1) biochemically for changes in expression of the autophagy markers LC3-II & p62, 2) via confocal microscopy for co-localization with the early autophagy marker ubiquitin and the late autophagy and lysosomal marker LAMP-1.

Results: Biochemical analyses of autophagy markers LC3-II and p62 revealed that autophagy could be induced in macrophages through chemical means and by amino acid starvation and these treatments were used as positive controls. Both wild-type *F. tularensis* and the *wbtA2* mutant also stimulated autophagy. By confocal microscopy the *wbtA2* mutant co-localized more frequently with ubiquitin and other early autophagy markers than did wild-type *F. tularensis*. Inducing autophagy by starvation or drug treatment in conjunction with *wbtA2* mutant infection did not result in greater co-localization of autophagy markers with mutant bacteria.

Conclusions: The results of previous studies suggest that *F. tularensis* may induce autophagy in macrophages to increase nutrients available to sustain infection, but whether autophagy contributes to clearance of mutant bacteria is unclear. We show here that the *wbtA2* mutant co-localized with ubiquitin but not LAMP-1, which suggests that enhanced autophagy induction that did not progress to fusion with lysosomes. We therefore conclude that O-antigen plays a role in the avoidance of autophagy detection, but *F. tularensis* uses separate mechanisms, such as phagosome escape, to avoid targeting to lysosomes.

Overcoming Inhibitors Using piggyBac in factor VIII Deficiency

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Abstract

Background: Hemophilia A is caused by a deficiency in coagulation factor VIII and is the most severe inherited bleeding disorder. Hemophilia A affects roughly 1 per 5,000 males. The current treatment is to administer recombinant factor VII (rFVIII), but due to its high cost it is estimated that less than 25% of those affected are adequately treated worldwide. Furthermore, roughly 30% of severely affected patients develop inhibitory antibodies that neutralize the rFVIII activity. For this subset of patients, treatment options are limited and expensive. Gene therapy holds promise for hemophilia A because a small increase in factor VIII levels can alter the clinical phenotype. Previous studies have shown that products that contain von Willebrand factor (vWF) reduce the risk of inhibitor development. The purpose of this study is to investigate the effect of vWF and FVIII co-delivery to a preclinical model of hemophilia with pre-existing inhibitors.

Hypothesis: Co-delivery of vWF and FVIII will achieve long-term expression of FVIII and alleviate pre-existing inhibitors.

Methods: FVIII null mice were used in the experimental group. Mice were given rFVIII(Baxter) once weekly for four weeks, a well-established method for FVIII null mice to develop inhibitors. These mice were then injected with two plasmids via hydrodynamic tail vein injections (HDTVI). The first plasmid used was the *piggyBac* vector with variants of FVIII and vWF. The second another plasmid containing the transposase iPB7. Plasma was collected at three days post injection and then every two-four weeks thereafter. FVIII activity in plasma samples was quantified using the Coamatic FVIII Assay as well as a FVIII ELISA and samples were read on a microplate reader.

Results: Co-delivery of FVIII and vWF demonstrated similar results 24 weeks post gene transfer compared to codon-optimized, B-domain-deleted FVIII alone. Post injections with Advate (to induce FVIII antibodies), animals receiving both codon-optimized B-domain-deleted FVIII and vWF demonstrated persistent FVIII levels. After successful induction of FVIII inhibitors, hemophilia A null mice expressed FVIII antigen and activity measured by ELISA and Coamatic activity assay compared to animals receiving FVIII cDNA alone.

Conclusions: While this study is still on going, the data show promise that we can overcome the pre-existing inhibitors in the treatment of hemophilia A. The preliminary results demonstrate that cis delivery of FVIII and vWF cDNA is improved compared to FVIII alone. These data demonstrate that we can prevent inhibitor development which suggests potential treatment for hemophilia patients with inhibitors.

Modeling Bacterial Attachment in the Cystic Fibrosis Airway

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ABSTRACT

BACKGROUND: Airway mucus defends the respiratory tract by trapping inhaled pathogens and facilitating their removal by ciliary transport. Submucosal glands and goblet cells contribute by secreting mucins, the major proteins of airway mucus. Mucins are hydrated and self-assemble in the airway surface liquid (ASL). In cystic fibrosis (CF), ASL is relatively acidic, abnormally viscous, and mucus detachment is impaired. Patients with CF develop chronic bacterial airway infections with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but it remains unclear how these bacteria initially attach and subsequently adapt to the CF airway. **Our aims were to determine whether these bacteria attach better to mucus at the low pH observed in CF and to determine which bacterial factors are essential for this interaction.**

HYPOTHESES: 1. *P. aeruginosa* and *S. aureus* have greater adherence to mucus at lower pH.
2. *P. aeruginosa* attachment to mucus requires pili and flagella.

METHODS: We adapted an *in vitro* binding assay to quantify the number of *P. aeruginosa* and *S. aureus* that bind to mucus under different environmental conditions. 100 µg of Type III pig gastric mucus (Sigma-Aldrich) or methacholine-stimulated tracheal mucus was deposited in triplicate in 96-well plates and incubated overnight at 37˚C. Wells were washed 6 times with PBS, and 1 x 10^6 - 3 x 10^7 bacteria were added in PBS for 1 hour at 37˚C. Wells were washed again 6 times with PBS to remove unattached bacteria. For studies examining the effect of pH, the bacteria were incubated with PBS with pH ranging from 6.1-8.7. We also compared wild type *P. aeruginosa* to isogenic strains lacking pili (Δ*pilA*) or flagella (Δ*fliC*). Adherent bacteria were removed from microtiter wells with 200ul of a .05% trypsin solution (*S. aureus*) or 0.01% Triton X-100 (*P. aeruginosa*) and quantified by standard CFU assay. Binding ability is expressed as fraction bound (FB), calculated by taking the number of CFUs counted for an individual microtiter well, and dividing it by that well's initial inoculum.

RESULTS: The study revealed that wild type (WT) *P. aeruginosa* binds with greater efficacy to pig gastric mucin (FB=1.9 x 10^{-2}) as compared to the flagellar mutant Δ*fliC* (FB=3.92 x 10^{-4}) and the pilus mutant Δ*pilA* (FB=1.40 x 10^{-3}). These results were significant between WT *P. aeruginosa* and Δ*fliC* (p=.0475). This study also discovered that *P. aeruginosa* binds in greater numbers when incubated at pH 6.1 (FB=2.52 x 10^{-2}) as compared to pH 7.2 (FB=1.30 x 10^{-2}) and pH 8.7 (FB=1.32 x 10^{-2}), a result with significance of p=.0334. *S. aureus* demonstrated no optimal pH for binding to mucus.

CONCLUSIONS: *P. aeruginosa* demonstrates a greater binding ability to porcine mucus at lower pH, an interaction that is dependent upon both pili and flagellar components for maximal efficiency.

Alterations in Nighttime Systolic Blood Pressure Dipping is Associated with Aortic Stiffness and Inflammation Among Middle-Aged/Older Adults with Obesity

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Background: Blood pressure (BP) fluctuates considerably throughout the day, with a clear decrease in systolic BP by 10 - 20% at night during sleep. This physiological 'dipping' process appears to be important for cardiovascular health, as failure of an individual's systolic BP to appropriately decline 10% or more (e.g. 'non-dipping' profile) during the night is associated with increased cardiovascular target organ damage and elevated risk of developing cardiovascular disease (CVD). Furthermore, individuals whose systolic BP increases during sleep (e.g. 'reverse dipping' profile) are at the greatest CVD risk. However, the mechanisms that contribute to a blunted or reverse BP dipping profile remain unclear. Aortic stiffness, as measured by the gold standard carotid-femoral pulse wave velocity (PWV), is an independent predictor of adverse CV events in middle-aged and older adults with and without hypertension. In individuals with untreated hypertension, a 'reverse dipping' profile is associated with elevated aortic stiffness compared to normal BP dippers. However, there are no studies investigating whether aortic stiffness is associated with alterations in BP dipping in middle-aged/older adults with obesity. Therefore, the objective of this study was to investigate the association between alterations in nighttime systolic BP dipping and aortic stiffness in obese adults at higher CVD risk.

Hypothesis: Blunted nighttime systolic BP dipping will be associated with elevated aortic stiffness, independent of mean arterial pressure (MAP) and other CVD risk factors, in middle-aged and older obese adults with at least one additional CVD risk factor.

Methods: Baseline data from 107 men and post-menopausal women was analyzed in a retrospective cross-sectional design. Participants were between the ages 40-75 years and obese (body mass index, BMI $\geq$ 30 kg/m^2) with no documented CVD or pulmonary disease. Participants did have one or more of the following CVD risk factors: 1) treated hypertension or BP > 140/90 mmHg, 2) treated diabetes or fasting glucose > 110 mg/dL, 3) treated hyperlipidemia, or LDL $\geq$ 130 mg/dL, or non-HDL $\geq$ 160 mg/dL, or HDL < 40 mg/dL, or TG $\geq$ 200 mg/dL, or 4) metabolic syndrome with three or more milder CVD risk factors. Carotid-femoral PWV was measured to assess aortic stiffness by recording carotid and femoral artery pressure waveforms sequentially with an applanation tonometer. Time delay (t) between waveforms were R wave-gated to ECG and PWV was calculated as the path length (L) between carotid and femoral artery sites divided by 't.' Twenty-four-hour BP was recorded via an ambulatory BP monitor (Space Labs, Inc.) on the right brachial artery. Nighttime systolic BP dipping was defined as the percent change of mean daytime (06:00 – 23:00 hours) systolic BP to mean nighttime (23:00 – 06:00 hours) systolic BP. Normal BP dipping was defined as a 10-20% decrease (n = 52), non-dipping as 0-10% decrease (n = 43), reverse dipping as <0%, i.e. increased night BP (n = 5), and extreme dipping as $\geq$ 20% decrease (n = 7). Systolic and diastolic BP were recorded twice per hour during the day and once per hour at night. Venous blood samples were obtained under fasting conditions and assays were performed for circulating metabolic factors and C-reactive protein (CRP), a biomarker of systemic inflammation. Significance was set at a p-value < 0.05. Analysis of variance (ANOVA) and covariance (ANCOVA) with least significant difference post-hoc tests were used for analyses.

Results: Groups did not differ by sex, BMI, 24-hour heart rate, MAP, or daytime systolic BP; but reverse dippers were slightly older ($p = 0.016$). In the unadjusted analysis, reverse dippers demonstrated higher carotid-femoral PWV (ANOVA $p = 0.009$) compared to both normal dippers ($p = 0.002$) and non-dippers ($p = 0.011$). PWV among extreme dippers was not significantly elevated compared with normal dippers (ANOVA $p = 0.137$). After controlling for sex, BMI, MAP, and anti-hypertensive medications, the difference in PWV between reverse dippers and normal dippers remained significant ($p = 0.003$). However, further adjustment for age abolished the difference in PWV (ANCOVA $p = 0.196$). Twenty-four-hour pulse pressure, a surrogate measure of aortic stiffness, remained significantly higher for all dipping categories compared to normal dippers after adjusting for age, sex, BMI, MAP, and medications (ANCOVA $p = 0.01$). Furthermore, reverse dippers had significantly elevated serum concentrations of C-reactive protein compared with the other three groups (all $p < 0.001$) after controlling for age, sex, BMI, MAP, medications and additional adjustment for 24-hour mean systolic BP (ANCOVA $p = 0.007$).

Conclusion: A reverse nighttime dipping profile was associated with elevated aortic stiffness that could not be explained by sex, BMI, MAP, or participant's antihypertensive medications; but was explained by the older age of reverse dippers. In contrast, pulse pressure, a surrogate expression of aortic stiffness and robust predictor of CVD risk, remained significantly higher in non-dippers, extreme dippers, and reverse dippers compared with normal dippers. This suggests that alterations in the physiological nighttime dipping process (reverse, blunted, and exaggerated) are associated with elevated CVD risk in middle-aged/older adults with obesity. Furthermore, the elevated CRP in reverse dippers suggests that inflammation may be one potential mechanism contributing to the reverse dipping phenomenon in our cohort. However, because our study is cross-sectional, a cause-and-effect relation between reverse dipping, elevated aortic stiffness, and inflammation cannot be determined; and must be further investigated in future research. This study identifies a subset of middle-aged/older adults with obesity who are at higher CVD risk based on elevation of nighttime systolic BP and inflammation, who otherwise would not have been identified based on office systolic BP alone.

Mixed State and Suicide:
Is the Effect of Mixed State on Suicidal Behavior More than the Sum of its Parts?

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Abstract

Introduction: Mixed episodes are experienced by an estimated 20% of individuals with bipolar disorder. Individuals who experience mixed states are known to be at high risk for suicide; however, it is not known if this excess burden of risk is due to an additive effect of experiencing symptoms associated with both mania and depression, or if there is excess burden of risk during mixed state beyond that which can be explained by the additive contributions of depression and mania. To address this question, the aim of this study is to determine whether individuals experiencing mixed states are at an excess burden of risk of suicide attempt than would be expected by the components attributable to manic or depressive symptoms alone.

Hypothesis: The risk of suicide attempt during mixed state is greater than can be explained by the additive effect.

Methods: This study uses data from 429 participants of the National Institute of Mental Health Collaborative Depression Study (CDS) with prospectively-defined bipolar disorder and at least 1 year of follow-up. Weekly symptom severity and onset of mania/hypomania and depression were captured using the Longitudinal Interval Follow-up Evaluation (LIFE) and suicidal behavior was captured initially using the Schedule of Affective Disorders and Schizophrenia (SADS) and across follow-up using the LIFE. Frailty models were used to assess time-to-onset of suicidal behavior, with mixed episodes modeled using an interaction term.

Results: We found no increased risk of suicide attempt during mania/hypomania (HR: 1.19, 95% CI: 0.82-1.74, p=0.36) and an increased risk of suicide attempt during depression (HR: 5.28, 95% CI: 4.26-6.54, **p<0.0001**). The inclusion of an interaction term showed no suggestion of increased suicide risk during mixed state beyond the individual contributions of mania/hypomania and depression alone (p=0.80). In follow-up analysis, we found that individuals with a history of mixed state spend a greater proportion of time depressed (46% vs. 28%, **p<0.0001**) and have an overall higher occurrence of suicide attempt (β=0.69, SE=0.21, **p=0.0012**) relative to individuals without a history of mixed state.

Conclusion: Although this study fails to detect evidence of an excess burden of risk for suicide attempt during mixed state beyond the risk contributable to the depressed component, we do find suggestion that individuals who experience mixed states are a particularly vulnerable population to depression and suicide, due largely to greater burden of depression.

Sex Differences in the Consequences of Prenatal Stress on Neurobiology and Behavior: Habit Learning and Locomotion

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Background: Prenatal stress (PS) has been identified as a risk factor for neuropsychiatric disorders. Risks are elevated for disorders in which the caudate putamen and the behavior it controls are implicated; including: autism spectrum disorder (ASD) and Tourette syndrome. The impact of PS on developmental mechanisms and how these relate to long term effects on behavioral outcomes are not well understood. Further, neuropsychiatric disorders associated with PS occur more in males than females, a phenomenon that is not understood. Utilizing mouse models, this project investigates whether behavior dependent on the caudate putamen and the neurobiology of the caudate putamen are changed by PS in a sex-specific way. By administering an antioxidant to mice during development, we will also identify possible mechanisms responsible for effects of PS. This may be translatable, in the longer term, to prevention of risk to the finely tuned circuitry of the brain.

Hypothesis: The effects of PS on developmental mechanisms within the caudate putamen will differ between male and female offspring. This will differently affect motor and habit control. We hypothesize these effects will be due to oxidative stress mechanisms, ameliorated by antioxidant administration.

Methods: Pregnant CD1 females were singly housed beginning on embryonic day 12. On embryonic day 12, half of pregnant female mice were placed in a plexiglass restraint for 45 min under bright lights, three times daily. From the day of birth until weaning, nursing mothers and therefore also offspring received an antioxidant, N-acetylcysteine (NAC), through drinking water (8-12 mg/day). Behavioral testing during the animals' light cycle was performed at 3 weeks and 2 months of age:

- **Open Field:** In a rectangular plastic arena, mice were tested for locomotor activity for 30 min on one day (3 week old mice) or two consecutive days (adult mice). Test mice were placed in the corner of the arena and their movements recorded using an overhead camera and Anymaze software. ANOVA was used to evaluate group differences in total distance and time spent in the center.
- **Water T-maze:** Pilot mice were trained in a water T-maze with proximal and distal cues, 10 trials per day until 5 trials without error occurred. Then, the maze was turned 180° for a probe trial. The direction of the first turn in the maze was recorded. Habitual behavior was defined as turning left or right, as trained. 3 more probe trials were completed, each after 2 more days of training.
- **Brain tissue:** Mice were perfused and brain tissue was fixed at least 7 days after completion of behavioral testing. Tissue was frozen in OCT and sectioned. Volume and stereological cell count were made with a Zeiss Axioskope 2 Mot Plus equipped with a digital camera after fluorescent immunocytochemistry with anti-GAD67 and anti-parvalbumin antibodies.

Results: Only male mice at 3 weeks of age displayed a significant difference in open field locomotor activity, but not behavioral inhibition, due to PS. This effect was rescued by NAC.

- **Open field:** At 3 weeks old, control and NAC-treated, PS male mice both were significantly more active compared to PS males. Females showed no difference in activity. At 2 months of age, display of a significant difference in motor and habit control across each group was absent.
- **Water T-maze:** Pilot testing demonstrated that the rates of habit-based behavior changed as expected over repeated training and testing, with 80% of mice initially displaying habitual behavior. This rate fell to 60% on the 3rd probe trial and increased again to 100%. Testing is ongoing for prenatally stressed mice, with and without NAC treatment.
- **Brain Tissue:** Adult caudate putamen volume was significantly increased after PS only in males. Also only in males, GAD67 neuron density showed a trend increase after prenatal stress. Analysis is ongoing for mice treated with NAC.

