Does Cancer Center Volume Effect Overall Survival in Soft Tissue Sarcoma? A review of the National Cancer Data Base
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Abstract
Background:
Soft tissue sarcoma (STS) is a rare malignancy with an incidence of over 12,000 new cases in the United States annually. The optimal treatment of soft tissue sarcoma requires a multidisciplinary team of experts with experience in surgery, radiation, and systemic treatment. Although other cancers and conditions have shown superior outcomes in high volume centers, the relationship between institution volume and outcomes in sarcoma has yet to be defined.

Aims:
The purpose of this investigation is to determine the effect of hospital volume on treatment decisions (amputation or limb salvage, use of radiation), treatment results (positive margins, 30-day readmissions), and overall patient survival.

Methods:
The National Cancer Database (NCDB) was used to identify patients ≥ 18 years of age with non-metastatic STS of the extremity treated with surgery. We divided the cohort into high (institutions that treat >10 sarcoma patients annually) and low (institutions that treat <10 sarcoma patients annually) volume cancer centers. Propensity scores were determined based upon patient (age, sex, race, socioeconomic status, insurance) and tumor (grade, size, site) factors. Patients were then matched by propensity score and placed into two equal comparative groups of 2,380 patients each. Bivariate methods (chi square, t-test) were performed to determine univariate measures of association. A Cox proportional hazards analysis, controlling for hospital volume, patient, tumor, and treatment factors, was performed at 2, 5, and 10 years.

Results:
There was no significant difference in the rates of limb salvage surgeries at high-volume cancer centers compared to low-volume (92% vs. 92%, p =0.956). However, radiation treatment was used at a higher rate in high-volume cancer centers compared to low volume (55% vs. 51%, p =0.027). There was a lower incidence of positive margins in high-volume centers compared to low-volume centers (12% vs 17%, p<0.001). Although there was a slight increase in rates of 30-day readmissions for patients of high-volume cancer centers (4% vs. 3%), this difference was not statistically significant (p =0.113). In a multivariate Cox proportional hazards model, high-volume cancer centers demonstrated superior overall survival at all time points (hazard ratio for low-volume at 5 years = 1.31, 95%CI 1.16-1.47).

Conclusion:
High-volume cancer centers were associated with fewer positive margins and increased overall survival at 2, 5, and 10 years. Continued efforts should focus on optimizing the balance between patient access to specialty care and experience of the treating center
Novel Methodology to Visualize Mouse Choroidal Vessels

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**Background:** The choroid is a thin layer of vascular tissue that underlies and nourishes the retinal pigment epithelium (RPE) and outer retina. Age-related macular degeneration (AMD) is a leading cause of blindness among people age 50 and older. Excessive loss and/or thinning of choroidal endothelial cells occurs early in the pathogenesis of AMD, and that loss is associated with the formation of drusen, collections of lipid and protein that increases a patient’s risk for developing advanced vision-debilitating AMD. Current methods for visualizing the mouse choroidal vasculature require expensive and relatively inaccessible technology but also fail to image the choriocapillaris in detail. In this study, we describe an approach to reliably and rapidly visualize the choroid and choriocapillaris from whole mount in albino mice.

**Methods:** The albino mouse strain B6(CG)-TYRC-2J/J (Jackson Laboratories) were cardiac perfused within 30 minutes with either the mixture of Isolectin GS-IB4 Alex Fluo 647 and FITC conjugated Albumin or the mixture of specially formulated aqueous solution containing 1,1’-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) with FITC-Albumin. They were set for about 20 minutes before bilateral enucleation and fixing of the eyes in 4% fresh paraformaldehyde solution overnight at 4°C. The eyes were dissected to isolate the choroid. Fluorescence microscopy was performed to directly visualize the blood vessels. The choroid tissues were then washed and stained with alkaline phosphatase (BCIP/NBT) substrate stain to confirm vessel formation under bright field microscopy.

**Results:** Perfusion with DiI and FITC-Albumin directly labeled choroidal capillaries in mice without the need for adjunct staining. The intensity of fluorescence signal obtained from both rhodamine and FITC channels was robust and specific for choriocapillaris under high-power microscopic observation. Alkaline phosphatase substrate stain yielded visualization of mouse choroidal vessels in bright field microscopy at low-power magnification.

**Conclusion:** Due to the pathologic changes that occur in the choroid in AMD, having a reliable method to visualize these vessels in mice is an essential tool. This methodology provides a novel solution to visualize choroidal capillaries with fluorescence and bright field microscopy in a relatively quick and reliable manner.
LJA5: A Novel Population of Neurons in the Brainstem

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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) or saline to selectively activate the LJA5 dynorphin neurons during experimental sensory conditions. These experimental conditions included intradermal nape injections of histamine or radiant heat testing (Hargreaves) test of hind paws to test LJA5 effect on itch and thermal pain, respectively.

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.
Title: Synaptic Phenotyping of the Amygdala—pontine projection

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Background: The emotional significance of a stimuli or situation can modify our behaviors. In order to enable an appropriate behavioral or motor response, neural systems must be able to integrate incoming signals, including context and past experiences. For example, the constant stream of transient threats that are negotiated crossing a busy city intersection requires accelerated processing of the approaching threat stimuli to avoid harm. Two key brain areas relevant in executing the successful outcome are the amygdala and cerebellum. Using animal models, several of the neural pathways involved in the fear memory system and motor memory system have been identified using amygdala-mediated fear conditioning and cerebellum-mediated eyelink conditioning. Previous work has identified a projection from the amygdala central nucleus (CeA) to the basilar pontine nucleus (PN). It is hypothesized that this projection is critical to assigning sufficient amount of attention to a threatening stimulus. The goal of this study was to identify, with immunohistochemical methods, if the projection is inhibitory or excitatory.

Methods: Eight male Long-Evans rats underwent stereotaxic surgery for delivery of AAV5-hSyn-eYFP to the right CeA. After 3-week survival period, rats were perfused with a low/high pH buffered paraformaldehyde. Five one-in-five series of frozen coronal sections (30 μm-thick) were sliced on a sliding microtome and stored at -20°C in cryoprotectant until processing. Free-floating sections were incubated with three primary antibodies of vesicular transporter proteins (vGAT, GABA vesicular transporter; vGLUT1 and vGLUT2, two isoforms of the glutamate vesicular transporter) in 0.02 M KPBS containing 0.3% Triton X-100 and blocking goat serum for approximately 48 hours at 4°C. Sections were then mounted on subbed slides and cover-slipped with aqueous mount.

Results: Fluorescent microscopy of the PN revealed terminals from the medial division of the CeA (CeM) intermingled with dense vGAT labeling. vGLUT staining was absent in the in area of CeM afferents and was relatively light in the pontine as a whole. These results suggest that the nature of the CeA—PN projection is inhibitory. These results will help interpret future experiments using optogenetic inhibition or excitation of this pathway in classical conditioning experiments.
Correlation between Arterial Stiffness and Aortic-to-Radial Pressure Gradient after Cardiopulmonary Bypass

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Background
Development of an aortic-to-radial pressure gradient is not uncommon following cardiopulmonary bypass (CPB) during cardiac surgery. However, this can lead to misleading hemodynamic management, especially when a significant gradient develops. A recent study by Kanazawa, et al. suggested aortic stiffness might contribute to this phenomenon. In this study, patients that develop an aortic to radial pressure gradient after CPB have an increased aortic pulse wave velocity (PWV), compared with those that did not develop a gradient measured using an intraoperative invasive technique. The aim of the current study is to determine if non-invasive preoperative measurement of aortic stiffness measured by carotid-femoral PWV can be an independent predictor for developing an aortic-to-radial pressure gradient following CPB.

Methods
The study was approved by IRB. Patients between the ages of 40 and 80 undergoing elective coronary artery bypass grafting (CABG) surgery were eligible for enrollment. The electronic medical record was used to collect demographics as well as to identify study candidates. Once informed consent was obtained in the day of surgery admissions area (DOSA), the patient’s hemodynamic profile was assessed using a non-invasive cardiometer from Osypka Medical (La Jolla, California) and the patient’s PWV was measured using the SphygmoCor XCEL device (AtCor Medical, Sydney, Australia). Central aortic pressure was measured using either the antegrade cardioplegia cannula or the aortic cannula, radial pressure was assessed using a right arterial line, and cardiac output/index were assessed using the Swan-Ganz catheter with thermodilution technique. The hemodynamics including central aortic pressure, radial arterial pressure, central venous pressure and cardiac output were measured at: 1) prior to initiation of CPB and 2) 10 minutes after termination of CPB.

Results
Twenty-one patients were enrolled between the months of June and August of 2017. Three patients were excluded from analysis due to protocol violation. Of the 18 patients used for analysis, six developed a central to radial systolic pressure gradient of more than 20% after the discontinuation of cardiopulmonary bypass. The average PWV for the group that developed a gradient of more than 20% and the group that developed a gradient of less than 20% was 8.6 m/s ± 2.3 vs. 10.1 m/s ± 1.5 (p=0.07). The average augmentation index, adjusted to a HR of 75 bpm, for the group that developed a gradient of more than 20% and the group that developed a gradient of less than 20% was 34.2 mmHg ± 4.7 vs. 26.0 mmHg ± 5.0 (p=0.005).

Conclusions
The current pilot data showed the trend that patients who developed a central to radial systolic pressure gradient had lower PWV values than the patients who did not develop a pressure gradient after the use of cardiopulmonary bypass. On the other hand, patients who developed a central to radial systolic pressure gradient had higher AI than the patients who did not develop a pressure gradient. We hypothesize that the patients who showed low PWV (likely due to anti-hypertensive medications, such as ACE-inhibitors) needed higher resistant artery tone to maintain systemic blood pressure by increasing sympathetic tone, which could have resulted in increased AI. In those patients, general anesthesia and CPB could lower resistant artery tone by suppression of sympathetic tone, which induces the pressure gradient between central to radial blood pressure. Although this study did not achieve a statistically significant result in its primary outcome with the current sample size, expected further enrollment with a more varied range of PWV values will likely improve statistical power and significance with the trends we have seen thus far.

Planned Use of Long-Acting Reversible Postpartum Contraception in Low-Risk Women in Group versus Physician Prenatal Care

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Background

Despite spending more money on medical care than many other countries, pregnancy outcomes, measured in maternal and neonatal mortality, are much higher in the United States. Poor pregnancy outcomes are associated with short interval pregnancies, as mothers are at an increased risk for preterm delivery, low birth weight, and increased mortality. Effective postpartum contraception is necessary to help women delay pregnancy until an appropriate time. While contraception is initiated at the postpartum visit, it has been found that only 50% of women present for their visit 6 weeks following delivery. Given that ovulation can return within 27 days of delivery in non-breastfeeding women or in women who decrease breastfeeding episodes, contraception should be discussed and a plan should be in place.

Purpose

Group prenatal care has been suggested as a model that helps foster increased exposure and time for counseling on important issues, including breastfeeding, contraception, labor/delivery expectations, and nutrition. This is in addition to providing a social environment that fosters feelings of community and accountability. CenteringPregnancy (CP), a standardized method of group prenatal care, is available to low-risk women at the University of Iowa. It is not known if this method of prenatal care can improve postpartum long-acting reversible contraception (LARC) use.

Method

a) Potential subject identification

Potential subjects include (1) women that participated in CP at UIHC between October 2012 and April 2016 and (2) low-risk women who choose individual OB/GYN physician care. Low-risk women include women without chronic HTN, pre-gestational diabetes, significant fetal anomalies, and others.

b) Data Collection

Each subject’s EPIC chart will be reviewed and demographic information, pregnancy history, and prenatal complications will be recorded. Delivery course, obstetric complications, discharge contraception plans, and infant feeding method will be recorded, as well as compliance with the 6-8 week postpartum visit. Data will be stored via a database into REDCap, a secure web-based information system. Postpartum LARC use will be compared between the 2 cohorts as the primary outcome. A chi-squared test and multivariable cox regression analysis will be used.

Results

Of all variables collected for demographic information, only (1) admission into the antepartum service, (2) number of prenatal visits, and (3) number of patients who visited at least 15 times differed significantly between the 2 cohorts. 32.8% of women who were in CP were admitted to the antepartum service, compared to 6.4% who chose to see an individual OB/GYN. The median number of prenatal visits for the CP group was 14, compared to 12 for the OB/GYN group. 41.9% of women who were in CP had 15 or more prenatal visits, compared to 16.1% of women whose prenatal care was with a generalist.

In terms of pregnancy outcomes, there were no variables that showed statistical significance between the 2 groups. Given that the null hypothesis is rejected at 5% significance, LARC as primary contraception at discharge could show statistical significance with a higher n value (more data collected), as this variable had a p-value of 0.072.

Conclusion/Discussion

LARC use postpartum has been associated with improved health outcomes for mothers, as mothers are better able to space pregnancies. Many findings recommend introducing LARC during antenatal care so that full education of its benefits can be offered. Our study showed no statistically significant differences between mothers who received CP care versus physician care. It is likely that more data will need to be collected to confirm that there is no difference between both cohorts.
Pediatric Pelvic Floor Dysfunction: Beyond Biofeedback
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Problem
In pediatric urology, we evaluate a significant number of children with recurrent UTIs, urinary incontinence and constipation. In fact, these types of patients may comprise upwards of 40% of the patients evaluated in a pediatric urology clinic.\(^1\) Commonly we identify that a large portion of these patients suffer from pelvic floor dysfunction, causing their genitourinary and gastrointestinal issues. Traditionally, these patients have been only treated with biofeedback, to rehabilitate their pelvic floor dysfunction. However, at the University of Iowa we have used a combination of pelvic floor physical therapy and biofeedback to treat these patients. This treatment approach is unique to the University of Iowa and has been utilized in treatment of our patients for the last four years. To understand the effectiveness and durability of this unique therapy and also to compare it to traditional treatments, we propose this study.

Hypothesis
The combination of pelvic floor physical therapy is successful in treating pediatric pelvic floor dysfunction. In addition, this treatment approach is durable after discharge from therapy and is more successful, compared to traditional biofeedback alone.

Aims
1. To determine the success of pediatric pelvic floor physical therapy and biofeedback in the treatment of our patient cohort
2. To compare our treatment approach to traditional treatments

Background Information
Urinary tract infection is one of the most common childhood infections and can cause permanent renal cortical scarring leading to complications later in life, such as hypertension and chronic renal failure.\(^2,3\) Constipation has been correlated with increase in UTI. Biofeedback and Pelvic floor physical therapy have shown to improve voiding dysfunction.\(^4\) The most common cause of voiding dysfunction appears to be an inappropriate contraction or relaxation of pelvic floor muscles.\(^5\) Biofeedback has become an important tool allowing patients to be more aware of the pelvic floor musculature and how to appropriately use them.\(^6\) Common treatments for include various medication and increased fiber intake, but these often do not address the underlying problems in these patients. Biofeedback in combination with Physical Therapy has shown to be beneficial to adult patients with urinary incontinence. With the high prevalence of Urinary incontinence in pediatric urology, treatments must improve to limit the burden on patients as they grow older. Pelvic floor PT in combination with Biofeedback provides a noninvasive way of treating these patients.

Methods
We performed a retrospective review of all pediatric patients who received pelvic floor physical therapy/biofeedback for the treatment of pelvic floor dysfunction at the University of Iowa. Patient data was gathered from epic including a voiding dysfunction questionnaire and urine flow rate test (Uroflow) data. Data was then analyzed. For the questionnaire patients were asked 18 questions and their answers were given a numerical number from 0 to 4 based on the severity of symptoms.

Results
After analyzing the voiding dysfunction questionnaire an average reduction of 5.083 with 95% CI, p <0.0005 was seen following start of treatment. From 43 patients that had pre and post treatment data, 32 patients saw a decrease in severity of symptoms, 10 experienced an increase in symptoms and 1 was unchanged. The uroflow analysis showed decrease in flow time and post residual value, however these data points were not significant as the p value was >0.05. In pediatrics post void residuals (PVR) values greater than 20ml is considered abnormal \(^7\). Of the 39 patients with pre and post treatment uroflow data, 16 had normal post void residuals prior and post, 8 improved from abnormal to normal, while 6 went from normal to abnormal PVR. Nine patients remained unchanged with abnormal PVR.

Conclusion
Based on these results, we conclude that there is a correlation between pelvic floor physical therapy with biofeedback and the degree of pelvic floor dysfunction. From the time that patients receive treatment there a decrease in severity of voiding dysfunction symptoms.

Since this is a retrospective study there were many patients that were missing various data points. Uroflow data is also difficult to interpret, as patients often urinate prior to appointments providing inaccurate tests. Patients often tend to terminate treatment once symptoms cease. This makes obtaining uroflow data of improved patients difficult.

A prospective study will allow patients to be contacted to ascertain longevity of treatment. Data from the Genesis Pediatric Therapy Center was also not able to be obtained. This would provide further data to assess the actual effects of this treatment.

While the data was unable to conclude without doubt that physical therapy in conjunction with biofeedback is effective at treating pelvic floor dysfunction a follow up study with updated data on patient’s symptoms to day may convey the viability of this treatment.

References
Vascular Correlates of Mood Change
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Background
Cardiovascular risk, mediated by arterial stiffness and pro-inflammatory states, has been independently associated with mood disorders. A variety of vascular measurements appear to be impaired in those with mood disorders, especially with a longer and more persistent course of illness. Numerous studies have demonstrated excess pro-inflammatory markers in individuals with mood disorders, including TNF-α and hsCRP. These associations are largely based on cross-sectional data and appear to be more strongly associated with abnormal mood states, such as depression or mania; longitudinal studies are lacking. Therefore, the purpose of this study was to longitudinally investigate the impact of mood and changes in mood on inflammation and the health of blood vessels.

Hypothesis
Improvements in depressive symptoms would be correlated with improved vascular function and reductions in hsCRP, as well as TNF-α, but not serum lipids.

Methods
With three assessments spanning 8 weeks, we prospectively assessed mood, arterial stiffness, arterial pressure wave reflection, levels of inflammatory markers (hsCRP and TNF-α), and serum lipids in a preliminary cohort of 7 participants diagnosed with a mood disorder. The Young Mania Rating Scale and Montgomery Åsberg Rating Scale were used to assess manic and depressive symptoms, respectively. Aortic stiffness and arterial wave reflection were measured by carotid-femoral pulse wave velocity (PWV), and augmentation index (AIX), respectively. All participants were undergoing treatment from their providers.

Results
Participants (n=7) were a mean (SD) age of 38 (11) years old and had 16 (2) years of education. The sample’s identifying gender was 71% female and 28% male. Racial distribution was 86% white and 14% black with 14% of the participants reporting a Hispanic ethnicity. The majority of participants were employed or students (71%). Four participants (57%) had bipolar I disorder, one (14%) had bipolar II disorder, and two (29%) had major depressive disorder. To date, a total of 16 assessments have been made, 7 at baseline, 6 at two weeks, and 3 at 8 weeks. In a preliminary analysis, using linear mixed modeling of the longitudinal data, there were no significant associations between mood symptoms and pulse wave velocity, radial augmentation index, or hsCRP (TNF-α pending). Exploratory analyses revealed that manic symptoms were significantly associated with higher HDL-c (p<0.01) and depressive symptoms with lower HDL-c (p<0.01).

Conclusion
Re-analyses are planned following recruitment of a larger sample to investigate the impact of changes in mood on inflammation and vascular function. A review of the literature has shown that the relationship between mood and HDL-c has been investigated previously. Persons et al. 2017 monitored serum lipid changes following the onset of depressive symptoms in 2,538 postmenopausal women, and no association was found between depression and subsequent changes LDL-c, total cholesterol, HDL-c, or triglycerides. It is likely that our findings of a relationship between HDL-c and depressive symptoms is due to type I error.
Anatomical tracing of efferent projections from glutamatergic neurons surrounding the novel nucleus LJA in the ventrolateral pons

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**Background:** The pontine tegmentum contains a heterogeneous group of neurons with different neurotransmitters and functions. The A5 catecholamine group has been studied for its descending sympathetic innervation, but there are also glutamatergic neurons adjacent whose targets and functions are unknown.

**Aims:** Here we sought to isolate and characterize the projections of this population of glutamatergic neurons in the ventrolateral pons which are adjacent to the A5 nucleus. Previous studies using nonspecific techniques have not distinguished between projections from different cell types in this region, and this project accomplishes that using updated genetic techniques. We also hypothesized that the glutamatergic group will have distinct efferent projection patterns from A5 neurons.

**Methods:** We used contemporary conditional tracing techniques to trace the efferent projections specifically from the glutamatergic neurons surrounding adrenergic A5. Using Vglut2-Cre recombinase expressing mice and a Cre-dependent viral tracer, we injected the tracer into the mice’s brains just rostral to the A5 nucleus. Upon perfusion of the mice and slicing of the brains, the slices were mounted and imaged. The images of the brain slices containing axons were then analyzed and the densities of axonal projections to various nuclei were quantified.

**Results:** We were able to visualize robust expression of the injected tracer at the site of injection and see its projections to other nuclei throughout the brain. Some of the densest targets of the glutamatergic neurons included specific thalamic, hypothalamic, cortical, and spinal targets that are not shared with A5.

**Conclusions:** We identified efferent projection targets of a population of glutamatergic neurons in the ventrolateral pons, and did so in a cell-type specific manner. The analysis demonstrated that the glutamatergic neurons surrounding A5 have a different projection pattern. Based on the unique projection pattern, we assume that the glutamatergic neurons adjacent to A5 also have a different function.
Targeting mismatch repair in aggressive basal-like breast cancer
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Background:
Basal-like breast cancer (BLBC) is an aggressive subtype of breast cancer with poor median survival. The elevated rates of genomic instability in BLBC is thought to contribute to increased development of resistance to therapy, as evidenced by a number of failures of clinical trials for targeted therapy in BLBC. Recently, inhibitors for Poly-ADP-Ribose Polymerase (PARP), single-strand DNA repair proteins, have shown promise for familial BRCA1/2-mutant associated cancers.

We recently found that the DNA mismatch repair proteins, MSH2 and MSH6, are highly elevated across BLBC samples compared to other breast cancer subtypes. Among BLBC samples, the highest protein levels of MSH2 or 6 were associated with poor survival outcomes in BLBC. With the potential of a new targeted therapy for BLBC, we investigated the underlying drive in BLBC tumors to increase mismatch repair proteins and conducted preliminary animal studies using genomic editing.

Methods:
Using CRISPR/Cas9 genetic editing, we knocked out (KO) MSH2 and MSH6 alleles. We used these cells in xenografts, observing variations in tumorigenic capacity by comparing the KO cell lines with controls. We have paired out in vivo and in vitro work with computational approaches to address the role of the upregulation of MSH2/6 with patient samples from the Cancer Genome Atlas (TCGA) and our KO cell lines.