Conclusion: Prenatal stress resulted in significant differences in only the behavior of 3 week old mice. Persistent changes into adulthood in caudate putamen neurobiology were also observed. This suggests that prenatal stress effects may capture the pathology present in developmental behavioral disorders in particular.

Functional Interactions of NIAM and RGS6 Tumor Suppressors in Breast Cancer

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Background: Breast cancer is the leading cause of cancer death in women worldwide. An improved understanding of the molecular pathways underlying breast tumorigenesis will facilitate earlier detection, predict which patients respond better to certain therapies, and provide new targets for cancer treatments. The p53 tumor suppressor plays a major role in preventing breast cancer; consequently, its regulators are potentially meaningful biomarkers and targets of the disease. Our research groups study two new activators of p53, named NIAM (Nuclear Interactor of ARF and Mdm2) and RGS6 (Regulator of G protein signaling 6). Each factor is down-regulated in human breast tumors and can suppress mammary tumorigenesis in mice. In addition, NIAM and RGS6 bind a common partner (the Tip60 acetyltransferase) and both proteins are upregulated by the anti-cancer drug, doxorubicin, to mediate tumor cell arrest and death. Other studies have shown RGS6 impairs Ras and Her2 oncogenic activity while NIAM binds to ARF, the key regulator of oncogene checkpoints. This collaborative project involving multiple HCCC laboratories will establish whether RGS6 and NIAM act through common or distinct mechanisms to prevent breast cancer.

Hypothesis: NIAM and RGS6 cooperate to suppress breast tumorigenesis.

Methods: Protein interaction studies were conducted in H1299 lung cancer cell lines. H1299 cells were transfected to overexpress tagged versions of the NIAM protein alone, the RGS6 protein alone, or both proteins. Presence of these overexpressed proteins were confirmed by direct lysate analysis through western blotting. Antibodies specific to the tags on each protein were used to immunoprecipitate and followed by a Western blot analyses to determine whether copreciptation of NIAM and RGS6 were seen. Protein half-life studies were also conducted in 293T cells to determine the impact of coexpression on each protein's half-life. 293T cells were treated with Cycloheximide, a protein synthesis inhibitor for five different time points. Lysates were collected and western analyses were done. Additionally, we have generated four different study groups of female mice (wild-type, *NIAM-/-*, *RGS6-/-*, and double *NIAM-/-; RGS6-/-*). Spontaneous and carcinogen-induced breast cancer formation and survival will be measured in these animals. Lastly, bioinformatics analyses of human breast cancer databases are being conducted to determine whether RGS6 and NIAM are independently or coordinately down-regulated in breast tumors, and if their loss correlates with changes in oncogenic drivers of breast cancer such as Her2.

Results: Initial bioinformatics analyses show there is coincident down-regulation of RGS6 and NIAM mRNA expression in a subset of breast tumors designated HER2-enriched. Binding studies demonstrated that exogenous GFP-tagged RGS6 interacts with HA-tagged NIAM in H1299 cells. The half-life of RGS6-GFP in 293T cells decreases when coexpressed with mNIAM (mouse NIAM). However, the half-life of mNIAM does not change when coexpressed with RGS6-GFP. Aggressive tumors indicative of advanced breast cancer were seen in 1 of 7 NIAM-/- mice (14%) compared to no tumors in WT control.

Conclusions: Results support the notion that NIAM and RGS6 may be interacting functionally to suppress breast tumorigenesis.

Epidural Spinal Cord Stimulation: A Potential Novel Therapy in the Treatment of Restless Legs Syndrome

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BACKGROUND: Restless legs syndrome (RLS), or Willis-Ekbom disease, is a neurological disorder characterized by an irresistible urge to move the extremities, particularly the legs. The pathophysiology of RLS remains elusive; however, recent studies suggest that peripheral hypoxia is associated with RLS symptoms. Spinal cord stimulation (SCS) is a routine therapy in the treatment of peripheral neuropathic pain and is known to induce vasodilation in the legs and feet. The purpose of this study is to determine the extent to which epidural SCS decreases muscle sympathetic nerve activity (MSNA), improves lower limb blood flow in patients treated with thoracolumbar epidural SCS for chronic back pain, and reduces severity of RLS symptoms.

HYPOTHESIS: Thoracolumbar epidural SCS will diminish MSNA, increase blood flow to the lower extremities, and will reduce RLS symptom severity in patients diagnosed with RLS.

METHODS: Subjects without RLS who had previously undergone thoracolumbar SCS implantation for chronic pain conditions underwent measures of femoral and brachial artery blood flow (Doppler ultrasound) at baseline and at 30 and 60 min following administration of SCS, and at 30 min following the secession of SCS. MSNA was also measured from the peroneal nerve using microneurography. To examine the clinical efficacy, RLS symptoms were evaluated using the International Restless Legs Syndrome Scale (IRLSS) in a RLS patient pre- and post-SCS implantation for primary treatment of chronic back pain.

RESULTS: In a RLS patient receiving SCS implantation, IRLSS score was 33 (very severe) prior to implantation and 0 (mild) after implantation. In subjects without RLS and receiving SCS implantation (n=1), femoral artery blood flow was acutely increased +16% at 30 min and +16% at 60 min of SCS administration and returned to baseline following the secession of SCS (30 min post: +0.2% of baseline). Likewise, MSNA was reduced -28% at 30 min and -53% at 60 min of SCS administration and returned to near baseline following the secession of SCS (30 min post: -7% of baseline). As expected, no change in brachial artery blood flow was observed during SCS administration (baseline: 39 ml/min; 30 min: 38 ml/min; 60 min: 35 ml/min).

CONCLUSION: These preliminary data suggest that thoracolumbar epidural SCS may relieve symptoms of RLS. In addition, these initial data indicate that SCS reduces MSNA and subsequently increases leg blood flow in patients with chronic back pain without RLS. Additional studies are ongoing to test whether SCS-induced reductions in MSNA and blood flow to the lower limb may represent potential mechanisms by which SCS relieves clinical symptoms of RLS.

Effects of Long Distance Cycling on Median Nerve Sonographic Appearance

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Background: Injuries, both traumatic and overuse, are common in long distance cycling events. Dannenberg et. al. found that overuse injuries occur at 13.7 injuries per 100,000 person-miles with some of the most common being nerve deficits of the hands and wrists. Median neuropathy at the wrist has been documented as a result of prolonged pressure on the hands and wrists from the handlebars. Previous studies have used nerve conduction studies and muscle strength tests to examine the effects of long distance cycling on the median nerve at the wrists. These studies have shown that cyclists can experience the onset of Carpel Tunnel Syndrome (CTS) like symptoms or exacerbate symptoms in those with already diagnosed CTS. With ultrasound (US) becoming more prevalent as a diagnostic tool, this study aims to use US to examine the appearance of the median nerve in cyclists before and after a multi-day long distance cycling event.

Hypothesis: The ultrasonographic cross sectional area of the median nerve will change during a 6-day endurance cycling event.

Study: Prospective Cohort

Methods: After approval from the Institutional Review Board was obtained, 15 subjects were recruited from the Register's Annual Great Bicycle Ride Across Iowa (RAGBRAI) participants. Of those subjects, 4 were lost to follow up either due to an accident unrelated to the study or by not returning to the camp site on day 6. Each subject went through the informed consent process with a member of the research team before participating in the study. Subjects were excluded from the study if they were not at least 18 years of age, if they already had a diagnosed neuropathy in either wrist, or if they were not riding all six days of the event. The cross sectional area of the median nerve at the inlet to the Carpal tunnel was evaluated in each wrist of the 11 subjects (22 wrists) on day 1 and day six of RAGBRAI. This was done using a Philips Lumify, a hand-held ultrasound device. The measurements were taken at the subject's camp site while the subject sat with their arm resting in the supine position. Two questionnaires were also given. The first was given before the event started and gathered demographic and riding style information from each subject. The second was a nerve function questionnaire (Levine-Katz Questionnaire), and that was given both before and after the event. Each subject was given a score based on their answers to the nerve function questionnaire.

Results: A two-tailed paired T-test was used to analyze the 44 data points collected from the 11 subjects. The p-value from the t-test was .97 with a t-value of -.04. Two subjects reported an onset of new or exacerbated neuropathy in the hand via the Levine-Katz Questionnaire with one raising their questionnaire score from 11 to 12 and the other 11 to 13.

Conclusion: We found no difference in the cross-sectional area of the median nerves before and after the 6 days of cycling. Only 2 of the 11 subjects reported any median nerve symptoms, and these were mild. We conclude that it is unlikely that endurance cycling causes sub-clinical structural changes to the median nerve in most cyclists.

Novel Targets for Obesity: Gut Microbiota & Anaerobic Resting Metabolic Rate

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Background: Weight gain and obesity are the result of a chronic imbalance between energy intake and output. The obesity epidemic in the United States is the result as little as a ~7.2 kcal/day imbalance between intake and output, a fact which underscores how tightly energy balance is regulated – and how *any* and *all* of the biological mechanisms that contribute to energy balance might be targeted to treat obesity. Therefore it is surprising that although resting metabolic rate (RMR) accounts for between 60 to 100% of total energy expenditure for endothermic organisms such as humans, *none* of the five current FDA-approved anti-obesity drugs work through the stimulation of RMR. Thus, the long-term goal of our research program is to clarify biological mechanisms that control RMR and thereby identify potential novel therapeutic targets to treat obesity and its sequelae such as diabetes and hypertension.

The gut microbiota refers to the consortium of bacterial species which live within the gastrointestinal tract. Increasing evidence supports a role for the gut microbiota in the control of weight gain, but there remains a critical lack of mechanistic understanding with regard to how changes in the composition of the gut microbiota modulates host weight gain. Although many groups have *qualitatively* demonstrated effects of gut microbiota changes upon digestive efficiency, *quantitative* bioenergetics assessments have not been performed. Studies in humans and animal models using respirometry-based technologies (which only assess aerobic, oxygen-dependent processes) have failed to detect direct effects of gut bacteria changes upon aerobic RMR, however, these studies fail to acknowledge that gut bacteria survive and function in a largely anaerobic environment. Clearly, the use of quantitative methods, which are capable of assessing both aerobic plus anaerobic RMR in vivo, are needed to fully characterize the contributions of the gut microbiota upon energy homeostasis.

Our team recently demonstrated that anaerobic RMR contributes a large fraction of total RMR in mice (between 7-10% of total energy expenditure), and that changes in the gut microbiota can specifically and robustly modulate this type of energy output. We have demonstrated that the antipsychotic drug, risperidone, uniquely shifts the gut microbiome in both humans (Bahr *et al*, Transl Psychiatry 2015) and mice. Further, fecal transplant from risperidone- versus vehicle-treated animals to naïve animals results in major and specific suppression of anaerobic RMR and increased weight gain (Bahr *et al*, EBioMedicine 2015). In addition, we have demonstrated that exposing wildtype mice to a shift in diet from a chow-based diet to a high-fat diet results in the suppression of anaerobic RMR (Burnett & Grobe, Mol Metab 2014). We thus hypothesize roles for bacterial composition and mass in RMR control:

Hypotheses: (1) Surgical modulation of the biomass of the gut microbiota (reductions via cecectomy; and increases via Roux-en-Y gastric bypass, RYGB) will result in a concomitant shift in anaerobic, and thereby total, RMR in mice.

(2) Dietary modulation (various chow, high-carb/low-fat and low-carb/high-fat diets) will change the composition of the gut microbiota and its contribution to total RMR in mice.

Methods: To explore the first hypothesis, C57BL/6J mice were obtained from Jackson Labs, acclimated to the laboratory for at least one week, then treated for two weeks with vehicle- or risperidone-supplemented drinking water. Baseline RMR of each mouse under general anesthesia (ketamine+xylazine) was measured using a combined calorimetry system. Following baseline analysis, mice were either subjected to cecectomy or a sham surgery, and RMR was measured again. In a separate cohort, obese C57BL/6J mice (45g) underwent either sham- or RYGB surgery and then recovered for one week before awake, unanesthetized assessment of RMR by combined calorimetry.

To explore the second hypothesis, age- and weight-matched C57BL/6J mice were acclimated to the laboratory for one week, then fed either a high-fat diet (HFD, 45% fat, 35% carb), high-carb diet (HCD 10% fat, 70% carb), or standard chow diet (SCD's 2920 16% fat, 60% carb or 7913 18% fat, 59% carb) for one week. Body weights and compositions were determined using NMR both before and after the change in diet. RMR was assessed under anesthesia as above.

Results: Addressing hypothesis 1; within the vehicle-treated group of mice, cecectomy resulted in an immediate drop in RMR (~8%) which was disproportionate to the reduction in body mass (~1%). As previously, risperidone treatment suppressed baseline RMR, and surprisingly this treatment also abolished the effect of cecectomy upon RMR. RYGB surgery caused a large increase in total RMR, entirely due to an increase in anaerobic RMR.

Addressing hypothesis 2; both HCD and HFD mice had elevated body mass compared to chow-fed mice. HCD mice had elevated proportions of lean mass while HFD mice had decreased proportions of lean mass and elevated proportions of fat mass compared to SCD's. In both HFD and HCD, cecum mass was significantly lower than that of SCD's. Baseline RMR of HCD mice was decreased compared to SCD's, though HFD did not differ from SCD's. Following cecum removal, both SCD and HFD mice demonstrated a drop in RMR. HCD mice did not have a significant drop in RMR. Metagenomic sequencing of cecal contents from all groups is ongoing, to characterize changes in the gut microbiota composition.

Conclusion: (1) Anaerobic RMR is a major contributor to total energy expenditure, (2) the biomass of the gut microbiota should be considered an anaerobic thermogenic "tissue," and (3) the gut microbiota may represent a novel therapeutic approach to obesity, through the promotion of metabolically-active species, and/or bacterial biomass.

Evaluation of Carpal Tunnel Syndrome with Ultrasound: Reliability of Medical Trainees and Physicians

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ABSTRACT

BACKGROUND: Nerve conduction studies (NCS), or electrophysiological studies, have long been considered the test of choice to assess Carpal Tunnel Syndrome (CTS). Although this condition remains the most common nerve entrapment syndrome in the peripheral limb, the current technology used to study them lacks efficiency, causes patients discomfort, and costs more than the alternative: ultrasound (US). As the use of US as a diagnostic tool continues to expand, some research studies have demonstrated its similarity, and in some cases superiority, in specificity and sensitivity when compared to NCS. Unfortunately, inconsistencies do arise when employing US as a diagnostic tool for CTS. Currently, no consensus exists on the cross sectional area (CSA) thresholds for a positive CTS diagnosis and varying levels experience among sonographers calls the precision of measurements into question. Furthermore, experts disagree about the standard of procedure and training for sonographers to diagnose CTS. With these pitfalls in mind, this study aims to determine the intra-rater and inter-rater reliability of US assessment for median nerve CSA at the carpal tunnel inlet and the proximal third of the pronator quadratus muscle. Determining reliability among sonographers with different levels of experience will help identify the significance of experience in the ability to assess nerve entrapment and establishing a standard diagnostic protocol.

HYPOTHESIS: Intra- and inter-rater reliability of assessing the median nerve with US at the carpal tunnel and proximal third of the pronator quadratus muscle are high when comparing sonographers of varying skill levels.

METHODS: After obtaining approval from the Institutional Review Board for the study, 11 subjects were recruited (22 wrists, 44 measurement sites) from the community and Orthopedic Sports Medicine and Rehabilitation Clinic to participate in the study. Each subject went through the informed consent process with a member of the research team before participating in the study. The average age of the subjects was 25 (range, 23-35). Subjects for the study had to meet the following inclusion criteria in order to participate: 1) Must be 18 or older 2) No previous diagnosis of CTS 3) No previous nerve injury at the hand, wrist, or forearm 4) No previous hand or wrist fractures 5) No previous diagnosis of neuropathic diseases, such as diabetes. We developed the inclusion criteria in order to obtain subjects that most likely display normal anatomy. The group of four examiners consisted of physicians and trainees with varying levels of US experience: an attending Orthopedic Sports Medicine and Rehabilitation physician with over 6 years of US experience, a new Orthopedic Sports Medicine and Rehabilitation fellow with minimal US experience, but that just started an US course, and two medical students with limited US experience. The different levels of medical trainees and experience were selected to determine the variability and reliability of US measurements across different levels of training and experience. Before subjects were scanned, the attending physician participating as an examiner in the study gave the other examiners a quick tutorial of the equipment, a lesson regarding the identification of anatomy in the forearm, and instruction about how to record measurements. Ultrasound scans were done while the patient sat in a chair, with their arms placed on top of a pillow in a supinated position, and extended across an exam bed. Each examiner performed their scans independently using a Phillips iU 22 cart, a L15-7 IO transducer set to the musculoskeletal preset, and the continuous trace caliper function of the cart to measure CSA. Measurement sites included the left carpal tunnel inlet (LI), proximal third of the left pronator quadratus muscle (LPQ), right carpal tunnel inlet (RI), and proximal third of the right pronator quadratus (RPQ). With the measurements recorded for each examiner at the different sites, the inter-rater and intra-rater reliability was calculated using the Intraclass correlation coefficient (ICC) with a consistency definition and 95% CI. The SPSS 23 software package provided by the University of Iowa was used to calculate the ICC. Finally, the scale for interpreting our results were taken from Fundamentals of Biostatistics (Rosner, et. al). According to this source, interpreting the strength of agreement, or reproducibility, between examiners and the ICC (ρ) can be understood as follows: $\rho < 0.4$ indicates poor reproducibility; $0.4 \leq \rho < 0.75$ indicates fair to good reproducibility; $\rho \geq 0.75$ indicates excellent reproducibility.

RESULTS: The total data set for this project included 176 discrete data points from 11 different subjects. All calculations used an ICC definition of single measurements and consistency. After the first scans, the inter-rater reliabilities were: LI- ρ=.690, LPQ- ρ=.345, RI- ρ=.800, RPQ- ρ=.449. The inter-rater reliabilities after the second were: LI- ρ=.709, LPQ- ρ=.493, RI- ρ=.752, RPQ- ρ=.646. Intra-rater reliability using the same statistical definitions were: Sonographer 1—LI- ρ=.774, LPQ- ρ=.498, RI- ρ=.812, RPQ- ρ=.582; Sonographer 2—LI- ρ=.570, LPQ- ρ=.396, RI- ρ=.832, RPQ- ρ=.461; Sonographer 3—LI- ρ=.782, LPQ- ρ=.526, RI- ρ=.672, RPQ- ρ=.552; Sonographer 4—LI- ρ=.708, LPQ- ρ=.662, RI- ρ=.826, RPQ- ρ=.683.

CONCLUSION: Inter-rater reliability among sonographers was fair to good, sometimes even excellent. Greater inter-rater reliability exists based on measurement location and marked improvement was observed on the second occasions of scanning. Meanwhile, intra-rater reliability classified as fair to good, with excellent achieved at least once by every sonographer, and remains greater than inter-rater reliability throughout all measurement sites. Thus, sonographers with different levels of experience can produce statistically similar CSA measurements by using the same US procedure.

Asymmetrical PET Hypometabolism in Temporal Lobe as a Predictor of Neuropsychological Outcomes for Patients with Language-Dominant Temporal Lobe Resection

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BACKGROUND: Approximately one-third of individuals with epilepsy will have drug resistant seizures that are refractory to antiepileptic drugs, with anterior temporal lobectomy (ATL) being the standard surgical treatment. In individuals with focal epilepsy, surgical resection of the epileptogenic cortex produces positive outcomes in terms of seizure freedom, however cognitive outcomes are a concern. Fluorodeoxyglucose (FDG)-positron emission tomography (PET) is a commonly used functional neuroimaging technique in patients with drug-resistant focal epilepsy prior to surgical intervention. Two studies have evaluated the prognostic value of PET in predicting post-resection cognitive outcomes. One study looked asymmetry in hippocampal FDG uptake on preoperative PET as a predictor of verbal memory decline in patients post-ATL. This study (N = 14), using quantitative methods to analyze degree of FDG-uptake, found that asymmetry in hippocampal FDG uptake was not significantly predictive of change in verbal memory, an aspect of cognitive functioning. Another study (N = 60) found that the difference in preoperative FDG uptake between the right and left temporal lobes may predict post-resection verbal memory functioning. However, this study established degree of asymmetry qualitatively by using visual PET assessment, which can vary widely. The current literature remains unclear on whether FDG-PET can be used as a reliable predictor of cognitive functioning post-ATL and no studies to date have looked specifically at naming ability post-ATL.

HYPOTHESIS: Temporal lobe hypometabolism, as illustrated by FDG-PET, is a positive prognostic indicator for confrontation naming ability in patients who have undergone left hemisphere anterior temporal lobectomy.

METHODS: All included subjects underwent left-ATL (L-ATL). The following additional inclusion criteria were applied: (a) preoperative and postoperative examination of naming performance and an Wechsler Adult Intelligence Scale (WAIS-IV) greater than or equal to 70 ((b) were at least 16 years old at time of L-ATL (d) preoperative assessment of speech dominance showing left lateralization based on fMRI, or intracarotid amobarbital testing with alternating selective anaesthetization of the left or right hemisphere and speech and memory testing of the isolated (Wada testing) (c) first-time execution of brain surgery (e) English was their first language. Following these criteria, an archival sample of 22 participants from the patient registry of the Division of Cognitive Neuroscience at the University of Iowa were selected. Demographic data were collected from EPIC for the 22 patients including: gender, age of seizure onset, age at surgical intervention, handedness, employment status, antiepileptic medications, psychiatric comorbidities, mesial temporal sclerosis (MTS) on MRI, FDG-PET seizure lateralization, EEG seizure lateralization, presurgical seizure frequency, and seizure freedom. PET scans were analyzed using NeuroQ software, co-registered to a standard templated brain, and normalized to whole-brain, comparing the FDG uptake in the region of interest (medial anterior region within the temporal lobe) relative to the whole brain. The degree of left-right asymmetry was calculated as ratio. We examined naming ability using the 60 item Boston Naming Test (BNT), and postoperative change scores were dichotomized into decline and stable groups based on reliable change score of greater than 5-point raw score reduction in performance. Age of epilepsy onset, degree of left-right asymmetry, and the presence of MTS were examined as potential predictors of pre- versus postoperative naming change.

RESULTS: Logistic regression analysis showed degree of hypometabolism in the medial anterior region within the temporal lobe to be the best predictor of decline in BNT performance. Age of epilepsy onset and the presence of MTS were not significant predictors of decline.

CONCLUSIONS: Postoperative change in naming ability is associated with degree of hypometabolism in the medial anterior region within the temporal lobe. The potential interpretations and implications of these findings are discussed.

Patient outcomes and satisfaction in treatment for Pectus Excavatum

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ABSTRACT

BACKGROUND: Pectus excavatum is a congenital malformation of the anterior chest wall. It is characterized by a malformation of the sternum and ribs resulting in a sunken in or "funnel" chest appearance. The condition varies in severity but can lead to reductions in cardiac output by compression of the right heart, decreased pulmonary function through lower vital capacity and body image problems. Although the cause of pectus excavatum remains unclear there appears to be a genetic component with multifactorial inheritance.

The Nuss procedure, described in 1987, has been used to treat pectus excavatum. In this procedure a metal bar is inserted through the ribs and underneath the sternum. The bar is then "flipped" to move the sternum out into a position that is flush with the natural contour of the chest. The operation takes approximately 2 hours and the recovery time varies but hospitalization generally lasts 3-5 days and the patient will require approximately a month of opioid pain control and recovery before resuming normal activities as possible. The procedure's main drawback is the lengthy recovery period punctuated by a large amount of pain following bar insertion. The bar is left in place for 2-3 years and then removed in a smaller operation.

METHODS: This was a retrospective study that evaluated the clinical outcomes of patients who underwent the Nuss procedure at the University of Iowa Children's Hospital from 2000 to 2016. We reviewed the data from 100 patients that met study inclusion criteria. 2 patients were excluded due to conflict of interest (students under the supervision of study staff). Key elements of data review include primary outcome, pre-surgical testing and health status through CT based Haller index measurements, pulmonary function testing, and echocardiography. Patients were followed through their inpatient follow-up course and for the 2-3 year follow up period until bar removal. Other data were also obtained from medical records, such as patient demographics, medical history, hospital course and complications, and final discharge disposition. Data was analyzed using SAS statistical software, Microsoft Excel, and REDCap database software

RESULTS: A total of 71% of the patients were male and 29% were female. 91% of the patients were non-hispanic Caucasians. The mean age at time of procedure was 15.9, the mean operating time was 139 minutes, the mean length of stay was 4.21 days. A total of 5 patients received two bars. No patients sustained cardiac injury or evidence of pericarditis. Postoperatively, 7 patients developed an infection, either cellulitis or a local abscess requiring incision and drainage and/or antibiotics. In one of these instances the bar was removed due to complications. One patient required removal of a stabilizer bar due to discomfort. A total of 2 patients required either repositioning of the bar due to rotation or chronic discomfort. 4 patients had incomplete repair of their defect.

CONCLUSION: In this prelimary examination of patients receiving treatment for pectus excavatum at the University of Iowa Children's Hospital the nuss procedure is a safe and effective treatment.

Cortical Morphology in Children with the Gene Expansion for Huntington's Disease. Philip Ryan, Peg Nopoulos. Department of Psychiatry, University of Iowa Hospitals and Clinics, Iowa City, IA 52242

BACKGROUND: Huntington's disease (HD) is progressive, fatal autosomal dominant disorder that manifests through behavioral, cognitive and motor disturbances and typically presents between 25-45 years of age. HD is caused by a trinucleotide repeat mutation in the Huntingtin gene (*HTT*). Subjects with CAG repeats ≥40 are termed Gene-Expanded (GE) and will develop HD. Subjects with <40 repeats are termed Non Gene-Expanded (NGE) and will not develop the disease.

Current dogma holds that etiology of HD is that the mutant Huntingtin (*mHTT*) results in a gain-of-function toxicity that causes neural damage and degeneration. However, there is compelling evidence that in addition to this mechanism, loss of function of normal *HTT* may also play a role. Normal *HTT* plays a vital role in neuronal survival and stability throughout brain development. Given *HTT*'s key role in development, a partial loss of function mutation could manifest in abnormal neural development.

Studies have shown that preHD adults exhibit abnormalities in brain structure up to 20 years before clinical diagnosis. One interpretation is that this is due to early degeneration, but another possibility is that *mHTT* leads to abnormalities in growth of brain circuits. This theory posits that the partial loss of function of *HTT* results in a developmental etiology of a neurodegenerative disease. There is evidence that normal *HTT* affects brain development. A study of NGE children found a significant dose-effect relationship of CAG repeats on brain structure where longer repeat length was associated with thicker cortex measurements in female subjects, but with minimal effects on boys.

We do not know how *mHTT* affects brain development. HD affects primarily the striatum, which receives input from throughout the cortex, but has very specific outputs to the frontal lobe. Therefore, *mHTT* might be expected to preferentially affect frontal lobe and motor strip.

Most studies of preHD subjects are limited to adults. Studying children allows for the evaluation of brain structure 30-40 years before the onset of HD. If abnormalities are identified at this stage, the etiology may be considered developmental in nature.

AIM/HYPOTHESIS: To evaluate the thickness and surface area of cerebral cortex in GE children compared to healthy controls (HC). In addition, differences in cortical morphology in GE subjects based on sex will be evaluated. The study will also evaluate the dose-effect of CAG repeats on the degree of abnormal cortical morphology in each sex. Our hypotheses were:

1. GE children will demonstrate abnormal cortical development, in particular in the frontal cortex and motor strip.
2. Sex will play a role in cortical development abnormalities. Given that *HTT* affects cortical thickness more strongly in females, the effect of *mHTT* is expected to be greater in GE girls compared to GE boys.
3. There will be a dose-effect of CAG repeat such that increasing CAG repeats will be associated with greater abnormality.

METHODS: Subjects**:** The Nopoulos lab's Kids-HD study recruited children at risk of HD throughout the USA. Children who tested GE (n=45) were compared to controls (n=204) consisting of normal healthy children and children at risk for HD who did not inherit the gene.

Brain imaging: Subjects underwent brain imaging via MRI on a 3.0T scanner and multimodal sequence of T1 and PD/T2 weighted scans were obtained. Image analysis was done using FreeSurfer, a sophisticated method for automatic parcellation of the cerebral cortex. Output measures for each distinct region (43 total) include gray matter cortical depth (in mm) and surface area (in mm^2).

Data Analysis: The sample size of 45 GE subjects (31female, 14 male) provided adequate statistical power (> .80) to detect significant differences between GE and HC groups. Analysis was performed using SPSS software. Measurements of cortical regions were compared between GE and HC groups using ANCOVA to account for normal structural differences related to other variables (age, etc.) that were not our variable of interest. In order to reduce the chance for Type II error, regional analysis at the lobe level (frontal, parietal, etc.) was only done if the initial overall cortical measures were found to be significant. To evaluate the relationship between cortical measures and CAG length (dose effect), Pearson Partial Correlations were calculated, which also allowed the study to control for confounding factors. This was done only on the regions in which a significant group differences were found.

RESULTS: After controlling for sex, age, and total brain size (ICV or intracranial volume), we found no significant difference in cerebral cortex surface area between the GE group and the HC subjects. However, we did observe that *mHTT* had a statistically significant effect on cerebral cortex thickness, which showed differences based on sex. Male GE subjects were found to have thinner (p=.012) cortical measurements than male HC subjects when looking at the entire cerebral cortex. However, differences in cortical thickness of female GE subjects compared to female controls were insignificant. The correlation in males was found to be significant in the frontal (p=.014), parietal (p=.016), and temporal (p=.006) lobes. The distinct brain regions that showed significantly thinner cortical measures in GE subjects compared to HC subjects were then identified. The correlation between cerebral cortical thickness and number of CAG repeats was not significant, suggesting no 'dose effect' of repeats.

CONCLUSION: The results of this study provide insight into the neural development of preHD children, some of whom will not develop HD for 30-40 years. The presence of significant abnormalities at this stage of development supports the theory that the etiology of HD is developmental in nature. This is possibly due to the partial loss of function of the protein. Contrary to the original hypothesis, males were found to be more affected by *mHTT* than females. Given that the male brain is more vulnerable to developmental aberration, this was not completely unexpected. The thinning of the cortex in preHD boys appears to be a global cortical effect, rather than preferentially based in the frontal cortex as hypothesized. The absence of a significant 'dose effect' indicates that the thinning of the cortex occurs when males reach the threshold of 40 CAG repeats.

Contraceptive use postpartum at an academic medical center

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INTRODUCTION
Short intervals between pregnancies are well-known to be associated with increased fetal and maternal morbidity. While women are typically asked about postpartum contraception after delivery, postpartum visit attendance is highly variable and many women wishing to delay subsequent pregnancy are using low or moderately effective methods of contraception at 6 months postpartum. This study seeks to determine rates of follow-through and satisfaction with contraceptive methods included prenatally or immediately post-partum in our previous patient population.

METHODS
Women receiving prenatal care at University of Iowa Hospitals and Clinics, previously involved in the Postpartum Contraception Acceptance and Readiness study were contacted via phone 4-6 months postpartum to inquire about contraceptive use regarding their current use of contraception and satisfaction with their current method.

RESULTS
117 women were contacted postpartum via phone an average of 25 (±4.2) weeks postpartum. Of women who were using contraception, 90.4% of women were either very satisfied or satisfied with their chosen method and 94.4% would recommend their current method to a friend. Most common methods used was intrauterine contraceptives (31, 26.5%), oral contraceptive pills (26, 22.2%), and barrier methods (17, 14.5%). 83.5% of women reported that the method they were currently using was discussed with them during their prenatal care. 66.1% of respondents chose their method during their prenatal, 11.3% chose during their hospitalization postpartum and 16% chose at their postpartum visit. 70.1% of women responded that postpartum contraception should be discussed in 2nd or 3rd trimester of their prenatal care.

CONCLUSION
Women are most likely to definitively choose a postpartum contraceptive method during their prenatal care. Discussion of postpartum contraception is thus most successful, as well as preferred, by pregnant women.

Cloning Porcine Programmed Death 1 (PD-1)
for the study of checkpoint blockade in Porcine Lymphoma Model

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ABSTRACT

BACKGROUND: The use of checkpoint blockade in cancer immunotherapy is rapidly expanding with FDA-approved monoclonal anti-programmed death 1 (PD1) antibodies demonstrating efficacy in treating an increasing number of cancer types. Programmed death 1 (PD1) is a receptor expressed by T cells which, upon binding to its ligand PD-L1, can inhibit the antigen specific activity of those T cells. Many labs have focused their efforts on developing monoclonal antibodies that block the PD1/PD-L1 immune checkpoint. The Weiner laboratory is exploring combinatorial approaches involving the PD1/PD-L1 and is interested in using a porcine model of lymphoma to further study and improve the efficacy of PD1 checkpoint blockade. Such a model is well suited for these studies due to the physiological and anatomical similarities between human and pig such as the high homology between human and pig PD-1 proteins. However, such studies are not currently possible due to the lack of porcine specific anti-PD1 antibodies.

OBJECTIVE: The objective of this project is to clone porcine PD1 to be used for the development of anti-porcine PD1 antibodies with the long-term goal of using the reagents to evaluate checkpoint blockade in a porcine lymphoma model.

METHODS: Total RNA was isolated from porcine PBMCs using a standard RNA isolation technique. Primers designed to amplify full-length porcine PD-1 were used to generate PD-1-coding cDNA and PCR product. PCR product was cloned into pcDNA4-HisMax C eukaryotic expression plasmid. Positive clones were confirmed through RE digestions and sequencing. Select clones were then transfected into Chinese hamster ovary cells (CHO) and human embryonic kidney cells (HEK 293). Expression of full-length PD-1 was detected by western blotting using anti-His tag antibodies.

RESULTS: Restriction enzyme digestion followed by DNA sequencing confirmed that we had successfully cloned the full-length porcine PD-1 PCR product into the plasmid with a 5' His tag in the reading frame**.** The PD-1 protein expression was detected in transfected HEK 293 cells around the 40 kDa molecular weight (expected protein size is 37 kDa). We were not able to detect PD1 protein expression in CHO cells.

CONCLUSION and FUTURE DIRECTIONS: Full-length porcine PD-1 protein was successfully cloned from porcine PBMC and expressed in HEK293. Future studies will include purifying the porcine PD1 expressed by HEK 293 cells and assessing whether human anti-PD1 antibodies cross react with this porcine PD1. If they do react, we can use the anti-human PD1 antibodies to study checkpoint blockade in the porcine model of lymphoma. If they do not cross-react, we will develop novel porcine anti-PD1 antibodies to be used for such studies.

The NAB2-STAT6 gene fusion in solitary fibrous tumor can be reliably detected by anchored multiplexed PCR for targeted next-generation sequencing.