Results:
We found that BLBC samples with the highest levels of MSH2 or MSH6 protein, had elevated levels of genes in other DNA repair pathways. In our xenograft model, we found the genetic KO of MSH2 or MSH6 reduced the tumorigenic capacity of BLBC cell lines. Our pathway analysis of TCGA patient samples found an enrichment in of impaired immune function in MSH2high BLBC samples. Furthermore, we also found a reduction in CD8+ T cell and macrophage signatures in the same MSH2high BLBC samples. In our initial exploration of the underlying cause, we found reduced mutational frequencies in MSH2high BLBC samples; a trend that was also mirrored MSH2 KO cell lines.

Conclusions
Mismatch repair is an intriguing potential target for BLBC therapy and further work is warranted. We are currently screening compounds for MSH2/6 complex inhibition. We will evaluate these as monotherapies, in combination with current chemotherapeutic regimes, and as an adjuvant for immunotherapy.
Impact of IL-1 on secondary inflammation following traumatic brain injury in murine model
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Abstract

Background: Aside from the immediate risks of death following head injury, a growing body of evidence suggests that traumatic brain injury (TBI) also initiates a long-term neurodegenerative process that puts patients at risk for developing Alzheimer-like diseases. Neuroinflammation mediated by microglias and astrocytes may be a key driver in chronic neurodegeneration following TBI. Interleukin-1 (IL-1) is a pro-inflammatory cytokine central to the initiation of the cellular inflammatory response. IL-1 signals through the type 1 interleukin-1 receptor (IL-1RI).

Aim: Evaluate the impact of IL-1 on the CNS inflammatory response through the measurement of cytokine expression and glial activation and the use of IL-1RI deficient mice.

Methods: Lateral fluid percussion injury (FPI) model was adapted to mice to induce moderate TBI. FPI utilizes a fluid pressure pulse to directly injure exposed dura mater and underlying brain following a parasagittal craniectomy. C57BL/6J and global IL-1 receptor (IL-1RI) knockout (C57BL/6J background) mice were used. To evaluate the expression level of cytokines qPCR is used at 6hr, 24hr and 21 days following TBI. Analysis of activated astrocytes was done using staining of coronal sections of mouse brain tissue for GFAP in ImageJ.

Results: 24 hours following TBI, in the left parietal cortex (L PCX) there was a significant decrease in the pro-inflammatory cytokines IL-1β and IL-6 in the IL-1RI -/- FPI compared to the WT FPI. Similarly, there was a significant decrease in expression of the same pro-inflammatory cytokines in the brainstem for the IL-1RI deficient mice compared to the WT FPI. When comparing the IL-1RI -/- FPI to the WT sham, there was no significant difference in expression of IL-1β or IL-6 in either L PCX or brainstem.

Anti-inflammatory markers were also measured at 24 hours following TBI. In the L PCX there was a significant decrease in the expression of Arginase-1 in the IL-1R1 -/- FPI compared to the WT FPI and only WT FPI mice had greater expression than sham control subjects. In the same region, there was no significant difference of expression of TGF-β between IL-1R1 -/- FPI mice compared to WT FPI. In the L PCX there was a significant decrease in expression of Arg-1 in the IL-1R1 -/- FPI and WT mice compared to shams, but no difference between the 2 genotypes following FPI TGF-β expression in the brainstem was increased in both IL-1R1-/- and WT FPI mice compared to shams but there was no difference between the 2 genotypes. In separate GFAP staining analysis, in both the right and left hippocampus, only the WT FPI mice had a significant increase GFAP staining compared to WT sham controls. However, the difference between IL-1R1-/- and WT FPI mice in this region was not significant. In the L thalamus, both IL-1R1 -/- and WT FPI mice had a significant increase in GFAP staining when compared to the WT shams. When comparing the IL-1R1 -/- FPI mice and the WT FPI mice, there was no significant difference between groups. In the R thalamus, there was a significant increase in staining in either IL-1R1-/- or WT FPI mice above shams. In the L corpus callosum, both IL-1R1 -/- FPI and WT FPI had a significant increase compared to the WT shams, but no difference between the 2 genotypes. In the R corpus callosum, there was no significant difference between any of the groups.

Conclusion: In IL-1R1 -/- mice, there was a significant decrease of measured pro-inflammatory cytokines in both the brainstem and L PCX 24 hours following TBI when compared to their WT counterparts. Surprisingly, IL-1R1 -/- mice also had a significant decrease in one anti-inflammatory marker, arginase-1, in the L PCX. A second anti-inflammatory marker, TGF-β, was not different significantly between WT and IL-1R1-/- FPI mice. The GFAP analysis shows a mild difference in astrocyte staining in IL-1RI deficient mice. In the hippocampus only, IL-1RI-/- FPI mice had no increase above sham levels whereas WT FPI mice were increased above shams. In other regions, there was no difference in astrocyte staining 21 days following TBI amongst IL-1R1 -/- mice and their wild-type counterparts. Further study of the impact of IL-1 signaling on neuroinflammation following TBI in underway.
Glioblastomas (GBM) are the most common type of malignant primary brain tumor in the United States. They are highly aggressive cancers associated with a dismal median patient survival of only 14 months following surgical resection, radiation therapy, and chemotherapy. Thus, a better understanding of mechanisms driving GBM pathogenesis is needed to identify new therapeutic targets and develop improved treatments of this disease. RABL6A is a novel oncogenic GTPase whose expression is a marker of poor survival for pancreatic and breast cancer patients. It functionally interacts with several key regulators of GBM development and is required for the growth and survival of astrocytes, the progenitors of GBM. As such, the Quelle lab investigated the role of RABL6A in GBM. Immunohistochemical analyses of patient tumors showed RABL6A expression is markedly increased in GBM (Grade IV astrocytoma) compared to less aggressive anaplastic astrocytoma (Grade III astrocytoma), implying an important role for RABL6A in promoting GBM progression. In agreement, silencing RABL6A in two GBM cell lines, U87 and U251, caused significant cell death and cell cycle arrest. The arrested cells displayed hallmarks of defective mitosis, including centrosome amplification and multinucleation, which coincided with dysregulated expression of several mitotic kinases. Together, these findings showed RABL6A is essential for GBM cell proliferation and survival.

To examine RABL6A function in GBM tumors in vivo, the current study sought to develop GBM cell lines with inducible RABL6A knockdown. Lentiviral constructs expressing RABL6A short-hairpin RNAs (shRNAs) under a tetracycline-inducible promoter were co-transfected with helper virus plasmids into human embryonic kidney (HEK293T) cells to generate knockdown lentiviruses. U87 and U251 cells were infected with empty vector control and inducible RABL6A shRNA viruses, selected in puromycin, and expanded for cryopreservation and testing. To evaluate knockdown, selected cells were treated with vehicle or doxycycline for 5-7 days. Cells were harvested, counted and RABL6A knockdown evaluated by western blotting. A minimal effect of RABL6A knockdown was observed by cell counting and viability assays, which correlated with limited downregulation of RABL6A protein following treatment with doxycycline. We repeated the study using a new preparation of inducible knockdown viruses on the assumption that the titer of the initial viruses was low; however, similar results reflecting ineffective RABL6A silencing were obtained. As a comparative control, non-inducible RABL6A viruses were generated and found to significantly reduce RABL6A expression in U87 cells. Importantly, the effective knockdown of RABL6A in these cells coincided with pronounced multinucleation, mitotic arrest and cell death, as expected. Moving forward, it may be beneficial to isolate clones from the puromycin-resistant populations to identify cells that have robust inducible RABL6A downregulation and associated biological phenotype. Once identified, those cells will be used for intracranial injections in mice to form GBM tumors and test the in vivo effects of RABL6A loss on tumor initiation, maintenance and sensitivity to clinically relevant anticancer drugs.
Assessment of Risk Factors and Litigation on rates of Subsequent Injury in Patients with Work and Non-Work Related Foot and Ankle Injuries
Gabrielle Bui, Yubo Gao, Andrea Pearson, Sean Boarini, Natalie Glass, and Lawrence Marsh

ABSTRACT:

Introduction:
Orthopaedic surgery has a long history documenting an association between workers’ compensation (WC) and negative outcomes. Previous studies have investigated outcomes after lower extremity, back, or shoulder surgery, but information on the foot and ankle is lacking. Subsequent injury may occur at various locations in the body during the postoperative period, potentially causing further disability. In this study, we investigated the rates and locations of subsequent injuries in WC and non-WC patients following foot or ankle surgery.

Methods:
After obtaining approval from the Institutional Review Board, we included all WC patients with a foot or ankle surgery performed by a single surgeon at the University of Iowa from 2009-2015. The most common current procedural terminology (CPT) codes from the WC population were defined as CPT codes of interest. A retrospective chart review was performed on WC and non-WC patients with CPT codes of interest during the same timeframe. Subsequent injury was defined as a new injury at a different anatomical location within 2 months – 2 years after the initial surgery. Chi-square and two-tailed t-tests were used to determine factors associated with subsequent injuries in both groups.

Results:
WC patients with subsequent injuries were older than WC patients without subsequent injuries 48.78 ± 7.30 versus 41.58 ± 12.40, p=.0137. In a double blinded process, WC patients with subsequent injuries were more likely to have legal representation than WC patients without subsequent injuries 10 (76.92%) versus 16 (37.21%), p=.0240. In the non-WC population, there were more males in the group without subsequent injuries than there were in the group with subsequent injuries 65 (42.48%) versus 1 (8.33%), p=.0287. The WC population was more male and had higher rates of subsequent injury than the non-WC population 37 (66.07%) versus 66 (40%), p=.000726 and 13 (23.21%) versus 12 (7.27%), p=.0011, respectively. While there were no significant differences in locations of subsequent injury between the WC and non-WC group, the WC group reported more back pain 6 (46.15%) versus 1 (8.33%), p= .0730.

Discussion and Conclusions:
WC patients with subsequent injuries were older and more likely to have legal representation than WC patients without subsequent injuries. Many WC patients have physically demanding jobs that may put them at increased risk for subsequent injury. Legal representation in WC cases may introduce confounding variables, putting WC patients at higher risk of subsequent injury. In the non-WC population, gender may mediate differences in reporting of subsequent injuries. Overall, WC patients had significantly higher rates of subsequent injury and were more likely to have legal representation than non-WC patients. Hip, knee and contralateral foot or ankle were common areas of subsequent injury in both groups.
Telemedicine Improves Timeliness and Appropriateness of Antibiotics in Rural Emergency Departments

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Background: Severe sepsis continues to have rising incidence and high mortality, and timely and appropriate early therapy remains key to improving patient outcomes. Rural patients continue to suffer from delays in care and higher mortality than urban patients. Emergency department (ED)-based telemedicine has been associated with improved outcomes in stroke care and with providing improved access to specialty care in underserved areas. The role of telemedicine in augmenting rural sepsis care, however, has yet to be examined.

Hypothesis: Telemedicine consultation in rural EDs will improve antibiotic appropriateness. Our secondary hypothesis is that telemedicine consultation will increase adherence to Surviving Sepsis Campaign bundles.

Methods: This study was a matched cohort study of patients seen in 16 critical access hospital EDs served by a large ED-based telemedicine network in the upper Midwest between February 2016 and May 2017. Participant records were obtained from an enterprise electronic medical record serving all participating hospitals and were abstracted by a single research assistant blinded to telemedicine use using a standardized case report form. Participants were included if they met the definition for sepsis or septic shock using the Sepsis 3 definition in the initial ED, and elements and timing of care were recorded in both the initial ED and all subsequent hospitals for the first 6 hours of care, including fluid resuscitation, blood cultures, lactate levels, antibiotic administration timing and appropriateness. Demographics and severity of illness (APACHE-2) metrics were also recorded. Telemedicine use was determined based on whether participant records were also present in the telemedicine call log kept by the telemedicine hub. Non-telemedicine cases were matched to telemedicine cases by presenting hospital, to limit confounding by hospital. The primary outcome was antibiotic appropriateness, adjudicated by a clinical pharmacist blinded to telemedicine group allocation. Secondary outcomes included antibiotic timing, inter-hospital transfer rate, total length of hospital stay, and mortality. Outcomes were evaluated using generalized estimating equations, clustered on presenting hospital. Final models were adjusted for APACHE-2 scores, dichotomized by above and below the median score in the cohort.