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ABSTRACT

BACKGROUND: Solitary fibrous tumor (SFT) is a mesenchymal tumor of fibroblastic origin, which can affect any region of the body. 10-15% of SFTs metastasize and metastatic tumors are uniformly lethal with no effective therapies. The behavior of SFT is difficult to predict based on morphology. Recently, an intrachromosomal gene fusion between NAB2 and STAT6 was identified as the defining driving genetic event of SFT and different fusion types correlated with tumor histology and behavior. Due to the proximity of NAB2 and STAT6 on chromosome 12, this fusion may be missed by fluorescence in-situ hybridization. We evaluated 12 SFTs from 10 patients. All tumors showed strong nuclear staining for STAT6 by immunohistochemistry (IHC).

HYPOTHESIS: An NGS-based sequencing assay can be used to reliably detect *NAB2-STAT6* gene fusions in SFT cases to a single-base resolution.

METHODS: The same formalin-fixed, paraffin-embedded blocks for IHC were used for gene fusion detection by a next-generation sequencing (NGS)-based assay. Targeted RNA fusion sequencing for gene fusions was performed using the Universal RNA Fusion Detection Kit, the Archer(™) FusionPlex(™) Sarcoma Panel and the Ion Torrent PGM, and data were analyzed using the Archer Analysis Pipeline 3.3.

RESULTS: All tumors were positive for NAB2-STAT6 fusion. Six types of fusions were detected: NAB2ex4-STAT6ex2, NAB2ex2-STAT6ex5, NAB2ex6-STAT6ex16, NAB2ex6-STAT6ex17, NAB2ex3-STAT6ex18 and NAB2intron6-STAT6Ex17. The NGS findings were confirmed by RT-PCR followed by Sanger sequencing. No STAT6 fusion was detected in selected morphologic mimics of SFT. The assay also allows for detection of novel fusions and can detect NAB2-STAT6 fusions at a single-base resolution.

CONCLUSION: This NGS-based sequencing assay using the Archer Anchored multiplex PCR approach and Archer Fusion Plex sarcoma panel can reliably detect *NAB2-STAT6* fusions from FFPE tissue. The assay also allows for detection of novel fusions and can detect NAB2-STAT6 fusions at a single-base resolution.

Medical Student Mistreatment: Is Our Problem Really That Big?

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Purpose

Determine why there is a discrepancy in medical student mistreatment reporting rates between the Association of American Medical Colleges Graduation Questionnaire (GQ) and our institution's survey.

Background

Institutions are increasingly aware of student-reported rates of mistreatment. In recent years, the GQ changed its questions to more accurately quantify the rates of mistreatment. The GQ rates of mistreatment at our institution are higher than the national average, while the internal survey given at the end of each clerkship demonstrates low rates of mistreatment.

Methods

Institution specific GQ data about student-reported mistreatment from 2000–2016, and post-clerkship surveys given to every student in clinical clerkships from 2011-2016 were eligible for analysis. Rates and classification of mistreatment were analyzed for each survey.

Results

GC data reported a mean (95% Confidence Interval) mistreatment rate of 23.39% ((-)4.30%-51.09%) from 2000-2016 and 39.25% (18.80%-59.70%) from 2011-2016. Rate of mistreatment on the internal survey from 2011-2016 was 2.15% (1.32%-2.98%). Classification of mistreatment was comparable, with "public belittlement or embarrassment" being the majority of reports for both surveys.

Discussion

Rates of reported mistreatment increased over the observation period and are higher for the GQ survey than our internal survey. The GQ does not use a gateway question to screen for mistreatment, but rather asks all students about types of behavior to determine an overall rate. Our internal survey has a gateway question, which may contribute to lower reporting rates. To better identify rates of mistreatment, it may be beneficial for our institution to remove its gateway question.

Genetic Suppression of Lamin-Associated Muscular Dystrophy

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ABSTRACT

Background: Lamins are intermediate filaments that form a network that lines the inner membrane of the nuclear envelope. Lamins provide structural support for the nucleus and regulate gene expression. The human genome encodes two types of lamins, A-Type and B-type, and while similar in primary structure, they differ in function. A-type lamins are products of alternatively spliced transcripts of the *LMNA* gene. They are expressed during cell differentiation and possess tissue specific functions. In contrast, B-Type lamins are the products of the *LMNB1* and *LMNB2* genes and are ubiquitously expressed. Dominant mutations in the *LMNA* gene cause a collection of diseases, including lipodystrophy, cardiomyopathy, muscular dystrophy, neuropathy, and progeria (early onset aging). The focus of our research is muscular dystrophy. We have generated a Drosophila model of lamin-associated muscular dystrophy that recapitulates many of the disease phenotypes observed in humans. Our prior studies show that mutant lamins cause reduced motility of Drosophila larvae and pupal lethality due to loss of muscle function. Furthermore, microarray analysis of muscle RNA showed up-regulation of several classes of genes, especially those related the redox homeostasis. It is not known which of these up-regulated genes contribute to the muscle pathology. To address this issue, we investigated the effects of RNAi knockdown of these misregulated genes on viability.

Hypothesis: RNAi knockdown of genes up-regulated by mutant lamins will suppress lethality.

Methods:
Immunohistochemistry: *LMNA* mutations that cause muscular dystrophy have been modeled in Drosophila Lamin C.. To determine where the mutant lamins localized within muscle, larval body wall muscle was dissected, fixed and stained with antibodies to Drosophila Lamin C , then analyzed by confocal microscopy.

Genetic Suppression Analysis: Genetic analyses were performed to determine which genes up-regulated by mutant lamins contribute to the loss of muscle function. Drosophila stocks expressing RNAi transgenes against the up-regulated genes are publically available. We utilized the tissue-specific Gal4/UAS system to co-express wild type or mutant lamins along with an RNAi transgene in muscle and assayed for adult viability. Multiple RNAi constructs of each gene were utilized to verify that the rescue results were due to the RNAi expression, rather than off target effects resulting from the specific RNAi strain. Crosses without RNAi and a *Luciferase* RNAi transgene served as negative controls.

Results: Using confocal microscopy, we found point mutations that cause amino acid substitutions in each domain in the Lamin C protein (head, rod, and tail (possessing an Ig-fold) result in the mislocalization of Lamin C. Mutations that alter the same domain do not appear to have a similar phenotype. Each mutant appeared to have a unique mislocalization patthern, showing lamin C aggregation in both the nucleus and the cytoplasm.

RNAi knock-down of 30 different genes was tested for suppression of lethality. We found that RNAi transgenes corresponding to four different genes, (*Arc1, CncC, Cyp6a17 and* ILP5) gave rescue to adulthood. Negative control crosses had no surviving adults. In contrast, knock-down of *Arc1, CncC, Cyp6a17,* and *ILP5* gave 6%, 16%, 17%, and 8%, respectively.

Conclusions: Mutant lamins have distinct mislocalization patterns within muscle fibers. Knock-down of *Arc1, Cyp6a17, CncC,* and *ILP5* partially rescued lethality caused by expression of mutant lamin. These genes are in pathways involved in redox homeostatasis and insulin signaling. Thus, these pathways are novel targets for potential therapeutic interventions.

Identification of amyloid chaperones in early Xenopus development

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Background: An amyloid is the aggregation of protein with a stacked beta sheet conformation. Amyloid formation may be part of normal cellular processes and is reversible; disaggregases and chaperones establish an equilibrium between a protein's native state and aggregated amyloid state. If equilibrium cannot be achieved, amyloids can accumulate and form plaques leading to diseases like Alzheimer's and Huntington's disease. However, when equilibrium is properly regulated, amyloids can be beneficial to a cell. Having a supply of already translated protein stored as an amyloid enables the cell to respond more quickly than if it had to synthesize new protein. Forming amyloids may also serve to concentrate proteins locally and ensure their selective distribution during cell division. All these properties would be advantageous for rapidly dividing embryos in early development.

Hypothesis: Our hypothesis is that there are essential, maternally inherited proteins that shift between a non-active amyloid state to an active native state by the action of tissue specific expression of chaperones.

Methods: We used the model organism Xenopus Laevis to study amyloid chaperones in early development due to the large quantities and size of oocytes produced. The panel of amyloid chaperones and disaggregases that were studied included TCP1, ABCF2, DNAJA1, HSPA8, HSPA9, MDN1, NPM1, NPM3, ANP32e, and HSPA1A. These candidate genes were selected due to the results of proteomic analysis and their functions. A DNA oligonucleotide complementary to each chaperone gene's mRNA sequence was used individually to knockdown the gene's mRNA in oocytes. The knockdown efficacy was measured by isolating the injected oocyte's RNA, turning it into cDNA followed by quantitation by using the polymerase chain reaction (PCR). The reduction in the targeted gene was compared to changes in the level of a non-targeted maternal RNA. The band intensity difference between the knockdown and internal control was quantified using Image J software. To test if there is tissue specific expression of chaperone and disaggregase mRNA, whole mount in situ hybridization was utilized.

Results: A panel of putative amyloid chaperones was cloned from maternal mRNA. In trials to determine effective target sites for oligonucleotide mediated destruction of mRNA encoding chaperone proteins, sites for both NPM1 and ANP32e were identified. Whole mount in situ hybridization revealed that the maternal mRNA pools of TCP1, ABCF2, NPM1, and ANP32e were localized to the animal hemisphere, indicating that during early development ectodermal precursors have increased ability to synthesize these chaperones.

Conclusion/Discussion: The in situ hybridization study enabled us to conclude that there is tissue specific expression of chaperones which facilitate maternally inherited proteins to shift between a non-active amyloid state to an active native state. With the effective knockdown sequences for NPM1 and ANP32e, modified oligonucleotides can be developed to prolong the effect of a knockdown, and then the developmental effects of loss of chaperone can then be assessed.

Women's Attitudes, Knowledge and Experiences with Misoprostol for Self-Induced Abortion

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Abstract

Background: Misoprostol is a prostaglandin E1 analogue that is used by the American College of Obstetricians and Gynecologists as part of a drug regimen to induce first trimester abortions in the United States. It has also been used to medically manage non-viable pregnancy with mixed results. One of the increasingly common ways that women are self-inducing their abortions worldwide is with illicitly obtained misoprostol. Recently, the United States has seen a rise in women self-inducing their abortions due to growing anti-abortion legislature which increase the cost, distance traveled and time required to access abortion care.

Aims/Hypothesis: This pilot study aims to quantify the prevalence of misoprostol use for self-induced abortion in eastern Iowa as well as women's knowledge and attitudes towards the drug in order to better protect, serve, and advocate for patients seeking medical abortion.
We predict that women who are actively seeking a physician-aided abortion will have knowledge of the drug misoprostol and its use as an abortifacient. Furthermore, some of our participants may have tried to obtain and employ misoprostol without the help of a physician in an attempt to self-induce an abortion prior to seeking medical treatment.

Methods: For this cross-sectional study a survey was offered to all women seeking physician-aided abortions at the Planned Parenthood of the Heartland Clinics (Des Moines and Iowa City) and The Emma Goldman Clinic (Iowa City).

Results: 212 women have participated in this pilot study while seeking physician-aided abortions between June 2016 and August 2016 in eastern Iowa. 61.1% of study participants had knowledge of misoprostol before appearing in clinic for their scheduled abortion and 51.3% of these women had learned about misoprostol from the internet. 77.6% of participants looked online for general abortion information and options before appearing in clinic for their scheduled abortion and 32.2% of these women found information online about "abortion pills". Only 2.1% of participants reported previous attempts to purchase misoprostol before seeking a physician-assisted abortion. When women were surveyed about their opinions on misoprostol availability over the counter, 55.7% responded that safe medication for early abortion should be available without a physician's prescription and 51.4% of women stated that over the counter abortion care would improve their access to abortion.

Discussion: The majority of patients participating in this study had knowledge of misoprostol prior to their scheduled abortion procedure. Most of the women with prior knowledge of misoprostol stated that their information came from online sources. Additionally, less than 5% of participants indicated that they had tried to purchase misoprostol themselves, but more than 50% of patients felt that medical abortion should be available over the counter and that OTC abortion care would have increased their access to abortion.

Managing the High Incidence of Genital Pain and Pathology in the Male Prison Population with Telemedicine

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ABSTRACT

Background

Stress has been shown to exacerbate testicular pain and likely few personal situation are more stressful than incarceration. Our medical center manages all of the urologic complaints in the state prison population and we have anecdotally noted a large number of incarcerated men reporting genital pain and pathology (GPP). Iowa prisoners with non-emergent urologic complaints are initially evaluated with telemedicine before an in-person clinic visit.

Purpose of the Study

The purpose of the study was to evaluate trends in urologic complaints in our male prisoner population and to determine the incidence, management, and outcomes of GPP.

Methods

We retrospectively reviewed the medical records of all prisoners in the state of Iowa who were evaluated by the Department of Urology at UIHC via telemedicine from Jan 2007 – Jul 2014 after obtaining IRB approval. Patient records were evaluated for urologic complaints, diagnoses, initial tests and treatments, outcomes, and eventual need for surgical treatment. To determine the incidence of GPP, we queried the state of Iowa prison database for average numbers of inmates per year during study dates and compared to the frequency of prisoner visits for urologic complaints.

Results

There were 361 prisoners with urologic complaints during the study period (incidence 7 per 1000 prisoner years) of which 30% (n=110) were for GPP. Tests were ordered in 78% of men presenting with GPP on the telemedicine encounter (73% US/radiology, 15% labs, 6% other). Medication was prescribed in 25%. Clinic visit followed telemedicine visit 49% of the time, of which the initial telemedicine visit diagnosis was confirmed in 98%. Follow-up revealed that 73% had resolution of pain or stabilization of the GPP, 26% were lost to follow-up, and <1% had worsening of their GPP. Nine patients (8%) ultimately required surgery (4 hydrocelectomy, 2 varicocelectomy, 1 spermatocelectomy, 1 spermatic cord block, 1 scrotal exploration) and no patients were found to have testicular cancer.

Conclusions

GPP represented nearly a third of the urologic complaints in this population, all of which were benign with few requiring surgery. It appears that these conditions could be mostly managed with telemedicine and testing in local facilities without compromising quality of care, potentially reducing healthcare expenditure by both the prison and healthcare systems.

Novel viral vector variants for improved delivery to airway epithelial cells

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ABSTRACT

BACKGROUND: More than 2 decades after the discovery of the cystic fibrosis transmembrane conductance regulator (***CFTR***) gene, the utility of gene therapy for cystic fibrosis (**CF**) remains unknown. Delivering a normal copy of *CFTR* cDNA to sufficient epithelia restores electrolyte transport and mucosal host defenses and has the potential to correct CF lung disease. However, there is no perfect viral vector for gene delivery to the airways. Adding to the toolbox of vector delivery options is of widespread interest to the gene therapy field. In Dr. Sinn's laboratory, viral vector systems based on adenovirus serotype 5 (Ad5) and lentivirus are being investigated for *CFTR* delivery. In collaboration with Dr. Anja Ehrhardt from Witten/Herdecke University in Germany, we screened 13 additional adenoviral serotypes. Several of these serotypes are known to bind to different receptors than Ad5. Additionally, Dr. Sinn's laboratory has developed a lentiviral-based CFTR delivery vector pseudotyped with the GP64 glycoprotein from baculovirus. The virus has undergone directed evolution to produce three double mutants on the GP64 protein. The goals of this summer project were to: 1) compare the transduction efficiency of Ad5 against other adenovirus serotypes and 2) determine if an engineered a lentiviral-based vector has airway transducing efficiency greater than the current state-of-the-art technology.

HYPOTHESIS I: Adenovirus serotypes with apical receptors will have a higher transduction efficiency than Ad5.
HYPOTHESIS II: Lentiviral gene transfer efficiency can be increased by directed evolution of the envelope protein.

METHODS:

Experimental protocol #1. Adenovirus serotypes expressing the reporter genes GFP and nanoluciferase will be delivered either to the apical or basolateral surface of primary cultures of human airway epithelia (HAE) at a multiplicity of infection of 50. The volumes will be constant at 100 ul for all samples. Four hours later, the cells will be washed 3 times with 1xPBS. Twenty-four hours later, the GFP activity will be imaged. Two days later, cells will be lysed and nanoluciferase activity will be used to quantify reporter gene expression.

Experimental protocol #2. FIV-secreted luciferase pseudotyped with the double mutants and wild-type GP64 will be delivered to the apical surface of HAE at a multiplicity of infection of 0.3, 3, and 30. The volumes will be constant at 100 ul for all samples. Four hours later, the cells will be washed 3 times with 1xPBS. Three days later, washings will be collected and a luciferase assay will be used to quantify reporter gene expression.

Experimental protocol #3. Western blots of FIV-GFP pseudotyped with each mutant and wild-type control GP64 will be performed using antibodies against GP64 and the viral protein p24. The goal of this experiment is to verify that incorporation of envelope protein into the budding virions is equal for each mutant.

RESULTS: The adenovirus experiment revealed that no serotype transduced the apical surface better than the basolateral surface. However, serotypes 16, 37, and 69 outperformed serotype 5 in both GFP and nanoluciferase assays.

The lentivirus experiment demonstrated that the GP64 mutants increased viral transduction compared to wild type GP64. A western blot comparing the virons indicated that the GP64 mutants displayed the same amount or less protein per viron when compared to wild type.

CONCLUSION: Adenovirus serotypes other than Ad5 may be better suited for *CFTR* delivery. In addition, mutations to the lentivirus GP64 glycoprotein increase the viral transduction compared to wild type GP64 protein despite having less GP64 protein per virion. These data suggest that novel serotypes or evolved envelope glycoproteins are effective strategies to improve airway gene transfer.

Novel Gene for Lacrimo-Auriculo-Dento-Digital (LADD) Syndrome with Glaucoma

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BACKGROUND: Lacrimo-auriculo-dento-digital (LADD) syndrome is an autosomal dominant disorder displaying variable expression of multiple congenital anomalies. Abnormalities of the syndrome include hypoplasia or aplasia of the lacrimal and salivary systems causing abnormal tearing and dry mouth. Patients with LADD syndrome have external ear abnormalities characterized by low-set, cup-shaped auricles that may also be associated with hearing deficits. Dental anomalies include microdontia, hypodontia, and enamel dysplasia. Finally, patients with LADD syndrome may have a variety of distal limb and digit abnormalities. Prior genetic studies of LADD syndrome have identified disease-causing mutations in the *FGF10*, *FGFR2*, and *FGFR3* genes. We recently identified a 30-year old male patient that had typical features of LADD syndrome along with novel findings of extremely thin corneas and open angle glaucoma.

OBJECTIVE: To identify the genetic cause of a case of LADD syndrome that includes thin corneas and open angle glaucoma among its clinical features.

METHODS: Whole exome sequencing was performed on the LADD syndrome patient's DNA with the Agilent v5 kit (Santa Clara, CA) and an Illumina Hiseq2500 next-generation DNA sequencer (San Diego, CA) using the manufacturer's protocol. DNA variations in within the coding sequences or the canonical splicing sites of known LADD genes (*FGF10, FGFR2, FGFR3*) were identified and analyzed. Next, variations present in the rest of the exome were filtered and prioritized to identify those that altered the coding sequences or canonical splicing sites of known genes. Top candidate mutations were further evaluated investigating their prevalence in the public database curated by the Exome Aggregation Consortium (ExAC) and the pathogenicity of these candidate mutations was also estimated using the Blosum62 matrix, PolyPhen2 and SIFT analyses. Mutations were assessed for alteration of conserved protein sequences by constructing homology tables using the UCSC browser. Finally, a structural analysis of the effects of the top candidate mutation in the tumor protein 63 (*TP63*) was conducted by superimposing the mutation over the solved crystal structure.

RESULTS: This patient was found to have no deleterious mutations in the genes previously described to be associated with LADD syndrome, *FGF10, FGFR2*, and *FGFR3*. When the rest of the genome was analyzed with our filtering approach, the top candidate for causing LADD syndrome in our patient was an Arg343Trp mutation in the *TP63* gene. The pathogenicity of this *TP63* variant was confirmed by a series of additional analyses. First, this mutation is absent from the ExAC database. Second, analysis of this mutation with the Blosum62 matrix, PolyPhen2, and SIFT suggest it is pathogenic. Third, the amino acid altered by this mutation (arginine 343) is highly conserved in all species with orthologous genes. Finally, analysis of TP63's crystal structure showed that the Arg343Trp mutation causes a significant alteration in the structure of TP63's DNA binding domain.

CONCLUSIONS: We report a LADD syndrome patient that does not have mutations in previously identified disease-causing genes (*FGF10*, *FGFR2*, *FGFR3*). Our analysis provides strong evidence that the Arg343Trp mutation in *TP63* causes LADD syndrome in our patient and that *TP63* is the fourth gene for this condition. *TP63* is known to have key roles in the development and differentiation of the tissues affected by LADD syndrome. Moreover, *TP63* has been implicated in several other autosomal dominant syndromes, many of which share anomalies with LADD syndrome. Finally, *TP63* is highly enriched in cornea and shown to be necessary for maintaining corneal limbal stem cell populations. Together these data suggest that *TP63* is a novel LADD syndrome gene and may also influence corneal thickness and risk for open-angle glaucoma.

Detection of IIH Vision Loss: The Outer Periphery

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Background:

Idiopathic intracranial hypertension (IIH) is an optic neuropathy that leads to increased intracranial pressure resulting in papilledema (optic disc edema). While IIH can occur in children and men, it is primarily seen in obese women of childbearing age. If IIH is left untreated, it can lead to progressive visual field loss and blindness in about 5% of cases.[1] It is very important for IIH patients to have visual field testing done early so that this visual field loss and blindness can be prevented. Common practice of monitoring changes in the visual field is done using standard automated perimetry which focuses on the 24° in the center of a patient's visual field.[2] Even though the peripheral visual field contains more than 3x the territory of the central visual field, it is rarely included in automated visual field testing. Even though peripheral visual field testing is rarely performed, this area of vision is commonly utilized by people and could provide very valuable information to better detect disease and monitor progress of treatments for visual disorders.

Aims:

1. Collect central and peripheral visual field data of patients with IIH with automated static testing, to understand how the disease affects the patient's visual field.
2. Determine whether peripheral visual field testing can be useful for diagnosis and monitoring response to therapy.

Methods:

We tested one eye of 48 controls ages 18-79 with central and peripheral visual field tests using the Octopus 900 perimeter that was running the Open Perimetry Interface (OPI) in our lab. We also tested one eye of 15 IIH subjects ages 18-79 with Grade 0-1 optic disc edema the same two visual field examinations. To compare cases (mostly young women) to controls (ages spread from 20 to 70 years), we standardized all of our collected subject data to the age of 50 using linear regression. This was necessary because of the the loss in visual field sensitivity with age. We then separated the nasal and temporal visual field data to determine the effect IIH had on both individually, then plotted the eccentricity vs. sensitivity of both the control and IIH subjects. A Mann-Whitney Rank Sum Test was then performed to compare the best fit lines between the control and IIH data.

Results:

The data shows that there is a more rapid decrease in visual sensitivity as the eccentricity increases for IIH patients compare with the controls. In both the nasal and temporal areas of the visual field this trend was deemed to be significant with a Mann-Whitney Rank Sum analysis ($p<0.001$). We also found a greater rate of loss in the periphery compared with the central visual field.

Conclusion:

Even though all subjects showed increasing visual field loss with increased eccentricity, our finding is that visual loss significantly ($p<0.001$) increases with eccentricity more in IIH subjects than controls in both the nasal and temporal visual fields. This shows that visual field testing in the periphery is likely to show defects for IIH subjects before defects appear in the central visual field. As such, performing visual field testing in the periphery may be important to incorporate into the protocol for tracking visual field defects in IIH subjects.

Citations

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Volumetric tumor segmentation on magnetic resonance imaging (MRI) in patients with primary and secondary glioblastoma (GBM)

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BACKGROUND: GBM is the most common primary brain tumor in adults. GBM can be further classified as either primary (de novo) GBM or secondary GBM (histologic progression from diffuse or anaplastic astrocytoma). Primary GBM occurs more frequently among older patients (mean age 55 years) and carries with it a worse prognosis. Secondary GBM occurs more frequently among younger patients (mean age 40 years) and has a higher chemoradiation sensitivity and better prognosis. Isocitrate dehydrogenase 1 (IDH1) is a genetic marker of secondary GBM with a sensitivity of 80%. However, there has been no definite imaging findings reported that would allow differentiation between primary and secondary GBM. IDH1 is a genetic maker of secondary GBM, with a sensitivity of 80%. There have been no definite imaging findings reported that would allow differentiation between primary and secondary GBM.

HYPOTHESIS: The volume of enhancing solid portion and necrosis on MRI is correlated with worse prognosis and IDH1 negativity, and volume of the non-enhancing solid portion with a better prognosis and IDH1 positivity.

METHODS: We retrospectively reviewed 49 patients with GBM from 2013 to 2016 who had an MRI done before treatment and had IDH1 mutation testing. Total tumor volumes and segmented tumor volume (enhancing solid portion, non-enhancing solid portion, edema, cystic, and necrotic portion) will be measured in the GBM before treatment. The genetic marker (IDH1) on pathological specimens is investigated in order to characterize the histological heterogeneity (primary or secondary glioblastoma). The tumor volumes of primary and secondary GBM will be correlated with IDH1 genetic analysis, progression free interval after temozolomide-radiation therapy and overall survival. Progression-free interval is defined as the time between completion of temozolomide-radiation therapy and the onset of tumor progression. The primary endpoint of this study is the diagnosis of progression. The secondary endpoint will be a patient's death for the evaluation of overall survival following a patient's death (Kaplan-Meier survival curves after the treatment). MRIs were performed using 1.5 or 3T imagers (Aera, Avanto, Espree, and Skyra, Siemens Medical Solutions, Erlangen, Germany). Segmental volumetric analysis of GBM was performed using brain tumor suite software (Neurosphere, Olea Medical) on post-contrast 3D volumetric interpolated breath-hold examinations (VIBE) T1-weighted images (TR/TE of 6.03/2.25ms, matrix of 256x216); fast spin-echo axial fluid attenuated inversion recovery (FLAIR) images (TR/TI/TE of 9000/2500/110ms, matrix of 384 x 384). Statistical analyses were made using correlation methods (cox regression), non-parametric statistical tests (log-rank tests), and Kaplan-Meier survival curves.

RESULTS: There were 26 males and 23 women. There were 4 IDH1 positive and 45 IDH1 negative cases. The median age at diagnosis of all patients was 57 years. The median overall and progression free survivals were 12.7 (95% CI = 8.4-17.0) and 8.6 (95% CI = 4.5-12.7) months respectively. The median overall survival for individuals with the IDH1 mutation and without were 38.1 (95% CI = 27.3-41.5) and 16.7 (95% CI = 6.3-15.2) months respectively. The median overall survival for primary and secondary GBM were 16.4 (95% CI = 13.5-19.3) and 38.1 (95% CI = 21.3-55.0) months respectively. Hazard ratios (HR) for segmental volumes were: enhancing tumor (HR = 1.023, P = 0.167), non-enhancing tumor (HR = 0.954, P = 0.051), necrosis (HR = 1.020, P = 0.288), and edema (HR = 1.007, P = 0.201). The only combination producing a significant prognostic factor was the ratio of non-enhancing tumor to total tumor volume (HR = 0.039, P = 0.020).

CONCLUSIONS: The findings suggest MRI segmental volume measurements can be used to predict GBM prognosis. Segmental volume measurements were found to show some correlation although single regions were non-significant. Other findings were consistent with the results found in literature. Prognosis of patients with GBM is poor. Secondary GBM was found to have a significantly better prognosis than primary GBM. Future studies involve recruiting more GBM patients who have had IDH1 mutation testing, IDH1 mutation testing on past patients, and waiting for current patients to reach endpoints of interest.

Outcomes of oral (interferon-free) treatments for HCV in patients with decompensated cirrhosis

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Background: Chronic Hepatitis C Virus (HCV) infection affects 80-160 million people worldwide, is one of the leading causes of chronic liver disease and an important cause of cirrhosis and hepatocellular carcinoma. Recently, new all-oral, interferon-free treatments have been developed that achieve high rates of Sustained Virological Response (SVR) among those treated, which some studies have shown to also include patients with decompensated cirrhosis. Decompensated liver cirrhosis is manifested in a variety of clinical presentations that can be evaluated through clinical lab tests, as well as physical findings. Ascites, encephalopathy and variceal bleeding are key signs of decompensated liver, while clinical lab tests include AST/ALT levels, albumin and bilirubin levels, and others. In a recent cohort study of the impact of Direct Acting Antiviral (DAA) therapy in patients with HCV and decompensated cirrhosis, MELD scores (based on clinical lab tests, i.e., bilirubin, creatinine, INR) improved in patients treated with all-oral, interferon-free regimens compared to those with decompensated cirrhosis who went untreated during the same time frame. However, it remains unknown whether ascites, variceal bleeding and/or encephalopathy resolve or improve following eradication of chronic HCV infection and whether overt clinical decompensation is reversible with virologic cure. The overall goal of this study is to address this question and to identify predictors of reversibility of decompensation after successful HCV treatment.

Hypothesis: Patients with decompensated cirrhosis who are cured of their HCV infection with DAA treatment will demonstrate improvements in liver function, clinical manifestations of decompensation, and decreased referral for liver transplant evaluation.

Methods: This was a retrospective analysis of patients with decompensated liver cirrhosis successfully treated for HCV using interferon-free DAA regimens at the University of Iowa Hospitals and Clinics (UIHC). Patients proven to have an SVR and a minimum of 6 months follow-up after the completion of antiviral treatment were included. Clinical indicators of liver decompensation were compared before and after SVR in order to determine effects on clinical and laboratory parameters of liver function. Parameters included AST/ALT levels, albumin levels, bilirubin levels, WBC, hemoglobin, platelet count, total cholesterol, PT/INR, creatinine, transferrin saturation, ferritin, and alpha-fetoprotein as well as the frequency of admission for encephalopathy and variceal bleeding. In patients with ascites, data was collected regarding changes in diuretics dosage, frequency of paracentesis and/or need for interventions such as transjugular portosystemic shunt for control of ascites. Patients were included irrespective of HCV genotype and DAA treatment regimens. Patients diagnosed with hepatocellular carcinoma during or after treatment were excluded from this analysis, as were patients with a history of liver transplantation.

Results/Discussion: Data collection still in progress. List of 904 patients treated with interferon-free DAA drugs between 12/1/2013 and 12/1/2015 were evaluated for signs of decompensation and subsequent SVR after treatment. Final number of patients to meet stated criteria was 25. Of these patients, 13 were reported as having mild/small ascites prior to transplant with 10 showing signs of improvement. Further analysis of improvement still required, including laboratory values of liver function and frequency of paracentesis. Patients from the Iowa City Veterans Hospital will also be evaluated and included to increase the power of the study.

A Single Cytoplasmic Lysine Residue is Sufficient for ENaC Regulation by the E3 Ubiquitin Ligase Nedd4-2

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BACKGROUND: The epithelial sodium channel (ENaC) plays a critical role in blood pressure control. ENaC is a channel that resides on the plasma membrane of epithelial cells in the collecting duct of the kidneys. Is composed of three homologous subunits, each with cytoplasmic N and C-terminal domains and extracellular loops. The N-terminal domains of each subunit contain lysine residues, which are substrates for ubiquitin. When ubiquitinated, ENaC is targeted for removal from the cell surface and subsequent degradation by the 26S proteasome or is recycled back to the cell surface. The E3-ubiquitin ligase Nedd4-2 catalyzes ubiquitination of intracellular lysine residues, which enhances ENaC endocytosis and degradation. Defects in ENaC regulation cause Liddle's syndrome, an inherited form of hypertension. The purpose of this study was to determine the individual role of lysine residues on each subunit, and to examine the extent to which they contribute to overall ENaC regulation by Nedd4-2.

HYPOTHESIS: Individual lysines independently contribute to ENaC regulation by Nedd4-2 and ubiquitin.

METHODS: To identify the lysines required for Nedd4-2 regulation, we mutated them to arginine and tested the effect of Nedd4-2 on ENaC cell surface expression and amiloride-sensitive current. HEK 293T cells were transfected with each ENaC subunit and increasing doses of Nedd4-2, cell surface proteins were labeled with biotin, and ENaC was detected via immunoblot. To examine ENaC current in the presence of increasing Nedd4-2, FRT cells were transfected as described above onto a polarized membrane. Short circuit Amiloride sensitive current was examined in an Ussing Chamber. Biochemical endocytosis and degradation assays were also performed in HEK cells.

RESULTS: Simultaneous mutation of all cytoplasmic lysines in α-, β-, and γENaC abolished the effect of Nedd4-2. Next, we analyzed ubiquitination of ENaC at the cell surface and found that only when all N-terminal lysines were mutated to arginine was ubiquitination nearly abolished. We then mutated γENaC lysines individually and in combination and identified differential roles for each. As a second strategy, we reinserted individual γENaC lysines in the context of an ENaC construct in which all N terminal lysines were mutated (αK- RβK-RγK-R) and analyzed the effect of Nedd4-2 on current and cell surface expression. A single N- terminal lysine residue at position 6 in γENaC was sufficient to reproduce the effects that Nedd4- 2 had on the wild type channel. Moreover, this single lysine was sufficient to induce both ENaC degradation and endocytosis. However, mutation of this lysine individually (γK6R) did not reduce the effect of Nedd4-2 on ENaC current.

CONCLUSIONS: Lysines on each ENaC subunit contribute to Nedd4-2 regulation. When all cytoplasmic lysines in ENaC are mutated to arginine, regulation by Nedd4-2 is abolished. A single lysine at position 6 in γ ENaC is sufficient for Nedd4-2 to reduce ENaC cell surface expression and ENaC current, though this lysine residue is not required for regulation by Nedd4-2. A single lysine at position 6 in γ ENaC is sufficient to induce ENaC endocytosis and degradation.

NEURAL CORRELATES OF DISPOSITIONAL MINDFULNESS IN OLDER ADULTS: A STRUCTURAL MRI STUDY

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ABSTRACT

BACKGROUND: Mindfulness is the state of being aware and attentive to the present moment without judgment. The topic of mindfulness has been an area of increasing interest to researchers and the public alike. To illustrate, a literature search of the PubMed database using the keyword "mindfulness" indicated that out of 3,585 total publications, 2,679 were published within the last five years alone. The interest in mindfulness is warranted as it has been found to have both mental and physical health benefits, which have important implications for clinical practice. For example, mindfulness has been identified as an effective intervention for a range of medical conditions, including depression, psychosis, post-traumatic stress disorder, cancer, multiple sclerosis, irritable bowel syndrome, and chronic pain conditions such as fibromyalgia. While neural systems involved in attentional control (cingulate cortex) as well as areas associated with self-awareness (insula) have been previously linked to mindfulness, several other brain areas have been identified as possible neural correlates. As such, there is no current consensus in the field. Additionally, virtually all of the prior mindfulness research has been conducted with younger adult samples, and there is little to no information on the older adult population.

AIMS: We sought to explore the neural correlates of dispositional mindfulness in older adults using a structural magnetic resonance imaging (MRI) of the brain approach. In addition, we took advantage of previously obtained psychometric data to investigate the relationship between mindfulness and various demographic, cognitive, and personality variables.