Results: The charts of a total of 114 patients (38 telemedicine, 76 non-telemedicine) were included. The odds of receiving an appropriate antibiotic within 3 hours was not statistically significantly different in the telemedicine group (aOR 2.65, 95% CI: 0.99-7.09). Time to appropriate antibiotics was 53 minutes shorter in the telemedicine group (adjusted mean difference, 95% CI: -115.38, 9.01). The odds of transfer to another hospital was greater in the telemedicine group after initial presentation at a critical access hospital emergency department (aOR 7.79, 95% CI: 2.89-21.05). There was no significant difference in serum lactate testing, time to first lactate draw, initial blood culture draw rate and timing, mortality or hospital length of stay between the two groups.

Conclusion: While there was no statistically significant difference, the data suggest telemedicine consultation may be more associated with appropriate and timely antibiotic use. Odds of transfer to a tertiary hospital were also greater among sepsis telemedicine patients. Telemedicine is a promising avenue for improving quality of early sepsis ED care. Future work will better adjust for severity of illness and measuring heterogeneity of treatment effects in a larger cohort.
Sex differences in the neuroanatomical correlates of trait personality in older adults: A structural MRI study

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Abstract

Background: The five-factor model of personality (FFMP) is a descriptive model that characterizes a seemingly universal pattern of covariation among personality traits. The way different personality facets tend to covary suggests that only a few factors account for the variation in personality traits (e.g., people who are more talkative tend to experience more positive emotionality than people who are quiet). This model divides personality into five trait factors: Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness (also known as the “Big 5”). Trait personality shows consistency across cultures and stability over the lifespan. Beyond serving as a descriptive model, the FFMP is implicated in psychiatric disorders as well. Extraversion and neuroticism may contribute to mood and anxiety disorder predispositions throughout one’s lifespan (e.g., low extraversion, high neuroticism, and low conscientiousness are associated with depressive symptoms). To explain this model, some have posited and have found evidence that the variations in trait personality may reflect differences in brain morphology. However, few have explored the role of sex on this relationship. As both brain morphology and personality is known to differ between sexes, this omission may contribute to the often equivocal results found in the literature. Additionally, the neuroanatomical correlates of trait personality have rarely been investigated in older adults.

Aim: We sought to explore the effect of sex on the neuroanatomical correlates of the FFMP in older adults using a reliable, self-report personality trait instrument and structural magnetic resonance imaging (MRI) of the brain.

Methods: Forty-four community-dwelling older adults (N = 44 (50% female); Mean age = 74.57, SD = 5.83, range = 60-88 years) took part in the study. All participants were neurologically healthy, and medical status was confirmed with an interview. Psychiatric illness, current or previous, was also absent in the sample. All participants had previously completed and passed a comprehensive battery of neuropsychological measures designed to exclude those with cognitive decline. The FFMP was assessed using the NEO Personality Inventory-Revised. Brain morphology was assessed using 3 Tesla structural MRI and analyzed with FreeSurfer, a suite of tools for the analysis of cerebral cortical thickness. First, FFMP was correlated with brain cortical thickness while separating participants by sex (22 women and 22 men) and controlling for age. Regions of significance were then evaluated for sex differences using the Fisher z-transformation for correlations from independent samples.

Results: We found four regions of correlation between cortical thickness and the trait personality Openness (refers to people who are curious, flexible, imaginative, artistic, and unconventional), and one region between cortical thickness and the trait personality Agreeableness (refers to people who are trusting, cooperative, modest, and straightforward). Furthermore, four of these five brain regions were found to differ significantly between sexes: 1) for the left lateral orbitofrontal cortex and trait Openness, men displayed a thicker cortex than women (z = 2.95, p = .002); 2) for the left supramarginal gyrus and trait Openness, women displayed a thinner cortex than men (z = -2.45, p = .007); 3) for the right postcentral gyrus and trait Openness, women displayed a thinner cortex than men (z = -2.60, p = .005); 4) for the right inferior temporal gyrus and trait Agreeableness, women displayed a thicker cortex than men (z = 2.34, p = .01).

Conclusion: Our findings support the notion that differences in sex play a crucial role in the way personality traits relate to brain morphology. The findings underscore the importance of investigating sex differences in this research area.
Identification of a Putative Mutation in MSX1 as a Cause of Left Ventricular Noncompaction: Early Characterization of a Cardiac-Specific MSX1 Knockout Mouse Model

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Background:
We have identified a multigenerational family with left ventricular noncompaction (LVNC), arrhythmogenic right ventricular cardiomyopathy (ARVC), aortic calcification, and sudden cardiac death. Clinical genetic panels for ARVC and LVNC failed to identify variants in known causative genes. Whole exome sequencing identified a putative mutation, E135D, in muscle segment homeobox 1 (MSX1). This variant is located in a highly conserved region and has not been reported by the 1000 Genomes Project or NHLBI Exome Sequencing Project. MSX1 is expressed in the endocardium of the left ventricle, which is most affected in LVNC.

The primary role of MSX1 is as a transcription factor, but MSX1 also participates in protein-protein interactions. Several known cardiac-related functions of MSX1 include regulation of the expression of connexin 43, the primary gap junction in cardiac myocytes, and involvement in the endothelial to mesenchymal transition of the atrioventricular valves during cardiac development. MSX1 has also been reported to have an association with aortic valve calcification.

Objective:
To generate and characterize a cardiac-specific MSX1 knockout mouse line.

Methods:
Mice containing floxed alleles for the second exon of MSX1 were purchased from a commercial supplier (Jackson Labs). Floxed mice were crossed with mice expressing cre recombinase driven by an alpha myosin heavy chain promoter (Jackson Labs). Cardiac specificity of the knockout was confirmed by qRT-PCR (Sybr Green, ThermoFisher). Age- and littermate- matched knockout and control mice were subjected to electrocardiography (EKG) and echocardiography.

Results:
Generation of a cardiac-specific knockout line was confirmed. Haploinsufficient mice demonstrated a 50% reduction in cardiac MSX1 mRNA levels, while showing no difference in cardiac MSX2 mRNA levels. In addition, two instances of unexpected early death at 42 days of age in male homozygous knockout mice were noted.

EKG analysis showed no difference in the QRS complex duration or QT interval. However, mice demonstrated a shortening of the PR interval in homozygous knockout mice (n=10) compared to controls (n=11) (P = 0.04). Echocardiographic analysis of cardiac morphology is currently underway.

Conclusion:
The putative mutation in MSX1 may be causative for cardiac disease in this multigenerational family. A shortening of the PR interval was not expected. This may indicate a potential role for MSX1 in regulation of the cardiac conduction, potentially through previously-reported interactions with cardiac T-box transcription factors (Boogerd et al., Cardiovasc Res, 2008). Further in vitro studies will examine this in light of the putative MSX1 mutation. Further in vivo studies will use aged mice to elucidate the long-term effect of MSX1 knockout on cardiac function. Together, these studies will allow for further determination of the effect of MSX1 knockout in the heart, and may allow for further understanding of the contribution of MSX1 mutations to the development of LVNC.
Probing the Identity of Central Chemosensitive Neurons Responsible for Hypercapnic Ventilatory Response

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Abstract

Background: Blood pH is maintained within a narrow range of 7.35 - 7.45, of which the respiratory system is a major regulator. As blood pH decreases, the rate of respiration increases in order to remove additional acid in the form of CO₂, thus raising blood pH. It is known that a group of intrinsically chemosensitive neurons located in the ventral medulla of the brainstem are responsible for hypercapnic ventilatory response. However, the exact mechanism of this response remains elusive despite decades of research. Previous research has produced two competing theories. One group espouses glutamatergic neurons of the retrotrapezoid nucleus (RTN), known to increase firing rate in response to acidosis, as central chemoreceptors. Our group believes the central chemoreceptors are serotonergic neurons with their somata in the Raphe Nuclei, and that the RTN functions as a relay from serotonergic chemoreceptors to the Central Pattern Generator of respiratory output. This rotation project aims to determine RTN neuron response to decreases in pH when serotonin has been depleted using PCPA (p-chlorophenylalanine), an inhibitor of serotonin synthesis.

Hypothesis: Cultured RTN neurons are not intrinsically chemosensitive and will show no response, or greatly reduced response, to decreases in pH when serotonin is depleted via PCPA treatment.

Methods: RTN neurons possess the transcription factor Phox2b, co-expressed with tdTomato in this model. Neurons of the Facial Motor Nucleus are also Phox2b+, however they also express choline acetyltransferase conjugated to GFP. Thus, RTN neurons are tdTomato+ and GFP-. The RTN and surrounding regions of the brainstem were dissected out and the constituent neurons were cultured for a minimum of 21 days prior to patch clamp recording. PCPA treated neurons were treated at 3mM for 1-2 days prior to recording. Whole cell and perforated patch clamp recording in current clamp mode was utilized in assessing the firing rate of these neurons. Cultured RTN neurons were recorded in Ringer’s solution maintained at pH 7.4 by bubbling with 5% CO₂ - 95% O₂. pH was reduced while recording to 7.2 by bubbling with 9% CO₂ - 91% O₂. These changes were repeated three times for each neuron to verify pH responsiveness.

Results: Patch clamp recording recapitulated previous findings that cultured RTN neurons increase firing rate when pH is decreased from 7.4 to 7.2. This change in firing rate increase ranges from 2.5 fold to 6 fold, returning to baseline as pH is returned to 7.4. Data for PCPA treated neurons is pending.

Conclusion: The identity of central chemoreceptors responsible for hypercapnic ventilatory drive remains a contentious topic. Previous findings have been repeated and confirm that RTN neurons have increased firing rate in response to physiologically relevant pH decrease. This response does not, however, establish their intrinsic chemosensitivity. Without data from PCPA treated cultures, the question of their ability to intrinsically sense pH changes without serotonergic input remains unanswered by this project.
BACKGROUND: Children are often sedated before neuroimaging studies such as MRI to ensure a successful scan. Children that are not sedated are liable to fail an MRI scan because of movement or they may become unwilling to complete the scan. However, sedation is costly and there are risks associated with administering anesthesia. Additionally, there is evidence in recent studies that anesthesia may have a deleterious effect on developing brain structure and function in children. Therefore, it may be more desirable to obtain non-sedated MRI scans. To avoid movement and anxiety without sedation, children can be trained and desensitized to the MRI machine before the scan. Preparing children for the MRI with behavioral desensitization programs and commercial mock scanners has been proven to lead to a high success rate for completing MRI scans and obtaining usable images without motion.

OBJECTIVE: The objective of this study was to assess how variables such as age and past history with non-sedated MRI scans may indicate successful completion of non-sedated MRIs in children. This study was part of a larger study examining neuroanatomical changes in children with type 1 diabetes (T1D) in comparison to healthy controls.

METHODS: Forty two children (4-9.9 years of age), 28 with T1D and 14 age-matched non-diabetic controls, participated in a longitudinal study evaluating the effects of T1D on the brain. After the completion of the 18 month long study, the study was extended for 24 more months. Thirty-two children out of the same cohort, (21 with T1D and 11 age-matched non-diabetic controls) returned to complete the follow-up study approximately two years later. Nine children were unable or unwilling to return, including 6 with T1D and 3 age-matched non-diabetic controls. Eleven children (7-13.9 years) not part of the initial study were newly enrolled in the follow-up study as replacements (8 with T1D and 3 age-matched non-diabetic controls). Anatomical (MP-RAGE, DTI, T2-FLAIR) and functional (working memory, response inhibition, and resting state) scans were performed without sedation on a Siemens 3-T Tim Trio MRI scanner using the standard 12-channel Siemens head coil. Subjects were offered behavioral training and practice sessions in a mock MRI scanner before each scan that they could choose to participate in if they felt nervous or uncomfortable before the scan. The MRI scans were performed at baseline, 18 months, and approximately two years later at the baseline and subsequent final 24 month visit of the follow-up longitudinal study. During the first baseline and 18 month scans, only anatomical scans were performed while the participants watched a movie. At the follow-up baseline scan, anatomical and functional scans were performed, and the participants did not watch movies during the functional scans but were performing memory tasks. For the T1D participants, their blood glucose levels had to remain between 70 and 300 mg/dL for the duration of the scans. The subject’s medical records and the studies’ source documents were accessed to obtain relevant information such as cognitive testing results, MRI scan dates and acquisition forms, MRI scan result summaries, and clinic notes detailing the outcome of the MRI scans performed. The information was analyzed to ascertain any statistically meaningful relationships between MRI success rate and age, cognitive testing results, or past history of MRI scan attempts.

RESULTS: In total, 35 children with T1D and 17 age-matched non-diabetic control children participated in the study. All of the participants attempted at least one non-sedated MRI scan and were included in the analysis. Scans were considered usable if they would be deemed acceptable for high-resolution anatomical studies. In total, 148 scans were performed, and 33 of the scans were unsuccessful. Twenty-two of the scans were unsuccessful due to motion, 7 due to subject refusal to complete the scan, 2 due to MRI malfunction, and 2 due to the subjects having braces. Of the 52 children that attempted a non-sedated MRI scan, 19 failed at least one scan (11 with T1D and 8 controls). Of the 19 subjects that failed at least one MRI, 6 of those subjects failed at least 2 MRI attempts, and 4 of those went on to fail a third attempt. Participants with T1D were less likely to fail MRI attempts than the healthy control participants- 68.6% of the subjects with T1D did not fail any of the attempted scans, while only 52.9% of the healthy control subjects did not fail any attempted scans. Of the MRI scans attempted by children between the ages of 4 and 5.9 years, 40.7% were unsuccessful, but only 28.6% of the MRI scans attempted by children between the ages of 8 and 9.9 years were unsuccessful. Children in all the other age cohorts experienced MRI scan failure rates between 9.1% and 13.6%.

CONCLUSION: Young children, specifically those between the ages of 4 and 6, are less likely to successfully complete non-sedated MRI scans than older children. Most likely this is due to being uncomfortable separated from parents and in a strange, loud environment. Past successful or unsuccessful attempts of non-sedated MRI scans do not have a predictive value for future completion of non-sedated MRI scans. Children with T1D are more likely to successfully complete non-sedated MRI scans than healthy control children of the same age, most likely due to the fact that children with medical conditions may be more comfortable in hospital settings and with hospital equipment.
Factors affecting \( \beta hCG \) levels in early pregnancy following In Vitro Fertilization

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Abstract:
OBJECTIVE: To determine clinical characteristics that influence early maternal \( \beta - \) human chorionic gonadotropin (\( \beta hCG \)) levels in pregnancies conceived by In Vitro Fertilization (IVF). Since obesity is a known risk factor for subfertility and lower pregnancy rates after IVF, we were particularly interested in the effect of maternal body weight on the \( \beta hCG \) levels. We also investigated the predictive ability of the first \( \beta hCG \) measurement (\( \beta hCG1 \)), the second \( \beta hCG \) measurement (\( \beta hCG2 \)) and the difference between \( \beta hCG \) measurements in predicting pregnancy outcomes.

METHODS: This was a retrospective analysis of all pregnancies conceived after transfer of a single embryo in IVF cases at the University of Iowa from June 2004 to May 2017. All pregnancies had two early \( \beta hCG \) levels obtained. We first examined clinical factors affecting the maternal level of \( \beta hCG1 \) by using generalized linear mixed models in pregnancies that resulted in the birth of a child. We next assessed the ability of \( \beta hCG1 \), \( \beta hCG2 \) and the difference between measurements to predict the outcome of all pregnancies, dividing pregnancies into those that resulted in delivery and those that resulted in either a miscarriage or ectopic pregnancy. Finally, patients were categorized by weight (<150 lbs, 150-200 lbs, and >200 lbs) to assess the positive predictive value of a low \( \beta hCG1 \) level (<50 mIU/mL) for adverse pregnancy outcomes.

RESULTS: A total of 2,043 pregnancies from 1,494 patients were analyzed. Clinical factors that influenced the \( \beta hCG1 \) level included cycle type (fresh versus frozen embryo transfer), inner cell mass grade and body weight. We calculated that for every 10 lb increase there is a 4% reduction in \( \beta hCG \). For \( \beta hCG2 \), the same clinical factors were found to be significant. In the modeling of the difference between \( \beta hCG \) measurements, the most important clinical factors included the log of \( \beta hCG1 \) and the number of days between measurements. For each additional day between measurements the \( \beta hCG \) rose by 75%. Weight had no independent effect on the rate of increase of \( \beta hCG \).

The most important predictors for a successful pregnancy resulting in delivery included log of \( \beta hCG1 \) and the rate of increase of \( \beta hCG \).

The positive predictive value of a low \( \beta hCG \) in predicting an adverse pregnancy outcome (miscarriage or abortion) decreases as the body weight increases (<150 lbs, PPV 87%, 150-200 lbs, 78%, >200 lbs, 70%).

CONCLUSIONS: We found a number of factors that influence the \( \beta hCG1 \) level in women who have a successful delivery of a child, including transfer of a cryopreserved embryo, quality of the embryo based on the inner cell mass, and maternal weight. These factors, particularly the maternal weight, are useful when advising patients of the significance of a low \( \beta hCG \) level in early pregnancy. Furthermore, we speculate that, in naturally conceived pregnancies, low initial \( \beta hCG \) levels could contribute to the sub-fertility found in obese women.
Bispectral EEG as Alternative to CAM-ICU for Delirium Screening in Emergency Department

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Background: Delirium requires a complex cognitive assessment to detect, and it remains under-diagnosed. We evaluated the diagnostic characteristics of bispectral EEG (BSEEG) as a tool to detect diffuse slowing waves, a pathognomonic finding in delirium in the ED.

Methods: A prospective cohort of hospitalized patients from the academic emergency department were recruited. These patients were assessed with cognitive assessment by the Delirium Rating Scale (DRS) and Confusion Assessment Method (CAM)- ICU, and BSEEG (measured using a handheld EEG device). The outcome variable of interest was the presence of delirium determined by a Delirium Rating Scale (DRS) score of 18 or greater. Other data that was analyzed included the median age of patients and BSEEG ratio. BSEEG and the CAM-ICU were compared to the reference standard for delirium (DRS score over 18). Means with standard deviations (SD) and medians with interquartile ranges (IQR) were used to report continuous variables. Student’s t-tests were used to compare means, the Wilcoxon test was used to compare medians, and a Receiver Operator Curve (ROC) was generated to identify a cutoff for the BSEEG ratio to find the optimal sensitivity and specificity to predict a DRS score of 18 or greater.

Results: A total of 98 hospitalized patients from the ED were approached, 67 consented and 58 had all study data collected at the time of analysis. We identified six of the 58 patients with delirium (10.3%) based on DRS score. The median age for the non-delirious group was 47 (IQR 36-56), and was 77.5 (IQR 57.0-80) for the delirious group (p=0.006). The mean BSEEG Ratio for the non-delirious group was 1.28 (SD 0.16), and was 1.40 (SD 0.07) for the delirious group (p=0.0029). ROC analysis determined that an EEG ratio of 1.3925 was optimal, with a sensitivity of 83.3% (95% CI 53.5-100), a specificity of 82.7% (95% CI 72.4-93.0), a positive likelihood ratio of 4.82 (95% CI 2.41-9.63), and a negative likelihood ratio of 0.20 (95% CI 0.03-1.21). The area under the curve (AUC) for BSEEG was 0.795 (95% CI 0.630-0.960), and the AUC for the CAM-ICU was 0.699 (95% CI 0.529-0.869).

Conclusion: BSEEG performed reasonably well for detecting delirium when compared to the DRS. It may be a viable alternative for delirium assessment in the ED when cognitive assessment is not feasible. A larger sized study is necessary to examine the validity of our findings.
Role of CTLA4 Expression on Regulatory T Cells in Helminth-induced Suppression of T Helper 1 Inflammation

Jeffrey Daniels, BS; M Nedim Ince, MD

Background: Intestinal helminth colonization suppresses T helper 1 (Th1) inflammation in various disorders. Acute graft-versus-host disease (aGVHD) is a frequent and lethal complication of bone marrow transplantation (BMT) or hematopoietic stem cell transplantation (HSCT) and is a robust form of Th1 inflammation. Acute GVHD is suppressed by helminths in mice (Li et al. J Immunol 2015). Although BMT/HSCT provide cure to patients with blood and bone marrow disorders—such as leukemia, myeloma, and lymphoma—lethal GVHD worsens the outcome of the BMT/HSCT. The graft that is transplanted in both these procedures constitutes immunotherapy against tumor (graft-versus-tumor; GVT). Although the Th1 response associated with GVT is beneficial, same donor, or graft-cell derived Th1 reactivity – associated with GVHD - is toxic to the host.

Helminth-induced regulation of Th1 inflammation in GVHD is associated with in vivo expansion of an important group of leukocytes that are called regulatory T cells (Tregs). The main purpose of Tregs are to discriminate self from non-self while suppressing abnormal immune responses. Tregs can be stimulated by affecting the immune system in the GI tract, which is the prime location in the initiation and regulation of GVHD. GVHD is associated with a marked decrease in number of Tregs in the gut. Tregs constitutively express proteins such as CTLA4 that is critical to in vitro and in vivo function of these cells. In this study, we wanted to test part of our hypothesis that functional Tregs are critical to suppressing Th1 response in GVHD.

Hypothesis: Determine whether Treg-specific expression of CTLA4 is critical to helminth-induced suppression of Th1 response.

Methods: To delete CTLA4 expression specifically on Tregs, we utilized a Treg-specific Cre-lox system. In this system, Cre recombinase expression is driven by the promoter of Treg-specific gene, Foxp3. These mice are called Foxp3-IRES-Cre (FIC) and back-crossed to mice that carry floxed CTLA4 alleles. Cre-mediated recombination of flox sites in Tregs lead to Treg-specific hemizygous (fl/wt) or homozygous (fl/fl) deletion of CTLA4. Due to difficulty in raising homozygous (fl/fl) mice, during this summer project, I focused on helminth-induced suppression of Th1 response in FIC x CTLA4 fl/wt mice with Treg-specific and hemizygous deletion of CTLA4.

The intestine of the experimental group of mice was colonized with helminths by administration of third stage larvae (L3) of Heligmosomoides polygyrus bakeri (Hpb), a mouse nematode. Mice were sacrificed 21 days later. Their mesenteric lymph node (MLN) Th1 response was analyzed by interferon-γ (IFNγ) ELISA of supernatants from anti-CD3 and anti-CD28 stimulated cell cultures. Furthermore, helminth-induced alterations in Treg population were analyzed by flow cytometric analysis of Foxp3+ Treg percentages. For flow cytometry experiments, cells were stained with CD4 FITC, Foxp3 APC, and CD3 PE/Cy7.