METHODS: Thirty healthy, community-dwelling older adults (*Mean* Age = 69.6; *SD* = 7.1; Range: 57-81 years) took part in the study. All participants were neurologically healthy, and medical status was confirmed with a semi-structured interview. Recruitment was based on MRI compatibility and completion of self-report measures. All participants had previously completed and passed a comprehensive battery of neuropsychological measures designed to exclude those with cognitive decline. Participants completed the Kentucky Inventory of Mindfulness Skills (KIMS) questionnaire to assess dispositional mindfulness during a single visit to the laboratory. On a separate visit, participants underwent structural imaging of the brain. In terms of approach, we first correlated dispositional mindfulness with existing neuropsychological data. Second, we correlated dispositional mindfulness with MRI-derived cortical thickness measurements.

RESULTS: 1) Interestingly, mindfulness was strongly correlated with several trait personality variables (e.g., conscientiousness, $r = .57$), while being relatively uncorrelated with demographic (e.g., education, $r = .12$) and cognitive (e.g., IQ, $r = .007$) variables. 2) In general, higher levels of mindfulness were associated with increased cortical thickness in several areas of the brain. Of particular interest, the right insula was highly and positively correlated with increased mindfulness. Several other brain areas were also implicated, including the cingulate gyrus, posterior cingulate, medial frontal gyrus, and parahippocampal gyrus (all $ps < .01$).

CONCLUSIONS: Our findings upheld the general notion that higher levels of mindfulness are associated with increased cortical thickness, even among a sample of older adults. Aging of the brain results in thinning of cortical regions as early as middle age; thus, the opportunity to influence this age-related process of cortical thinning is meaningful. Our finding of a thicker right insular cortex correlating with higher levels of mindfulness could reflect a greater capacity for the awareness of one's internal state, as the insula, particularly on the right side, plays a role in interoceptive awareness (defined as the sense of the physiological state of one's body). In terms of future directions, mindfulness as a clinical intervention has important implications for geriatric medicine by providing a non-pharmacological treatment option in a cohort that is commonly on multiple drug regimens. In addition, the issue of chronic pain, among both old and young, is a growing public health concern in the setting of the current opioid epidemic, and mindfulness has been shown to be effective across several clinical trials.

Proteomic approach to pathway analysis in chronic recurrent multifocal osteomyelitis

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Abstract

Background: Chronic Recurrent Multifocal Osteomyelitis (CRMO) is an autoinflammatory bone disease primarily occurring in children and characterized by chronic inflammation with negative cultures. Treatment is empiric, and there are safety concerns regarding the long term use of many of the medications in children. Although the precise cause of CRMO is unknown, the evidence suggests that the IL-1 pathway is critical in disease pathogenesis. A spontaneously occurring mouse model with symptoms similar to CRMO has been found and is called the cmo mouse. These mice have a spontaneous mutation in *Pstpip2,* which results in undetectable levels of protein in the cell. The mechanism as to how a defective PSTPIP2 protein leads to bone inflammation in cmo mice is not well understood. The protein is known to interact with PTPN12, PTPN18 (via the coil-coil domain), F-BAR protein-17, and actin, but little else is known about its interaction partners. The Ferguson lab has used a proteomic approach to suggest that TUBB6 (tubulin β, 6 class V) interacts with PSTPIP2. This protein is involved in microtubule function, which is the target of colchicine, a medication used to treat many autoinflammatory disorders.

Hypothesis: The proteins TUBB6 and PSTPIP2 interact in HEK293 cells.

Methods: HEK293 cells were incubated at 37°C and 5.0% CO_2 and grown in DMEM media mixture (500 mL DMEM, 50 mL fetal bovine serum, 5 mL penn/strep). The cells were transfected with either GFP, PSTPIP2-GFP, TUBB6, or PSTPIP2-GFP and TUBB6 using Polyfect Transfection Reagent. The cells were lysed using lysis buffer (2 mL NET 100, ¼ protease inhibitor tablets, and 20 μl 10% Tween 20). Immunoprecipitation was performed using Pierce Protein A/G Agarose beads and α-TUBB6 antibody with a FLAG tag. Laemmli sample buffer was used to release the beads. The western was loaded, with the first four wells containing the four cell lysis supernatants and the second four wells containing the four immunoprecipitation supernatants. The membrane was soaked in α-FLAG antibody as the primary antibody and α-mouse antibody as the secondary antibody.

Results: Of the wells from the cell lysis buffer, the wells with GFP and PSTPIP2-GFP did not show any results. The wells with TUBB6 and TUBB6+PSTPIP2-GFP had a positive result of about 50 kD**.** None of the wells containing the immunoprecipitation supernatant had any positive results.

Conclusion: The results failed to confirm the interaction of TUBB6 and PSTPIP2 in HEK293 cells.

Epileptic Seizure Activity Originating from Heschl's gyrus does not Disrupt Normal Electrophysiological Auditory Function

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BACKGROUND: Heschl's gyrus is part of the primary auditory cortex located in the superior temporal gyrus and is responsible for processing sound discrimination, volume and pitch. Recurrent epileptic seizures produce lesions that result in loss of brain function and electrophysiological dysfunction. However, epileptic seizures originating from primary auditory cortex are rare and effects of Heschl's gyrus epileptic activity on the electrophysiology of Heschl's gyrus are unknown.

HYPOTHESIS: Despite recorded electrophysiological recurrent seizure onset from Heschl's gyrus in primary auditory cortex, auditory function in the primary auditory cortex is preserved.

METHODS: We took advantage of an extraordinary clinical opportunity to study the auditory function in a research participant with medically intractable epilepsy while undergoing intracranial electrode monitoring for seizures. He was found to have an epileptic seizure focus in Heschl's gyrus after a three-week monitoring period. During this monitoring period, basic click mapping and multisyllabic word testing was performed to determine electrophysiological auditory function. Auditory electrode coverage consisted of electrodes recording from the superior, middle, and inferior temporal cortex and electrodes in the superior temporal plane including Heschl's gyrus. In addition to electrophysiological auditory tests, the patient received cranial nerve hearing and vestibular sense assessments and high-resolution brain imaging. *Basic Click Mapping:* Transient clicking noises were played to the patient while recording evoked potential responses in all intracranial electrodes to test for passive listening functionality. Evoked potentials were averaged over trials. *Multisyllabic Word Test*: The patient listened to a female speaker vocalize multisyllabic words (average duration per word: 0.7 s) that have overlap in the acoustic onsets or offsets of the words (e.g. "casket", "castle", "bristle", "brisket"). Intracranial electrodes recorded evoked potentials during listening.

RESULTS: A total of five electrographic seizures were recorded during intracranial monitoring all with onset in the Heschl's gyrus contacts. Seizure onset did not originate from any other site. Event related band power analyses of responses to clicks and multisyllabic words from right hemisphere electrodes in Heschl's gyrus, posterior planum temporale (overlapping into primary auditory cortex), and lateral superior temporal gyrus revealed high gamma band activity These auditory cortical responses are indicative of normal electrophysiological auditory functioning. All other electrodes were unresponsive. CN VIII assessment indicated normal hearing and vestibular senses. MRI showed no abnormal lesions and PET displayed normal metabolic uptake. Post-operatively, the CN VIII assessment demonstrated no hearing deficits.

CONCLUSIONS: Often, recurrent epileptic seizures contribute to cell death and abnormal neuronal pathways in the seizure focus area causing electrophysiological dysfunction and cognitive deficits. In this case, however, the patient presented no signs of electrophysiological nor auditory dysfunction despite having a detectable seizure focus and ongoing interictal discharges in Heschl's gyrus. This observation suggests that Heschl's gyrus and the primary auditory cortex in general may be more resilient to seizure activity. Further research in animal models and/or similar patient cases need to be studied to better define the resiliency of primary auditory cortex neural networks.

Mobile Phone Software Assessment of Pain and Opioid Use After Common Orthopedic Trauma Procedures

Alexander Volkmar BSE, Christopher Anthony MD, J. Lawrence Marsh MD, Matthew Karam MD, and Michael Willey MD, Apurva Shah MD MBA

BACKGROUND:

The ongoing opioid epidemic is a major problem in the United States. Prior authors have identified that unused opioid medication may contribute to the opioid addiction patterns of individuals the prescriptions were not intended for. Further complicating the issue, ineffective pain management after certain fracture patterns is associated with increased postoperative complications and decreased patient satisfaction scores.

Communication via mobile phone messaging interfaces has become increasingly common in the US over the last several decades, with cell phone ownership exceeding 90% in the adult population. Software algorithms can be used to directly contact mobile phones through traditional text messaging, social network instant messaging, and stand-alone messaging applications. Software driven mobile phone messaging platforms give healthcare providers a potentially cost-effective method to communicate with patients that requires relatively minimal use of healthcare resources compared to more traditional communication techniques.

PURPOSE:

The purpose of this investigation was to (1) evaluate the first 2 weeks of postoperative patient reported pain and opioid use after common orthopaedic trauma procedures; and (2) report results of communicating with postoperative orthopedic trauma patients utilizing an automated software and mobile phone messaging platform.

METHODS:

Fifteen adult patients who were capable of text messaging and were undergoing common orthopaedic trauma procedures (open reduction ankle fracture, open reduction tibial plateau fracture, operative fixation of hip fracture, intramedullary nail femur fracture, intramedullary nail tibia fracture) were enrolled in the study. Patients received a daily mobile phone message inquiring about their current pain level and amount of opioid medication they had taken in the past 24 hours from postoperative day (POD) 3 to POD 17. The following measurements were analyzed:

(1) Patient reported pain level (0-10 scale)
(2) Number and percentage of daily prescribed opioid medication patients reported taking
(3) Patient completion rate of mobile phone questionnaires

RESULTS:

Patient reported pain decreased over the initial two-week study period from an average of 4.22 on POD 3 to 2.44 on POD 16-17. Patients took an average of 70% of their prescribed narcotic on POD 3 compared to 13% of their prescribed pain medication on POD 16-17. Forty-six percent of patients reported no longer taking opioids by the end of the two-week study period. Patients responded to 88% of the pain and opioid medication inquiries they received over the two-week study period.

CONCLUSIONS:

Patient reported pain gradually downtrends from POD 3 to POD 17 after common orthopaedic trauma procedures. Patients report taking a small percentage of their prescribed opioid medication and this amount decreases consistently after discharge suggesting an opportunity for decreased amounts of prescribed opioid medication as well as altered prescribing regimens. We also find that in orthopedic trauma patients, an automated software and mobile phone messaging platform elicited a high patient response rate that improved upon prior methods in the literature.

Does Fluid Resuscitation Yield Increased Extravascular Lung Water in Emergency Department Patients?

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Background: Emergency department (ED) intravenous (IV) fluid resuscitation of sepsis patients is critical. However, fluid administration is a risk factor for the development of acute respiratory distress syndrome (ARDS), and sepsis complicated by ARDS has a mortality that exceeds 50%. This pilot study was conducted to better understand how IV fluids given in the ED influences extravascular lung water (EVLW) measured by lung ultrasound (LUS).

Hypothesis: IV fluids given in the ED increases EVLW measured by LUS, and the volume of fluid administered is proportional to increased EVLW.

Methods: A prospective observational study was conducted in a 65,000-visit Midwestern academic ED among patients receiving IV fluids for suspected hypovolemia. Participants underwent an 8-view standardized LUS examination before and 30-minutes after fluid administration. Sonographic B-lines have previously shown to predict EVLW, and a B-line score (BLS) is defined as the sum of the count of B-lines in an 8-view examination. The primary analysis (difference between pre- and post-fluid BLS) was performed with the paired t-test, and the secondary analysis (relationship between volume of fluid and change in BLS) was performed with linear regression. All statistical comparisons are reported with 2-tailed tests, and $p<0.05$ was considered statistically significant.

Results: Twenty patients (60% male) were recruited and had ultrasound scores before and after fluid administration. The mean fluid administered before the first examination was 360 ± 377 mL, and the fluid administered between the first and second examinations was 775 ± 323 mL. IV fluid administration seemed to be associated with increased number of sonographic B-lines (4.2 vs. 3.1 B-lines, $p=0.057$), although in this pilot study our finding did not reach statistical significance. Using linear regression, there was no relationship between the volume of fluid administered and the change in BLS ($p=0.459$).

Discussion: ED-based IV fluid administration seems to be associated with increased EVLW measured by sonographic B-lines, although with our small sample this did not reach statistical significance. In this pilot study, the change in BLS was not proportional to volume of fluid administered. Future research should focus on better titrating fluid administration to maximize organ perfusion while limiting pulmonary edema.

CMT1B: Transfection of RT4 Schwann cells with mutant Myelin Protein Zero DNA constructs induces quantifiable unfolded protein response

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Abstract

Background: Charcot-Marie-Tooth Disease (CMT) is one of the most common inherited, degenerative, peripheral neuropathy caused by irregularities in the myelin sheaths or neuronal axons. CMT type 1B (CMT1B) is dominantly inherited and caused by mutations in the myelin protein zero (*MPZ*) gene. This subtype of CMT is characterized by reduced motor nerve conduction velocities and segmental demyelination which lead to muscle weakness, atrophy, reduced sensation in distal limbs, foot deformities, and problems with balance and gait. In CMT1B, mutations in *MPZ* negatively impact the folding of MPZ – an abundant glycoprotein found in the peripheral myelin sheath, serving to enhance structural stability and integrity of peripheral nerve myelin. Misfolded MPZ can be retained in the endoplasmic reticulum (ER) of Schwann cells rather than properly transported to the myelin sheath after being processed by the Golgi apparatus. The aggregation of misfolded protein creates ER stress in the cell and activates the Unfolded Protein Response (UPR), a multi-cascade signaling pathway that functions to reduce the amount of misfolded protein by increasing protein degradation and attenuation of protein synthesis. This research will focus on identifying specific mutations of *MPZ* that activate the unfolded protein response in myelinating Schwann cells.

Hypothesis: Different mutations in myelin protein zero (MPZ) gene induce variable levels of unfolded protein response and protein retention as a result of aberrant protein trafficking in Schwann cells.

Methods: An RT4 Schwann cell line was stably transfected to contain a fused DNA sequence of Xbox-binding protein 1 (XBP1, a downstream product of the UPR signaling cascade), firefly-luciferase (fireflyluc), and nano-luciferase (nanoluc). This functions as a luminescence reporter system for UPR activation, as XBP1-fireflyluc-nanoluc will be produced instead of only XBP1. Wild type and mutant *MPZ* DNA constructs were generated by isolating total RNA from CMT1B patients and control skin biopsies. *MPZ* gene was amplified through RT-PCR. MPZ DNA sequence was ligated into pME-HA plasmid and transformed into E.Coli (E. Cloni 10G). MPZ cDNA was purified from colonies and transfected into the RT4 Schwann cell line using Lipofectamine 3000. Cells were lysed 24h post transfection procedure. Lysate was mixed with luciferase assay substrate to produce luminescence which was recorded by a Monolight 3010 luminometer. Fluorescence staining was performed on 48h transfected cells using antibodies to HA-tag, calnexin antibody to label ER, and giantin antibody to label Golgi apparatus.

Results: When normalizing to cell count, we observed that luminescence from mutant MPZ cDNA transfected cells was statistically greater than luminescence from wild type MPZ cDNA transfected cells in 21 of 26 (81%) of the generated mutant MPZ DNA constructs. Immunocytochemistry of transfected Schwann cells reveals increased retention of mutant MPZ protein in the endoplasmic reticulum than WT MPZ protein retention.

Conclusion: This experiment demonstrated a potential method of screening for unfolded protein response in Schwann cells of CMT1B patients. We were also able to relatively compare the degree of UPR response between different mutations in *MPZ*. Understanding the specific mutations that result in UPR and their impact on overall Schwann cell survival and viability in CMT1B patients will assist in development of future treatment options for CMT.

Racial/Ethnic Differences in Thyroid Cancer Incidence in the US: 2007-2013

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Background

Thyroid cancer incidence rates are projected to increase by 2030 from the 8th most common cancer currently to the 4th most common cancer. Trends show incidence rates stratified by race/ethnicity are highest for Whites followed by Asians, Hispanics, Native Americans, and Blacks. Past studies have argued that subclinical detection of thyroid carcinoma with ultrasound-guided fine-needle aspiration and computed tomography is the sole reason for increasing incidence, and that variation in race/ethnicity incidence is due to differing access to diagnostic technology, a difference stemming from socioeconomic and insurance coverage differences. Diagnosis of tumors less than 20mm, of papillary carcinoma histology, and of stage I carcinoma are affected by technology access; diagnosis of tumors greater than 20mm, of medullary, anaplastic, and follicular histology, and of stage II-IV carcinoma are much less affected by technology access. The purpose of this study is to evaluate age-adjusted incidence rates (AAIRs) by race/ethnicity across tumor and patient characteristics to determine their relationship to diagnostic technology access.

Methods

SEER 18 data were used to identify microscopically confirmed malignant thyroid carcinoma cases diagnosed from 2007-2013. SEER*Stat was used to calculate AAIRs and provide data for bivariate analysis that included gender, age at diagnosis, insurance coverage (uninsured, Medicaid, insured), histology (papillary, follicular, medullary), tumor size at diagnosis (0-40mm), and tumor stage at diagnosis (I- IV). Statistically significant 0.05 AAIRs per 100,000 person-years were obtained with adjustment to the 2000 US standard population using confidence intervals adjusted from the Tiwari modifications. SAS was used to develop logistic regression models to examine the association between race/ethnicity and tumor size and histology while controlling for other patients and tumor characteristics.

Results

Analysis included 80,658 cases. AAIRs varied significantly (<0.05) between race/ethnicity for all subcategories in histology, tumor size at diagnosis, tumor stage at diagnosis, and insurance coverage. Multivariate analysis found that after accounting other factors, race/ethnicity continued to be significantly associated (<0.0001) with tumors being of papillary carcinoma histology (compared to medullary, anaplastic, and follicular), and of tumors being less than 20mm at diagnosis (compared to greater than 20mm). After Whites, the greatest odds of having papillary carcinoma decreased from Asians (OR=1.4; 95% CI: 1.295, 1.529), Hispanics (OR=1.312; 95% CI:1.222, 1.409) and Blacks (OR: 0.685; 95% CI: 0.632, 0.744). In addition, Whites had the highest odds of having a small tumor size (<20mm) compared to all other races, whereas Blacks had the lowest odds of having a small tumor size (OR: 0.671; 95% CI: 0.631, 0.714).