Results: Although helminth-infection suppressed IFNγ production in mice with wild-type (WT) CTLA4 alleles (FIC Cre+ and CTLA4 wt/wt mice)(IFNγ in uninfected MLN cultures: 6.8±1.1 mg/ml vs. Hpb-infected cultures: 2.7±0.9 ng/ml; p<0.05), helminth-infection did not suppress IFNγ production in mice with Treg-specific and hemizygous deletion of CTLA4 (FIC Cre+ and CTLA4 fl/wt mice)(IFNγ uninfected MLN cultures: 5.7±0.7 mg/ml vs. Hpb-infected cultures: 10.5±3.3 ng/ml; p<0.05). Along with that, the percentage of Foxp3+ Tregs were not reduced after helminth infection (p>0.05 between uninfected and helminth-infected MLN cell Foxp3 percentage (n=2).

Conclusion: These experiments suggest that hemizygous deficiency of CTLA4 on Tregs is critical for helminth-induced suppression of Th1 response.
The Role of ATF4 in Protection Against Lipid-Induced Toxicity in Cultured Cardiomyocytes

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Abstract

BACKGROUND: Diabetic cardiomyopathy, a condition that is often characterized by cardiac fibrosis and increased apoptosis, is a major complication faced by \textasciitilde;30\% of diabetic patients. Although the exact pathophysiology is not fully understood, previous studies have suggested that dysregulation of cardiac autophagy, a self-degradative process that assists cells in the clearing of damaged proteins and organelles, contributes to disease development. Epidemiological studies have demonstrated a cardioprotective benefit of exercise, yet the mechanisms involved remain incompletely understood. Preliminary data has shown that cardiac autophagy is not only crucial for exercise, but that when it is impaired, diabetic cardiomyopathy progression is greatly increased. Further studies found that exercise training increased expression of known autophagy proteins, and that the Activating Transcription Factor 4 (ATF4) and its putative target Fibroblast Growth Factor 21 (FGF21) were transiently upregulated after an exercise bout. Chronic and robust upregulation of ATF4 has been associated with cardiac fibrosis and apoptosis in heart failure, but ATF4 has also been shown to induce select autophagy genes in response to amino acid deprivation. In addition, previous studies have indicated a protective role of FGF21 in the heart. Taken together, these findings suggest ATF4 may have an important cardioprotective role by regulating cardiac autophagy in parallel with FGF21.

HYPOTHESIS: Our hypothesis was two-fold. First, we predicted ATF4 overexpression would be cardioprotective against lipid-induced toxicity in cultured cardiomyocytes. Second, we predicted cardiac autophagy would be essential for the ATF4-mediated cardioprotection in parallel with FGF21 induction.

METHODS: H9C2 cardiomyocytes were infected with either Ad-GFP or Ad-ATF4-GFP for 36 hours. Subsequently, these cells were exposed to the following 6 hour-treatments: control (normal medium supplemented with BSA), control + chloroquine (CQ - 10\(\mu\)M), palmitate (200\(\mu\)M), palmitate (200\(\mu\)M) + CQ (10\(\mu\)M), oleate (200\(\mu\)M), oleate (200\(\mu\)M) + CQ (10\(\mu\)M). Each condition had separate sets of cells for protein analysis and mRNA analysis. Adding chloroquine allowed us to assess if autophagy was required for ATF4’s protective benefit. Cardiac autophagy gene expression analysis of several autophagy-related genes (Atg7, Beclin1, p62, LC3, GABARAP, ULK1, ULK2) and FGF21 was conducted using RT-qPCR. Autophagy flux was assessed by monitoring p62 and LC3-II/LC3-I via western blot. Lipid-induced toxicity was indirectly assessed via quantification of cleaved/full length Caspase 3 levels, which are indicative of apoptosis. Data was analyzed with ANOVA and Newman-Keuls post-hoc test and \(P < 0.05\) was considered statistically different.

RESULTS: Preliminary data showed ATF4 decreased apoptosis in control and oleate-treated, but not in palmitate-treated cells. Inhibition of autophagy with chloroquine increased apoptosis in all conditions, but did not seem to prevent the ATF4-mediated protection in the oleate condition. Expression of Atg7, LC3, and p62 were not altered by ATF4 overexpression, while ULK1 protein levels were upregulated. Likewise, mRNA levels of Atg7, GABARAP, p62, and LC3 were unchanged with ATF4 overexpression. Of note, increased ATF4 expression led to \(\geq 15\)-fold higher levels of FGF21 in all conditions.

CONCLUSIONS: ATF4 offers protection against lipid-induced toxicity in control and oleate conditions but is not protective in cells exposed to palmitate. Although preliminary, our results suggest that such protection appears to be, at least in part, autophagy-independent. However, the ATF4-mediated upregulation of ULK1 and FGF21 might indicate that these are important effectors in the protection observed. Further investigation is needed to fully understand the extent these might have in the ATF4 protection and the mechanisms involved.
Neither clinical judgment nor objective scoring systems can accurately predict the risk of alcohol relapse in patients with alcohol use disorder evaluated for liver transplant

De Nooy S (1), Gowda, A (2); Zimmerman, MB PhD (3); Jones, J PhD (4), Stille, B (5); Voigt, M (6)

**Introduction:** Alcoholic liver disease is one of the most common indications for liver transplantation. Many transplant centers in the US use pre-transplant data to predict risk of relapse to alcohol, and exclude those with highest risk from transplantation. We studied the accuracy of this risk prediction, as it is used to make life and death decisions.

**Methods:** We studied a retrospective cohort of 269 patients, all of whom had alcohol use disorder, referred for Liver Transplantation to University of Iowa, between December 2008 and August 2015. We evaluated the accuracy of the transplant committees, the social workers’, psychologists’ and the standardized instruments SIPAT (Stanford Integrated Psychosocial Assessment for Transplant) and HRAR (High Risk Alcohol Relapse) score assessment of the risk of relapse. We used Cox regression model to calculate hazard ratios for relapse to harmful alcohol use. We calculated the positive and negative predictive values for predicting relapse of each objective score and clinical assessment. We did a sensitivity analysis using the endpoint of relapse to any drinking.

**Results:** Of our 269 patients, 32 relapsed to harmful alcohol use and 237 did not. 93 did not have 2 years follow up and could not be classified. Of the 269, 43 received a transplant, an additional 38 were waitlisted for transplant but did not receive a transplant, and 188 were denied/deferred for liver transplant. A total of 97 patients died, 79 of the 188 (48%) patients not listed versus 18 of 81(22%) listed or transplanted.

Cox regressions showed that social work (SW), psychologist (PSY) and transplant selection committee assessment, and SIPAT were associated with risk of relapse (Table 1). However, HRAR, gender, presence of stable partner, tobacco use, family history of alcohol dependence, drug use, prescription narcotic use, history of alcohol treatments, hepatocellular cancer and age were not significantly associated with relapse.

Of those predicted that would relapse based on high risk scores from HRAR, SW, or PSY assessments, only 23%, 36%, and 25%, respectively, actually relapsed to harmful use (positive predictive value). Negative predictive value based on low risk scores/assessments for HRAR, SW or PSY for no relapse were higher, with 81% 96% 93% of those with low risk scores, respectively, correctly predicted to not relapse to harmful use.

**Conclusions:** Clinical and/or objective scoring assessment were somewhat predictive of the risk of relapse to high risk drinking but the positive predictive value was poor for all. Patient selection for liver transplant should not hinge on attempting to predict who is not safe to transplant because of perceived high risk for relapse. Patients who are not listed die at a higher rate than those who are listed. Patients with alcohol use disorder should not be ruled out from transplant solely on the assessment of their risk for relapse to drinking, as this assessment is intrinsically inaccurate.

<table>
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HRAR = High-Risk Alcoholism Relapse; SIPAT = Stanford Integrated Psychosocial Assessment for Transplant

*HRAR scores available for 165 patients

**SIPAT scores available for 78 patients
Contributions of MsrA Deficiency and Angiotensin II to Metabolic Syndrome Phenotypes

Summer Research Fellow: Sayantan Deb, BS
Mentor: Mark W. Chapleau, PhD
Collaborators: Rasna Sabharwal, PhD; Liping Yang, BS

Background: Metabolic syndrome (MetS) includes multiple disorders (e.g. hypertension, dyslipidemia, visceral fat deposition, impaired glucose tolerance), and markedly increases risk of developing cardiovascular and metabolic disease. Oxidative stress and activation of the renin angiotensin system fuel MetS. Polymorphisms in the antioxidant gene methionine sulfoxide reductase-A (MsrA) have been linked to central obesity, diabetes and hypertension. Furthermore, MsrA deficient (-/-) mice fed a high-fat diet exhibit insulin resistance. The Chapleau lab has reported that MsrA -/- mice exhibit decreased baroreflex sensitivity, sympathovagal imbalance, and mild hypertension.

Purpose of the Study: To test the hypothesis that autonomic dysregulation in MsrA -/- mice is accompanied by metabolic dysfunction under basal conditions, and that chronic infusion of a low, non-pressor dose of angiotensin II (Ang II) will exacerbate cardiovascular, autonomic and metabolic abnormalities.

Methods: Male and female C57BL6 (control) and MsrA -/- mice were studied. In the first set of experiments, cardiovascular, autonomic, and metabolic phenotypes were measured at baseline and during infusion of Ang II (120 ng/kg/min, SC) delivered by osmotic mini-pump. Blood pressure (BP), heart rate (HR) and locomotor activity were recorded by radiotelemetry. Mean BP (MAP), BP variability (BPV, SD of systolic BP), baroreflex sensitivity (BRS, sequence method), and cardiac sympathetic tone (HR response to beta-blocker propranolol) were measured. Metabolic phenotypes including fasting blood glucose, glucose tolerance, and body fat percentage (NMR) were measured in the FOE Diabetes Center Metabolic Phenotyping Core before and after 2-3 weeks of Ang II infusion. In the second set of experiments, only metabolic phenotypes were measured, at baseline and after 3-4 weeks of Ang II infusion. In group 2 experiments, male and female mice were analyzed separately, while data from males and females were combined in group 1 because of limited numbers. Furthermore, the age at the time measurements were taken was younger and less variable in group 2 (11-18 wks) than in group 1 (20-32 wks).

Results – Group 1: At baseline, MsrA -/- mice exhibited higher MAP (115±2 vs. 103±2 mmHg), BPV, sympathetic tone and blood glucose; lower locomotor activity and BRS; and impaired glucose tolerance (vs. C57BL6 mice, P<0.05). Ang II infusion increased MAP, sympathetic tone and blood glucose in MsrA -/- mice (P<0.05), but failed to affect these phenotypes in control mice.

Results – Group 2: Unlike group 1, baseline measurements of blood glucose and glucose tolerance did not differ in C57BL6 and MsrA -/- mice. Glucose levels were higher and glucose tolerance worse (P<0.05) in males than in females in both genotypes. Ang II infusion increased blood glucose and impaired glucose tolerance in C57BL6 and MsrA -/- mice, particularly in males (P<0.05). Interestingly, Ang II infusion increased body fat percentage in male MsrA -/- mice only.