Conclusion

These findings reveal that AAIRs vary by race/ethnicity in categories unaffected by and affected by ultrasound fine-needle aspiration and computed tomography, and argue against subclinical detection as the only cause of AAIR racial/ethnic variation. There are racial/ethnic differences between AAIRs and insurance coverage, which argues for further investigation into racial/ethnic risk factors for thyroid cancer. Furthermore, results suggest that Black race is associated with more advanced tumors compared to Whites, which may be indicative of poorer access to health care and/or delay of seeking treatment.

Development of Aortic Aneurysms in Angiotensin II-infused Methionine Sulfoxide Reductase-A Deficient Mice: Contributions of Blood Pressure and Sympathetic Tone

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BACKGROUND: Oxidative stress and inflammation associated with hypertension and aging are implicated in aortic aneurysm formation and rupture, an important clinical problem with substantial morbidity and mortality. Methionine sulfoxide reductase A (MsrA) confers protection against oxidative damage by reversing methionine oxidation. The Chapleau Lab reported previously that MsrA deficient (-/-) mice infused with angiotensin II (Ang II) for 4 weeks exhibit increased sympathetic tone, exaggerated hypertension, and aneurysms in the ascending aorta (dilatation) and abdominal aorta (Sabharwal et al., FASEB J, 2012). Furthermore, intracerebroventricular (ICV) infusion of the sympatho-inhibitory drug rilmenidine (RIL) for two weeks completely reversed the hypertension and ascending aortic dilatation in MsrA-/- mice (FASEB J, 2015). The aims of this study were to: (1) Compare and contrast the degree of dilatation in ascending vs. abdominal aorta in Ang II-infused MsrA-/- and C57BL6 mice in vivo, and define the relationship between aortic dilatation and blood pressure (BP); (2) Determine if systemic subcutaneous infusion of RIL at the same dose (2.5mg/kg/day) as administered ICV previously also reverses Ang II hypertension and aortic dilatation; and (3) Relate the changes in aortic diameter and BP measured in vivo to changes in diameter and histopathology measured in the same mice post-mortem.

HYPOTHESIS: (1) BP does not correlate well with changes in aortic diameter; and (2) Subcutaneous administration of RIL will elicit similar physiological effects compared to ICV infusion.

METHODS: Male C57BL6 and MsrA-/- mice (n = 35) were studied at 10-18 weeks of age. Groups of mice were infused with Ang II (1000ng/kg/min) for 4 weeks. Some of these mice were co-infused with RIL during the final 2 weeks. Untreated mice were also studied. Ang II and RIL were infused subcutaneously via osmotic mini-pumps (Alzet). Systolic BP (tail-cuff) and diameters of ascending and abdominal aorta (ultrasound) were measured in vivo, before and after drug treatments. Mice were euthanized by either isoflurane or carbon dioxide inhalation. The arterial vasculature was perfusion fixed. The diameter of ascending aorta, aortic arch, and abdominal aorta was measured at multiple defined locations and histopathological changes noted.

RESULTS: Ang II infusion increased systolic BP to a greater extent and more consistently in MsrA-/- compared with C57BL6 mice. Ascending aortic diameter also increased during Ang II infusion although the magnitude varied in individual mice. Interestingly, abdominal aortic diameter increased minimally or not at all in both genotypes. Correlations between changes in BP and aortic diameter were modest or poor. RIL infusion decreased BP minimally or not at all in both genotypes, which is very different than the marked decreases in BP observed in previous studies where RIL was infused ICV. Substantial variability in systolic BP within the same mice and between mice was observed and raises questions as to the reliability of tail-cuff measurements as performed in this study. Effects of RIL on the diameter of ascending and abdominal aorta in vivo and their relationships to post-mortem analyses are still being analyzed. Analyses of ascending aorta and aortic arch suggest that the presence of aneurysms is associated with lengthening of aorta in this region.

CONCLUSIONS: (1) The diameters of the ascending and abdominal aorta are differentially affected by Ang II infusion; (2) Changes in aortic diameter are not well correlated with changes in systolic BP during Ang II infusion in C57BL6 and MsrA-/- mice; and (3) The failure of subcutaneous infusion of RIL to lower BP in Ang II-infused mice suggests that ICV administration more effectively targets the central cardiovascular regulatory sites responsible for inhibiting sympathetic activity, despite the view that RIL freely crosses the blood brain barrier in either direction.

FOLLOW UP STUDY: PULSE WAVE VELOCITY AS A PREDICTOR FOR POSTOPERATIVE CARDIAC EVENTS

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Background: Vascular stiffness increases as a person ages, due to the repetitive stress that is put on the vascular system which causes changes in the elasticity of the vessel walls.[1] The increased stiffness of the arteries puts added stress on the circulatory system. This rise in stiffness has been shown to be associated with an increased risk of cardiovascular events, in both presumably healthy patients, as well as elderly patients.[2] The current method for assessing perioperative cardiac risk is the Goldman's Revised Cardiac Risk Index (RCRI). This method, however, does not include a direct measurement of arterial stiffness. Applanation tonometry is a non-invasive technique that has been shown to reliably provide indices of arterial stiffness.[1] While the use of applanation tonometry has been widely studied in general medicine, it is has not been studied for pre-operative risk assessment in surgical patients.

Purpose: The purpose of this investigation is to examine whether aortic stiffness is an independent risk factor for developing cardiovascular related adverse events in patients who are having major surgery under general anesthesia.

Methods: This study was conducted at the University of Iowa Hospital and Clinics and was approved by the University of Iowa Institutional Review Board. This study was conducted between June 2015 and August of 2016. A total of 100 patients who were having major surgery were enrolled. Patients' pulse wave velocity (PWV) was measured using a SphygmoCor in the Day of Surgery Clinic, before the patients went to surgery. Patients' medical history, intraoperative hemodynamics, and any postoperative complications were recorded to determine significant correlations and relationships. Data was analyzed using SPSS. The primary out was comparing PWV between the groups with or without measured cardiovascular adverse event postoperatiave within a week, univariately as well as risk adjusted with RCRI.

Results: A total of 139 patients were included for final analysis. In two patients, PWV was not able to obtained. 19 patients had cardiovascular adverse events within a week after surgery. The results of this preliminary study found average PWV was significantly higher in patients who developed postoperative cardiovascular events compared with patients who had uneventful postoperative course. (9.8 ± 2.8 versus 8.7 ± 1.9, $p=0.024$) Average PWV was also an independent variable in risk adjusted analysis with RCRI for postoperative cardiovascular events. ($p= 0.04$, OR = 1.3 (95% CI 1.006 – 1.6)).

Discussion: These preliminary results suggest PWV could be an independent predictor of adverse cardiac events following major surgery. This simple non-invasive assessment of arterial stiffness, measured by PWV, can be easily performed for preoperative risk stratification. More subjects are needed for further analysis with other potential covariates.

1. *DeLoach, S. S., et al. (2008) Clinical Journal of the American Society of Nephrology 3(1):184-192.*
2. *Mattace-Raso, F. U., et al. (2006) Circulation 113(5):657-663.*

Role of transient receptor potential channels in Marfan syndrome-induced aortopathy and cardiomyopathy

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ABSTRACT

BACKGROUND: The primary cardiovascular presentation of Marfan syndrome (MFS) includes aortic aneurysm/dissection and cardiomyopathy. Advances in studying TGF-β and AT1R signaling have led to promising therapeutic targets for the treatment of aortopathy; however, clinical studies have tempered this optimism. In particular, these studies suggest additional signaling pathways that play a significant role in aortic disease progression. Furthermore, even less is known with respect to effectors involved in MFS-induced cardiomyopathy. Presently, the study of MFS-induced disease progression has been gravely hampered by a lack of an accelerated disease model. As a result, while transient receptor potential (TRP) channels have been implicated in vascular smooth muscle and cardiomyocyte pathologies, the investigation into their mechanistic roles has been virtually absent.

PURPSOSE: In these studies, we investigate the importance of TRP channels in the pathogenesis of MFS-induced aortopathy and cardiomyopathy by evaluating differential expression in a novel murine model of accelerated MFS etiology.

METHODS: B6.129 (Wild-type [WT]) and Fbn1C1039G/+ (MFS) mice were used to create accelerated aortic aneurysms and cardiomyopathy via subcutaneous osmotic mini-pump installation in 4 treatment groups: WT + 0.9% saline (vehicle); WT + angiotensin II (4.5mg/kg/day); MFS + vehicle; MFS + angiotensin II (4.5mg/kg/day) (accelerated group). Echocardiogram was utilized with flow analysis at day 1, day 14, and day 28 to assess cardiac function and dimensions, aortic insufficiency, and aortic dimensions. Mouse hearts and aortas were harvested at 28 days to assess by VVG staining and TRP channel differential expression by quantitative PCR, RNA sequencing, and western blotting.

RESULTS: Aortic root diameter in the accelerated MFS model demonstrated significantly enlargement leading to an elevated degree of elastin fragmentation. Dilated cardiomyopathy was demonstrated within 14 days, with 60% penetrance (based upon <80% ejection fraction and an indexed end-diastolic volume >1.75 (µl/g of body mass)) and correlated with histopathologic changes. Greater than 40% of the accelerated MFS mice with mild to no aortic insufficiency presented with an intrinsic dilated cardiomyopathy. None of the vehicle treated mice met the dilated cardiomyopathy criteria. The severity of aortopathy and cardiomyopathy was readily apparent with increased mortality in the accelerated MFS model (63% at 28 days versus 0% for non-accelerated MFS mice). Differential gene expression of TRP channels reflected these findings in aortic tissue through quantitative PCR revealing a 9.9-fold increase in TRPC4 when comparing the accelerated MFS model with WT+vehicle mice. In cardiac tissue, TRPC6 expression was enhanced in the accelerated model at the DNA (2-fold increase), RNA (1.7-fold increase) and protein (5-fold increase) level.

CONCLUSION: This new accelerated murine model creates consistent and accelerated MFS-induced aortic aneurysms and primary cardiomyopathies to aid in the expedited investigation of the disease. This study suggests a potential role for TRPC4 in MFS-induced aortic aneurysm formation and TRPC6 in MFS-related cardiomyopathy.

Posthumous assisted reproduction and patient perspectives of the consent process prior to embryo cryopreservation

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ABSTRACT

BACKGROUND: There are thousands of frozen embryos currently in storage in the United States with the potential to be stored for years. Because of this, there have been cases where a person who has frozen embryos in storage has died. Posthumous assisted reproduction (PAR) is a term used to describe assisted reproduction using the genetic material (gametes or embryos) of the deceased. This process has been subject to much ethical debate. Studies have shown that even with couples actively seeking fertility, 1 in 5 did not agree to PAR, and a small percentage believed PAR should be illegal. In order to help determine the wishes of the deceased, some in vitro fertilization (IVF) clinics have instituted a consent process prior to undergoing IVF on what to do with frozen embryos in the event of death. However, as these consent forms are signed prior to the IVF process and most couples are focused on getting pregnant, the fate of a cryopreserved embryo following an unexpected death is most likely not at the forefront of their minds. There is little known research about the consent process and if it is adequate in helping patients make the difficult decision of what they or their partner would want regarding disposition of frozen embryos and PAR if they died while their embryos were in storage.

OBJECTIVES: To determine what the most common chosen embryo disposition is on the required consent form signed prior to egg retrieval regarding possible choices for frozen embryo disposition in the event of unexpected death and to analyze differences in responses based on patient characteristics.

METHODS: We reviewed the charts of all patients who underwent egg retrieval and had at least 1 embryo to freeze at UIHC between January 1, 2011 and January 1, 2015. We collected data on the following variables: MRN, age, parity, gravidity, sex of partner, IVF outcome, and questions regarding disposition of embryos in the case of death of one or both partners. Options included discarding the embryo, donating to research, or direct or anonymous donation to a different IVF user.

RESULTS: 520 patient consent forms were analyzed, 11 excluded because scanned consent form was missing pages in EMR. 30 patients used donor oocytes, 6 used gestational carriers, 8 were single, 5 couples were homosexual, and 17 used donor sperm. The average number of embryos frozen per consent form was 6.21. In response to the question of disposition of embryos in the event of death of one partner, 95.2% chose to revert to surviving partner, 1.3% chose to discard, 0.6% chose to donate to research, 1.2% chose to donate to another couple. In the event of death of both partners, 66.9% chose to donate to another couple, 32.7% chose to discard. Data is currently being reviewed by the obstetrics and gynecology department statistician, set to be done by August 31st. Analysis includes looking for significant differences in consent form answers between women of differing gravidity, parity, ethnicity, age, single, homosexual, heterosexual, and differences depending on the use of donor vs. partner sperm, donor vs. partner oocytes, and if they inseminated/ICSI all oocytes or a limited number.

DISCUSSION: The overwhelming majority of patients chose to revert to the surviving partner in the event of one partner death, while in the event of both partners death 66.9% chose to donate while 32.7% chose to discard. Data review is in progress to assess differences in patient responses based on patient characteristics and final results will be included in the poster presentation. A second, qualitative arm of the study is also in progress involving one-on-one interviews with patients and partners (if applicable) to determine patient perspectives of the consent process and the disposition of frozen embryos in the event of death.

A Systematic Review of Intralesional Treatments for Keloids and Hypertrophic Scars

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BACKGROUND: Keloids and hypertrophic scars are dermatologic conditions that occur after trauma, surgery, or spontaneously develop. Individuals with these scars can undergo psychosocial stigmatization as well as physical impairments such as pain, pruritus, and restricted range of motion. There is no gold standard for the treatment of keloids and hypertrophic scars and clinicians often rely on prior experience to guide their treatment and management approach. Both keloids and hypertrophic scars are known to recur after surgical excision alone; however keloids are more likely to recur. Since the 1960s, intralesional corticosteroid injections of triamcinolone acetonide have shown success in treating pathological scars. Many other intralesional modalities have developed in recent years.

OBJECTIVE: The aim of this systematic review was to examine current intralesional therapies for keloids and hypetrophic scars.

METHODS: In July 2016, we searched PubMed MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), and EBSCO CINAHL for any randomized controlled trials and quasi-experiments (controlled clinical trials) comparing intralesional therapies for the treatment of keloids or hypertrophic scars with any other therapies, no treatment, or placebo. A search strategy based on Cochrane's Highly Sensitive Search Strategies yielded a total of 760 articles. After examination of titles and abstracts by two independent reviewers, 595 articles were excluded because they were not relevant. Disagreements were resolved by a third reviewer. Of the 165 articles remaining, 35 were included for data extraction for having met all inclusion criteria. We included full-text articles of randomized controlled trials and quasi-experiments involving at least 10 human participants, published in English, and that mentioned any intralesional intervention into existing keloids or hypertrophic scars. We excluded articles that solely discussed injection techniques. References of relevant reviews were screened for additional articles, but yielded no further papers for examination. Data were first extracted by one reviewer, then verified by a second reviewer. Standard data items on study characteristics, study participants, intervention arms, outcome measures, and results were captured. The Cochrane Collaboration tool for assessing risk of bias within individual studies was used to check for selection bias, performance bias, detection bias, attrition bias, reporting bias, and other issues.

RESULTS: We included 35 trials involving 1,531 participants, ranging from the ages of 3 to 81. Twenty-five of the trials were randomized controlled trials and the remaining ten were quasi-experiments (controlled clinical trials). Article publication dates ranged from 1977 to 2016 and spanned 18 countries, with most of the trials conducted in Asia (21 studies). Twenty-nine out of 35 of the trials involved triamcinolone acetonide either alone or in combination in comparison with another treatments or controls. Four studies showed that triamcinolone in combination with 5-fluorouracil, an antineoplastic agent, was more effective than treating with triamcinolone alone. Four other studies showed that intralesional cryotherapy was more effective than contact cryotherapy.

CONCLUSION: Many intralesional treatment options are available for patients. However, there is no consensus on one particular treatment. Results should be considered carefully as many of the papers included have high risk of bias either with randomization or blinding of participants. Some articles report conflicting results, thus more high-quality, randomized controlled trials are needed.

Exercise is associated with a decrease in brown adipose tissue temperature in mice

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Background/Introduction: Due to a high surface area to volume ratio, mice can easily and quickly lose heat in cold environments. Therefore, mice must use several compensatory measures to maintain a consistent core body temperature. Brown adipose tissue (BAT) can produce heat for the mouse by combusting glucose and lipids. Located in the interscapular region, BAT is an essential component of nonshivering thermogenesis. Low ambient temperature, feeding, and stress can all activate BAT. There have not been any studies looking into how BAT temperature regulation in mice responds to exercise. We sought out to see how both the core body temperature and BAT temperature change with exercise in mice. IPTT-300 transponders have been used to measure temperature in many studies but never during exercise.

Hypothesis: Temperature measured using IPTT-300 temperature probes of mouse BAT will decrease during exercise.

Methods: Temperature was measured in mice using IPTT-300 temperature transponders produced by Bio Medic Systems. A radio frequency reader system read the temperature from the transponder. Mice were anesthetized before using an injector to place transponders in three different locations: interscapular, midback, and intraperitoneal. Knowing that intraperitoneal transponders were closely measuring core temperature, we first looked to determine how the interscapular and midback temperatures compared to intraperitoneal. Each mouse was injected with an intraperitoneal transponder and either a midback or an interscapular transponder. Temperature was measured to determine the normal baseline temperature for both locations in each mouse. Next, the mice were exercised in a sprint protocol. Baseline temperatures were taken every 1 minute for 20 minutes while the mouse was placed on an unmoving treadmill. The treadmill was then started at 5 m/min with the speed increasing by 1 m/min every 1 min and temperature readings continued to be taken every 1 minute.