Conclusions/Discussion: We conclude: (1) MsrA -/- mice exhibit cardiovascular and autonomic dysregulation under basal conditions, confirming previous findings; (2) While group 1 experiments demonstrated impaired glucose tolerance in MsrA -/- mice at baseline, the results were not confirmed in group 2; and (3) Infusion of a low, non-pressor (in controls) dose of Ang II increases BP, sympathetic tone and metabolic dysregulation in MsrA -/- mice. Further studies are needed to explain the differences observed in group 1 and group 2 studies. We speculate that differences in sex, age and duration of Ang II infusion may have contributed to some of the disparate findings. In addition, mice in group 2 were housed in a room infected with pinworms and were subsequently treated prior to release of quarantine which may have affected our results (project supported by UI Coll of Med SRF, HL14388, U.S. Dept of Vet Aff).
CTGF Expression as a prognostic marker in sarcomas
Student: Chandni Desai, DDS, MS, M2
Mentor: Munir Tanas, MD

Study Objectives: Sarcomas are malignant mesenchymal neoplasms which can arise in bone as well as various soft tissues and can be very difficult to treat. Very few effective medical therapies are available for metastatic sarcoma; the average five year survival is 16%. Moreover, these tumors can approach large sizes and approximate crucial anatomic structures. For this reason, intermediate to high grade sarcomas often receive neoadjuvant radiation therapy to decrease the size of the tumor prior to resection and reduce the risk of local recurrence. While grading according to the French grading scheme has been adopted for many sarcomas, there is a subset of sarcomas for which the grading scheme does not adequately predict clinical behavior. Additional biomarkers are required to predict future clinical behavior and the need for neoadjuvant therapy. We anticipate the Hippo pathway, an emerging signaling pathway in cancer, contains such biomarkers. Its dysregulation, via inactivation of oncoproteins TAZ and YAP, is an important impetus for uncontrolled cell growth or neoplasia. However, compared to other signaling pathways involved in cancer, few somatic or germline mutations have been discovered in Hippo pathway genes. One exception includes epithelioid hemangioendothelioma, a vascular sarcoma, which demonstrates a WWTR1-CAMTA1 gene fusion. The resulting TAZ-CAMTA1 fusion protein represents a constitutively activated form of TAZ that abrogates negative regulation by the Hippo pathway. Study of the TAZ-CAMTA1 fusion protein suggested that TAZ and YAP are frequently activated oncproteins in sarcomas. Though canonical transcriptional targets have been identified, their role in promoting TAZ/YAP mediated hallmarks of cancer is not well understood. The objective of this study is to evaluate one of the canonical transcriptional targets of TAZ and YAP, connective tissue growth factor (CTGF), which appears in most gene expression signatures of TAZ/YAP.

Methods: A total of 159 untreated sarcomas that have previously been incorporated into a tissue microarray were obtained from the University of Iowa Department of Pathology with prior approval from the Institutional Review Board. CTGF/CCN2 (mouse monoclonal antibody; Novus Biologicals catalog # MAB660) immunohistochemistry was utilized for the evaluation of TAZ and YAP activation. To confirm specificity of the antibody, an expression vector containing the CTGF insert (pDNA3-CTGF) was successfully transfected into HEK293 cells and the protein probed with the aforementioned antibody. In addition, Western Blots were performed in 3T3 cells expressing the TAZ-CAMTA1 (TC) and YAP-TFE3 (YT) gene fusions (the constitutively active forms of TAZ and YAP). When evaluating the tissue microarray, blood vessels in the sarcomas were used as internal controls and the intensity of staining was classified as strong (3+), intermediate (2+), and weak (1+). Tumors were defined as positive if greater than 70% of the cells demonstrated intermediate levels of cytoplasmic/membranous CTGF, reflecting previously published parameters. Clinical data including time from initial diagnosis, survival status, and presence/absence of metastasis was collected.

Results: Western Blots were performed in 3T3 cells expressing the TAZ-CAMTA1 and YAP-TFE3 gene fusions. A distinct band at approximately 38 kDa was identified which represents the CTGF protein. Kaplan-Meier (survival curve analysis) performed as a function of CTGF expression at the protein level, histological grade and the presence of metastasis evaluated as a function of CTGF positive vs. negative tumors are pending.

Conclusion: CTGF expression, a transcriptional target of TAZ and YAP, can be used as a biomarker at the RNA level in addition to TAZ and YAP immunohistochemistry to identify sarcomas that have a worse prognosis and may benefit from neoadjuvant radiation therapy. It also raises the possibility that anti-CTGF therapeutic agents could be developed to target TAZ/YAP activated, CTGF positive sarcomas.
Background: Cystic fibrosis (CF) is caused by a mutation in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), an apical membrane anion channel expressed in epithelial cells. Defective CFTR function leads to epithelial surface dehydration and acidic luminal pH, which then dehydrate mucus, causing mucus plugs. Excessive mucus production contributes to obstruction, inflammation, and severe lung disease in CF. In CF lungs, two large mucin glycoproteins, MUC5AC and MUC5B, are overproduced and likely contribute to the obstructive process. Pancreatic ducts normally express MUC1 and MUC6, but mucin expression can be altered in disease states. For example, in pancreatic duct adenocarcinoma, MUC4 is overexpressed, whereas MUC1 is moved from membrane to the cytoplasm. MUC4 is also upregulated in CFPAC-1 cells, a pancreatic adenocarcinoma cell line lacking CFTR. It is not known whether mucins are overexpressed and contribute to the disease process in CF pancreas. Investigating pancreatic mucin gene expression in CF is possible due to development of the CFTR knockout pig model. CF pigs develop pancreatic disease early in life similar to humans with CF.

Hypothesis: MUC5AC, MUC5B, MUC4, and MUC1 are differentially expressed in CF pig pancreas compared to non-CF.

Methods: Pancreata were harvested from 8 newborn pigs with CFTR°/° genotype, and 8 newborn pigs with CFTR°/° genotype. RNA was isolated from the pancreas specimens for qRT-PCR analysis.

Results: qRT-PCR analysis showed no change in the transcriptional levels of MUC5AC, MUC5B, or MUC4 in CF pig pancreas. MUC1 expression was significantly reduced in CF pig pancreas compared to non-CF (p=0.0002).

Conclusions: In contrast to lungs, aberrant mucin expression is not increased in CF pancreas. Our results suggest that mucins do not contribute to pancreatic pathology in CF. Down-regulation of MUC1 is unexpected and needs further study. We will next explore MUC6 expression and cellular localization of mucins in CF pig pancreas.
Incidence and Risk Factors of Posterior Fossa Syndrome in Children with Posterior Fossa Tumors

Demi Eble, BS, Toshio Moritani, MD PhD, Fatimah Alazron, MS, Aaron Boes, MD PhD, M. Bridget Zimmerman, PhD, Mariko Sato, MD PhD

Background: Posterior fossa syndrome (PFS) is characterized by mutism, ataxia, hypotonia, and irritability that usually develops immediately or within several days following surgical resection of posterior fossa tumors in children. The incidence of PFS is reported as high as 25% of children with medulloblastoma. Duration of recovery from PFS varies, ranging a few days to several years with no evident clinical signs to predict its prognosis. The exact cause of PFS is still largely unknown, and its unpredictable outcome makes the management of PFS very challenging.

Hypothesis: (1) Duration of symptoms, brain stem compression, or operative parameters may predict incidence, severity, and clinical course of PFS. (2) Volumetric measurement of MRI image may predict incidence, severity, and clinical course of PFS.

Methods: Retrospective chart review from 1976 to March 2017 was conducted. Medical records of 87 out of the 111 children with posterior fossa tumors were analyzed. Medical records were reviewed for patient demographics (age, sex), diagnosis (tumor type, histological type, location), clinical course (physical exam, time before surgical procedure, neurological exam), operative record (operation time, surgical approach, splitting of vermis, blood loss), treatment (surgery, radiation therapy, and/or chemotherapy), presence or absence of posterior fossa syndrome, (symptoms, symptom delay onset, length of symptoms), and neuropsychological outcome. Pre and post surgical MRI imaging review and analyses using voxel-based lesion symptom mapping was performed.

Results: Of the 87 charts reviewed 48 (55%) had medulloblastoma, 26 (30%) had low grade glioma, 10 (11%) had ependymoma, and 3 (3%) had ATRT. Fifteen patients (17%) developed PFS after surgery. The median age of patients which developed PFS was 8.8 years (1.3-13.2). 10 out of 15 patients with PFS (67%) had severe PFS symptom severity. Of the 15 PFS patients, 10 (67%) had medulloblastoma, 2 (13%) had low grade glioma, 2 (13%) had ATRT, and 1 (7%) had ependymoma. MRI results, voxel-based lesion mapping, and statistical analyses are currently being gathered.

Conclusion: This study worked to elucidate the incidence and clinical outcome of PFS in UIHC patients with posterior fossa tumors. The 17% incidence of PFS in UIHC patients is lower than previously published data. Our data also revealed that patients who developed PFS not only had medulloblastoma, but other tumor types as well. This is still an ongoing investigation; voxel based data is still being gathered, and statistical analyses are currently being performed. Studies will need to continue to be performed and collaboration efforts through Children Oncology Group may elicit significant predictive values of PFS. The discovery of prognostic factors and volumetric changes will help us to prevent the development of PFS. These discoveries could greatly improve the postoperative outcome of children in the treatment of their posterior fossa tumors.
Effects of Patent Ductus Arteriosus Diagnosis on Nutritional Status and Growth of Preterm Infants

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ABSTRACT

BACKGROUND: The ductus arteriosus is a fetal blood vessel that connects the pulmonary artery to the descending aorta, which allows fetal blood flow to bypass the fluid filled lungs during gestation. This vessel commonly remains patent in preterm infants and can negatively affect cardiovascular function. Many methods have been used to treat patent ductus arteriosus (PDA) such as NSAIDs (ibuprofen or indomethacin), fluid restriction, and surgical ligation; all having advantages and disadvantages. There is no consensus on when it is appropriate to treat a PDA. Some studies have shown that fluid restriction can help facilitate spontaneous PDA closure in preterm infants; however, it may come at the cost of the infants’ growth and nutrition status.

HYPOTHESIS: We hypothesize that preterm infants who are diagnosed with a patent ductus arteriosus, and are not being treated, are experiencing nutrient restriction post-diagnosis leading to growth failure.

METHODS: We conducted retrospective data collection from the University of Iowa Neonatal Admissions Registry from January 2012 to December 2016 in neonates from 25 to 28 weeks gestational age diagnosed with a PDA. Subjects were then categorized based upon diagnosis of a PDA, which included: PDA that was ligated, PDA but not treated with NSAIDs, PDA that is treated with NSAIDs. Diagnosis of the PDA was determined from the echocardiogram reports. The parameters of most interest were growth following diagnosis and nutritional status during the 2 weeks post-diagnosis. This was assessed as energy (kCal/kg/day) administers.

RESULTS: We found no significant evidence that failure to treat a PDA, either medically or surgically, altered growth velocity in preterm infants 25-28 weeks gestation. There were no significant differences in growth among the following groups: 1) infants who received treatment with PDA closure; 2) infants who received treatment without PDA closure; 3) infants who underwent PDA ligation or 4) infants who received no treatment. Univariate analysis showed no difference in growth in the 2 months following PDA diagnosis between infants with a PDA duration of ≤14 days compared to >14 days. In addition, the size of the ductus arteriosus measured by echocardiography had no effect on growth.

CONCLUSION: The preliminary data analysis suggest that preterm infants who were diagnosed with a PDA, and were not being treated, were not experiencing growth failure from their parenteral nutrition regimen. However, nutrient density of the infants’ parenteral feedings could have been altered to maintain body weight while still restricting fluids. Therefore, fluid restriction may still be an effective treatment option if overall nutrition is being carefully monitored. Further multivariate statistical analysis will look at Z-scores of infants’ birth weights to get a more definitive picture of the effects of treatment methods on the infants’ growth.