Results: The average baseline temperature for interscapular, intraperitoneal, and midback pellets were 38.59 ± .1611 °C, 37.75 ± .2111 °C, and 38.03 ± .1467 °C, respectively. A paired t-test showed that the interscapular temperature was significantly greater ($p = .0235$) than the intraperitoneal (core temperature). An ordinary one-way ANOVA showed that the midback and intraperitoneal temperature was not significantly different than the midback temperature ($p = .5604$). Therefore, the midback was used to represent core temperature since the intraperitoneal transponder could not be read during exercise. The sprint protocol data showed that at time $t = 0$ the interscapular started at 39.0 °C and the midback started at 38.5 °C. As the mouse exercised the interscapular temperature dropped to around 38.7 °C and the midback increased slightly to around 38.7 °C.

Conclusion/Discussion: Our baseline experiments showed that the interscapular transponder was likely located within the brown adipose tissue since the temperature was significantly higher. Also the midback transponder was used as a measure of core temperature during exercise. Our exercise data showed that exercise was associated with a decrease in brown adipose temperature by about .03 °C. Since brown adipose tissue increases core body temperature during normal physiology, it is likely that as a mouse's core temperature increases during exercise, brown adipose tissue is selectively downregulated. Further tests are needed to explore these results. We saw a significant increase in temperature during baseline temperature readings, likely due to a stress response from the physical proximity of the transponder reader. Eliminating the stress response as a potential confounder, potentially by using a wireless telemetry system, will further suggest a causal relationship.

Cementing Constrained Liners into Secure Cementless Shells: A Minimum 15 Year Follow-Up Study

Grant Young, M2,
Advisor: John Callaghan, MD

Introduction: With the success of cementless acetabular components obtaining durable long term boney fixation, the surgeon can be presented with the scenario of a well-fixed, well-positioned shell in a low demand patient at high risk for instability. The authors have previously reported short term (minimum two year) results using the technique of cementing constrained liners into well-fixed, well-positioned cementless acetabular components. The purpose of the present study is to report the minimum 15 year results using this technique.

Methods: Prior to 2001, 31 consecutive tripolar constrained acetabular liners were cemented into secure, well-positioned cementless acetabular shells at three institutions. Sixteen cases were performed for recurrent instability and fifteen for intraoperative instability. The average age at surgery was 72.1 years (range, 31 to 91 years). Patients were evaluated for need of revision for failure of the constrained liners as well as revision for any other reason.

Results: At minimum fifteen year follow-up, 21 patients (21 hips) had died and 9 patients (10 hips) were living. Five hips required a revision over the follow-up interval. Three were revised for failure of the constrained liner. In one case, the liner was cemented proud and it pulled out. It was re-cemented with no further dislocations. A second liner fractured at the capturing ring of the constrained device when the patient had a grand mal seizure. It was successfully treated with a second constrained liner. Finally, a third liner failed by liner loosening and was successfully treated with another constrained liner. None of these three hips required further surgery prior to their death or final follow-up. There were two additional revision, one for infection and one for femoral component loosening.

Discussion and Conclusion: In the difficult revision population, cementing a constrained liner into a cementless acetabular shell demonstrated durable results with 9.7% of hips revised at fifteen years for constrained liner failure, but all were salvaged with a second constrained liner. Only one other case was revised for loosening which was femoral component loosening.

Summary: In the revision population, cementing a constrained liner into a well-fixed and well-positioned cementless acetabular shell demonstrated durable results with 9.7% revised at fifteen years for constrained liner failure. All were salvaged with a second constrained liner.

The Role of Sonic Hedgehog in Neuromuscular Development and Vestibular Functioning

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Background: The *Sonic Hedgehog* (*Shh*) signaling pathway has been suggested to direct organogenesis and control cellular differentiation during development. Inhibition of the pathway during development is linked to holoprosencephaly, neural tube defects, and congenital central nervous system malformations. Children with these disorders exhibit epilepsy, facial deformities and motor impairments. Case studies also associate the occurrence of extraocular defects, such as involuntary ocular oscillations characteristic of nystagmus, with the aforementioned diseases suggesting the involvement of *Shh* inhibition. Whether there is a causal relationship between *Shh* signaling and proper extraocular and vestibular function is not yet established. Preliminary work in the Fritzsch lab with *Shh* knockout models demonstrates disruptions in vestibular function observed through reduced neurosensory development of the inner ear and subsequent motor deficits. In this study, we built on our previous findings using vismodegib, a selective *Shh* antagonist, to examine the neuromuscular development of the extraocular muscles as well as the reticulospinal tract governing locomotion and postural control.

Hypothesis: *Shh* inhibition by vismodegib results in the formation of a diminished ocular system in terms of the development of extraocular muscles (EOMs), namely reduced myofiber cross-sectional area and hypoinnervation of EOMs, resulting in involuntary oscillations of the eyes. This combined with aberrant development of the Mauthner Cell, the cell body of the reticulospinal tract (reduced axonal diameter and dendritic branching) results in reduced visual input and problems in generating finely controlled movements and maintaining balance.

Aims: This study served to establish the effects of *Shh* inhibition via vismodegib treatment in *Xenopus laevis* on EOMs and reticulospinal tract development in the following ways: cross-sectional area of EOMs (aim 1); innervation patterns of the EOMs (aim 2); axonal diameter and degree of dendritic branching in the cell body (Mauthner Cell) of the reticulospinal tract (aim 3).

Methods: *Xenopus laevis* oocytes were acquired through induced ovulation of female frogs with an injection of human chorionic gonadotropin. Fertilized oocytes developed at 16 ^{o}C to start gastrulation. Twelve gastrulae were treated with 6.25 µM vismodegib, a concentration previously determined to perturb vestibular functioning. Untreated embryos were kept at 16 ^{o}C until the onset of the swimming stage (stage 46). Animals were introduced to an apparatus that delivered a mechanical stimulus to the tadpoles sufficient to activate the C-startle reflex and used a high-speed camera to render real-time, high-resolution images of tadpole movements. The number of frames required to elicit the C-start response was calculated. Following behavioral testing, all animals were anesthetized in 0.2% Benzocaine and immersed in 4% paraformaldehyde. Six animals were anesthesized and Texas Red dextran amine crystals were placed in their spinal cords at the level of hindbrain-spinal cord junction for three hours to promote labeling of the Mauthner Cell, the cell body of the reticulospinal tract. The brains were then subsequently imaged using a Leica SP5 confocal microscope. Additional fixed animals were dissected and brains were immunostained with 3A10, an antibody that labels the Mauthner Cell to assess for changes in cell body number. Heads were immunostained with an antibody against acetylated tubulin to label all neurons and an antibody against myosin VI to label extraocular muscle fibers. Secondary antibodies with distinct fluorophores were used to recognize the primary antibodies. Following immunohistochemistry, all animals were imaged using a Leica SP5 confocal microscope. Extraocular muscle cross-sectional area and innervation for all animals was determined and measured via full 3D volume rendering of serial confocal microscopy sections of the eye using Amira software.

Results: We show that a reduction in *Shh* signaling via vismodegib treatment results in a reduction of cross-sectional area of the extraocular muscles ($p = 0.005$) and aberrant innervation of the extraocular muscles compared to controls. *Shh* signaling inhibition results in reduced axonal diameter ($p = 0.0001$) and dendritic branching ($p = 0.01$) of the Mauthner Cell. Behavioral analysis in treated animals showcase a statistically significant latency to generate the C-startle response compared to controls ($p = 0.01$), demonstrating deficits in vestibular functioning and motor behavior.

Conclusions and Discussion: This provides evidence that *Shh* inhibition results in impairments of the neuromuscular development of the eye as well as spinal tracts responsible for the generation of finely controlled movements. These eye and spinal cord deficits could be responsible for the motor dysfunction and loss of balance observed in patients with *Shh* mutations, demonstrating the need to develop interventional treatments for these patients to increase their sense of balance and motor coordination.

MRI incidence of synovial hypertrophy in patients read as having no pathology

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ABSTRACT

BACKGROUND: The clinical exam findings and radiographic criteria for describing the presence of excessive synovial tissue that can lead to chronic recalcitrant anterior knee pain have been proven in patients for whom pain relief was achieved with surgical excision. Up to this point in time, however, it has not been common practice to evaluate the status of the synovium in the search for MRI evidence of pathology. The purpose of this study was to apply established radiographic criteria in order to investigate the incidence of patients with knee pain obtaining a "normal" MRI opinion, yet actually having evidence of synovial hypertrophy.

HYPOTHESIS: A significant number of patients with "normal" MRI findings actually exhibit evidence of excessive synovial tissue to the point of causing mechanical impingement sufficient to potentially result in anterior knee pain. Furthermore, the synovium often is not considered in the typical radiographic evaluation.

METHODS: This study was a retrospective analysis of the records of patients with knee pain who were referred by primary care providers to the University of Iowa Hospitals and Clinics in order to be evaluated by MRI. We reviewed 4,291 records of patients who received Tesla 1.8 MRI knee imaging between January 29, 2009 and January 29, 2013. Of these, 67 patients met the study criteria and were evaluated for evidence on MRI of synovial hypertrophy. The evaluation was performed by reviewing sagittal and axial T2-weighted MR images for evidence of synovial impingement. The Sakakibara grading method was used to identify the severity of the medial synovial shelf, if present. Three investigators evaluated MR images independently. Finally, the 67 patients in the study population were stratified into groups based on their ultimate clinical or surgical diagnosis. The MRIs of an additional 39 patients who had previously been surgically proven to have symptomatic synovial hypertrophy served as a control group.

RESULTS: 64 out of 67, or 95.5%, of patients were reported as having "normal" or "unremarkable" findings on MRI, yet actually showed evidence of synovial hypertrophy. The remaining three cases represent the only instances in which a problem with the synovium was described in the radiology report. 85.1% of patients also displayed evidence of synovial tissue impingement. Ultimately, 13 patients were clinically diagnosed with synovial pathology and 11 of these had evidence of medial synovial-plical complex impingement. The distribution of Sakakibara grades of the medial synovial-plical shelf in these 11 patients were identical to the grades of the control group with surgically proven medial synovial hypertrophy *(p=0.4178)*. A difference was observed between the medial synovial-plical shelf Sakakibara grades of these 11 patients and those of patients who remained undiagnosed at the time of the study *(p=0.0316)*, as well as between those of patients who eventually were diagnosed with some other knee condition *(p=0.0317)*. Medial synovial hypertrophy was present in 62.69% of subjects; the presence on MRI of medial synovial-plical shelf impingement was predictive of an eventual diagnosis of synovial pathology *(p=0.0267)*. Furthermore, hypertrophy of the femoral synovium also was likely to be associated with impingement and a presentation of knee pain, and was found in 50.74% of patients.

CONCLUSION: The status of the synovium should be considered in diagnosing a patient who presents with anterior knee pain, especially when MRI reveals no alternative suggestion as to the cause of the symptoms. A correct early diagnosis on MRI can be made using the radiographic criteria established by Nieto et al on sagittal and axial views of T2-weighted images. Given the lack of early radiographic diagnoses made in patients clinically diagnosed with the presence of high-grade synovial hypertrophy, there is substantial benefit to be had from radiologic evaluation of the knee for features consistent with the clinical presentation of synovial hypertrophy when a patient is referred for knee pain.

A Case-Control Study of Nipple-Sparing versus Skin-Sparing Mastectomy with Immediate Reconstruction: The Iowa Experience

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Background

Immediate reconstruction at the time of mastectomy for breast cancer treatment or prevention has been performed for decades to improve cosmetic outcome and preserve self-esteem. Skin sparing mastectomy (SSM) is a well-established procedure in which the nipple with or without the skin over the tumor is removed and the remainder of the skin is preserved to facilitate immediate reconstruction. Multiple single institution studies and meta-analyses with long term follow up have established the oncologic safety of SSM, although there is an increased incidence of post-operative wound complications compared to simple mastectomy without reconstruction. Nipple sparing mastectomy (NSM) is a newer procedure in which no skin is removed during mastectomy. Preservation of the nipple is felt to be important for body image, and the literature supports that women have a higher level of satisfaction with NSM and are better able to cope with the experience of breast cancer and breast loss. However, it is technically more difficult and is associated with a theoretical increased risk of skin flap necrosis and other wound complications. Long term follow up is limited and published studies are restricted to high volume institutions. We introduced NSM for both breast cancer treatment and prophylactic surgery at the University of Iowa in 2011. In this study we sought to evaluate our surgical outcomes in the setting of a teaching hospital with a surgical residency.

Hypothesis

Our null hypothesis is that NSM with immediate reconstruction (case group) has equivalent short term outcomes to SSM with immediate reconstruction (control group) among patients at UIHC.

Methods

An IRB approved retrospective chart review was performed of all female patients who underwent mastectomy for either cancer treatment or prevention at UIHC between January 1, 2008 and January 1, 2016. Patient and tumor characteristics, type of mastectomy, indication for surgery, intra and perioperative details, complications and time to adjuvant therapy were all abstracted from the medical record. These variables were then compared between SSM and NSM groups. Pearson chi squared test and two sample t-test were used to evaluate associations.

Results

We identified 518 mastectomies within the study period: 135 simple mastectomies without reconstruction, ,256 SSM and 127 NSM. Compared to SSM patients, those with NSM were more likely to be younger (mean age 48.7y vs 44.8y, $p<0.0006$), have a lower BMI (28.3 vs 25.3, $p=0.0001$) and were less likely to have diabetes (8.4% vs 0.8%, $p = 0.004$). Mastectomy was performed for cancer treatment in 65.2% of SSM and 40% of NSM ($p<0.001$). The recorded operating room time for mastectomy was significantly longer for NSM compared to SSM (mean 173+/-3 mins vs 163 +/- 3.3 mins, $p=0.0253$). There was no difference in the time required to perform reconstruction for either procedure (mean 207.6 +/- 8mins vs 200+/- 9.7 mins, $p=0.5633$). Patients with NSM were more likely to have a single stage reconstruction with a breast implant or flap compared to SSM patients (51.9% vs 42.6%, $p=0.002$). Intraoperative blood loss was higher for patients undergoing NSM (mean 245.8 +/-10cc vs 156+/-10cc, $p=0.0003$), and they were more likely to blood receive transfusion (8.6% vs 3.1%, $p=0.019$).). There was no significant difference in breast infection rate (9.8% NSM vs 5.5% SSM), skin flap necrosis (20.5% NSM vs 12.9%SSM), post-operative hematoma (3.1% NSM vs 4.7%), seroma (9.4% NSM vs 14.5%SSM) or wound dehiscence (8.9% NSM vs 7.4%SSM) between the two groups. Of all patients with immediate reconstruction (SSM+NSM), 78% required at least one additional reconstructive operation. Patients with NSM were less likely to require more than two additional procedures (3.1% NSM vs 16.7% SSM; $p=0.004$).

Conclusion

NSM is associated with similar rates of reconstructive complications as SSM at our institution. However the operative procedure time, blood loss and transfusion requirements are increased compared to SSM. It will be important to examine how patient selection affects these outcomes, and how they change over time in the teaching setting. Additionally, in this study NSM was associated with fewer subsequent reconstructive operations. Assessment of cosmetic outcome from both the surgeon and patient perspective is important to clarify the costs and benefit of this procedure in comparison to SSM.

Peptide similarity between human and mouse proteins predicts polymorphism conservation

Eliot Zhu and Adam Dupuy

Introduction: Inbred mice are often used for preclinical testing and to study the pathogenesis of human diseases due to ethical and practical reasons. However, can inbred mouse models provide useful information for diseases that are influenced by complex genomic variation? After all, humans and mice are separated by nearly 30 million years of evolution, so human and mouse biology may be influenced by vastly different genomic forces. Here, we address the concern that genomic variation in humans and mice are vastly different through the integration and analysis of heterogeneous publically-available data.

Hypothesis: The more similar a mouse protein is to its human protein ortholog, the more likely missense single nucleotide polymorphisms (SNPs) in the mouse protein will be conserved in the human protein.

Results: 1**)** The more similar a mouse protein is to its human protein ortholog, the more likely SNPs in the mouse protein are conserved. 2) 30% of mouse SNPs in orthologous mouse and human proteins are conserved. 3) The observed frequency of allele changes (*e.g.* A to T, G to C) was not significantly different between intronic and conserved missense SNPs. 4) 25% of conserved SNPs are function-altering while 22% of un-conserved SNPs are function-altering.

Conclusion: 1) A large fraction of mouse SNPs are conserved in human proteins. 2) Conserved SNPs may be split into two populations: one that is conserved due to biochemical forces affecting homologous DNA sequences across species and another that is conserved due to evolutionary pressures. With regard to both points, it is important to note that the mouse variation database is built from twelve strains of inbred mice and the human variation database is built from millions of individuals.

Methods: To map a SNP in a mouse protein to its human counterpart, we obtained a twenty-one amino acid long peptide centered on the mouse SNP and aligned it to orthologous human proteins using a global alignment algorithm. Peptide identity was taken as the alignment identity. SNPs were classified as conserved or un-conserved based on amino acid changes. Furthermore, conserved SNPs were classified as exactly, strongly, or weakly conserved based on amino acid properties. Raw information on mouse and human SNPs were extracted from ASC1 flat files obtained from dbSNP build 141. Protein sequences were obtained from RefSeq release 77. Orthologous pairings between mouse and human genes were obtained from Mouse Genome Informatics. SNP functional status was obtained using the SIFT tool. The data was processed and analyzed using custom scripts written in Bash and R programming languages.