Bovine Arch Anatomy is Not Associated with Flow-Mediated Aortic Arch Hypoplasia in Infants
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Divisions of Pediatric Cardiac Surgery1 and Pediatric Cardiology2, University of Iowa
Stead Family Children’s Hospital, Iowa City, Iowa
Division of Cardiovascular and Pulmonary Imaging3, University of Iowa Hospitals
and Clinics, Iowa City, Iowa

ABSTRACT
OBJECTIVE: Bovine arch is the most common variation of aortic arch vessel branching and is characterized by either the left common carotid artery (LCCA) arising from the innominate artery or by the two vessels sharing a common point of origin. Although believed to be a normal variant, previous data shows children with bovine arch anatomy are at a significantly higher risk of re-coarctation following surgical repair involving extended end-to-end anastomosis. Other studies have shown blood vessels reorganize their shape to optimize flow and regress if not appropriately perfused. We propose that a bovine arch alters perfusion throughout various sections of the aortic arch and thereby impacts vessel development. This study aims to determine the association of hypoplasia in a bovine arch at various points along the aorta.

METHODS: Thirty-four chest CTAs and 15 chest CTs performed on infants (<1 year old) at a single institution from 2012 to 2017 were analyzed. Vessel diameters were obtained at the sinotubular junction (STJ), ascending and descending aorta at the level of the right pulmonary artery, proximal transverse arch, distal to the LCCA/bovine trunk, distal transverse arch, aortic isthmus, and descending aorta at the level of the diaphragm. The diameter of the innominate artery/common bovine trunk was also obtained. Data were indexed to each of the STJ diameter, weight at time of scan, and body surface area (BSA) at time of scan and analyzed separately.

RESULTS: The diameter of the common bovine trunk in patients with bovine arch anatomy was found to be significantly greater than the diameter of the innominate artery (p<0.05) when indexed to STJ diameter, body weight, or BSA. However, there were no significant differences in the aortic diameters between patients with normal anatomy and patients with bovine arch anatomy at any point along the aorta (p>0.05), indicating no degree of aortic hypoplasia.

CONCLUSIONS: Though previous data demonstrates higher rates of re-coarctation in patients with bovine arch, this does not appear to be due to significant aortic arch hypoplasia, compared to normal arch anatomy. Future research may involve analyzing other anatomic characteristics of bovine arch and creation of a computational fluid dynamics model of bovine arch versus normal arch anatomy to determine the true impact of bovine arch on aortic fluid dynamics.
**Parkinson’s disease and temporal control**

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1-2. University of Iowa Carver College of Medicine, 3. University of Iowa Graduate Program in Neuroscience, 4. University of Iowa Department of Neurology

**BACKGROUND:** Parkinson’s Disease (PD) is a neurodegenerative disease of dopaminergic neurons that demonstrates both motor and cognitive dysfunction. The current absence of effective treatments for the cognitive symptoms of PD—which include working memory, executive function, and attention—is partly due to the undetermined mechanism of cognitive impairment in PD. It has been hypothesized that cognitive deficits in PD involve prefrontal dopamine circuits. This study focuses on temporal control of action, (the guidance of movements in time) an essential process impaired in PD patients that requires the prefrontal cortex. The impairment in temporal control seen in PD patients can be evaluated using an interval timing task. In this task, participants are asked to estimate a given time interval of several seconds and then give a motor response after the estimated time has elapsed—engaging working memory, attention, and other executive functions (Brown 2006; Parker et al. 2013). Patients with PD have demonstrated consistent impairments in these tasks (Merchant et al. 2008), reflective of temporal control dysfunction. The relationship between temporal control and cognitive deficits in Parkinson’s disease is unknown. In the present research, we propose to evaluate interval timing performance via the interval timing task and measure cognitive domains with the Montreal Cognitive Assessment (MoCA) in order to correlate cognitive function with interval timing performance.

**HYPOTHESIS:** Parkinson Disease is associated with decreased accuracy in time-estimation. Cognitive functions (assessed by the MoCA) modulate this effect.

**METHODS:** 31 Parkinson’s Disease patients were recruited from the University of Iowa Hospitals and Clinics. Subjects first underwent neuropsychological testing with the MoCA and afterwards completed the interval timing task on a computer. The task consisted of an initial training period, where patients were asked to estimate “short” (3 sec) or “long” (7 sec) time intervals following a visual stimulus. Patients then underwent 80 test trials divided over 4 blocks. In each test trial, patients were asked to estimate either a short or long interval by pressing down on the space bar just before and releasing the space bar just after they believed the interval had elapsed. Performance feedback was given after every third test trial. Patients were instructed not to count during this task and were unaware of the actual duration of either interval.

**RESULTS:** 23 males and 8 females were recruited. The median MoCA score was 25 (range: 13-30). We calculated a variance coefficient as a measure of variability in the start and release times for both the short and long interval. A Pearson product-moment correlation coefficient was computed to assess the correlation between MoCA scores and variance coefficients. There was a moderate negative correlation between all variance coefficients and total MoCA score: variance start short $r = -.400, p = 0.026$, variance stop short $r = -.429, p = 0.016$, variance start long $r = -.439, p = 0.013$, and variance stop long $r = -.395, p = 0.028$. Decreases in MoCA score were correlated with increased variability in time-estimation.

**DISCUSSION:** These findings suggest that decreased cognitive function across this PD population was associated with increased variability in time-estimation and thus, greater dysfunction in temporal control. Temporal control indexes executive resources; and deficits in executive functions (e.g., attention, planning, working memory, etc.) can predate the diagnosis of PD through motor symptoms as well as drive morbidity and mortality. Early identification of these cognitive impairments could potentially lead to earlier interventions and better prognoses. These results suggest that interval timing tasks may be useful in clinical settings to evaluate specifically for temporal control deficits, a marker of frontal/striatal dysfunction in PD.
A scalable, low-cost, portable platform for assessing balance and predicting fall risk in older adults

Nicholas Evans, Philip Polgreen

Background: Falls among older adults are a major cause of morbidity and mortality. Falls often cause disabling fractures and/or head injuries leading to further disability. Thus, preventing falls is a major public health priority. An important component of fall prevention is risk detection. Most previous work is survey-based and relies on self-report. Equipment to assess balance exists, however in many cases the cost to use such equipment is prohibitive. Recently, less expensive approaches have been developed. While some approaches (e.g., using force platforms or Wii Balance Boards) have been validated and compare favorably to alternative fall prevention measures, they have relied upon custom software that is not generally available in hospital or ambulatory care settings and are difficult to use by a general audience.

Purpose: The purpose of this project is to build a platform for rapidly detecting deficiencies in balance. This system needs to be easy to use, portable, and scalable.

Methods: We wrote custom software to (wirelessly) read data from the force sensors in an inexpensive balance board. Specifically, we wrote software to collect data via Bluetooth from a Wii Balance Board. The Wii Balance Board is an inexpensive controller associated with the popular Nintendo Wii game console. As soon as data from the force sensors is collected by a connected laptop, it is analyzed in real time to detect balance deficiencies. The developed software provides step-by-step instructions to facilitate data collection. All software was written in Python v3.5.

Results: Our system allows for entry of a subject identifier, then asks subjects to complete the following four tasks, each for 30 seconds: standing with feet together and eyes open, standing with feet shoulder-width apart and eyes open, standing with feet together and eyes closed, standing with feet shoulder-width apart and eyes closed. The subject is then asked to repeat the four tasks. The order of the tasks is randomly chosen automatically by the software for each subject. While the subject is standing on the balance board the researcher is able to see the real-time center of pressure. The raw force sensor data for each trial is timestamped and saved with the subject identifier for later analysis. The sample rate for each sensor varies between 30 and 100 Hz. A usability study demonstrated that our system can be used by non-technical personnel, and the entire data collection process takes approximately 4 to 5 minutes per subject.

Conclusions: We have successfully built a custom, low-cost system for detecting balance deficiencies. Our system is easy to use, and because of its affordability (requiring only a $40 Wii Balance board and Bluetooth-capable laptop or tablet) can easily be scaled for use in both research, and ultimately, clinical practice for safety and quality fall-prevention initiatives. The data we will gather over the next year will enable us to develop a machine learning approach to not only detect fall risk but also recommend assistive devices (e.g., cane or walker).
The Impact of Adverse Childhood Experiences (ACEs) on Burn Outcomes in Adult Burn Patients

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Introduction: Adverse Childhood Experiences (ACEs) are incidents that happen to a person in the first 18 years of life including emotional, physical, or sexual abuse and emotional and physical neglect. They also include household dysfunction such as parental divorce/separation, witnessing domestic violence, and having substance-abusing, mentally ill, or incarcerated family members [1]. Nationally 12.5% of the population have four or more ACEs; [2] that number is 14.5% within the state of Iowa [3]. Four or more ACEs have been linked to chronic social, behavioral, physical and mental health problems, including ischemic heart disease, cancer and psychiatric illnesses [1]. As burns are also associated with high risk behaviors and a disproportionately high rate of psychiatric comorbidities, we hypothesized that the burn population would also be characterized by a large number of ACEs and that those with more ACEs would have a more complicated recovery. The goal of this study was to characterize the prevalence of ACEs in the burn population, individual patient economic and social needs, and examine the relationship between burn outcomes and ACE exposure.

Methods: Adult (over 18-years of age) burn survivors who were either admitted to the hospital for their burns or seen in the outpatient burn clinic were approached for the study. Consenting participants completed surveys assessing childhood ACEs, family needs, family strengths and resiliency, pain, depression, post-traumatic stress disorder, and quality of life at 6-weeks post injury and again at one-month, 3-months, 6-months, one-year, and two-year follow up after discharge via email. Depression was assessed using the Patient Health Questionnaire (PHQ-9). Post-traumatic stress disorder was measured using the PTSD CheckList – Civilian Version (PCL-C). Quality of life was assessed using the Life Impact Burn Recovery Evaluation Scale. The Brief Resilience Scale (BRS), developed by Ohio State University, was used to measure resiliency. An institutional packet included the ACEs screening form and forms to assess the family strengths and needs. Other variables collected were demographics, comorbidities, length of stay (LOS), number of surgeries, burn total body surface area (TBSA), etiology of burn, and surgeries performed. Univariate analysis and Student’s t-test were used where indicated to describe the study sample and compare those with four or more ACEs to the rest of the population. Significance was assumed at the p<0.05 level. The study was approved by the Institutional Review Board.

Results: Of the 34 adult patients, 13 had four or more ACEs (38.2%). The only demographic variable that differed between the two groups was home ownership, with the high ACE population less likely to own their own home (54% ≥4 ACE vs 95% <4 ACE; p<0.005). In the area of family strengths and needs, patients with four or more ACEs were more likely to not have healthy coping skills, to feel under extreme stress, to have experienced a problem with drugs or alcohol in the past year, and to have had law enforcement involvement (all p<0.05). Individuals with four or more ACEs also rated their average and worst daily pain significantly higher (p<0.007) and were less likely to have always had controllable levels of pain during their treatment at UIHC (p<0.046). Moreover, the patients with four or more ACEs experienced more difficulties falling asleep, staying asleep or sleeping too much (p<0.003). This group was also significantly more likely to warrant a depression diagnosis (36% ≥4 ACE vs 0% <4 ACE; p<0.005) using the PHQ-9 depression scoring and more likely to screen as having probable PTSD (p<0.028).

Conclusion: The trauma informed assessment toolkit including the ACE screening tool may provide a powerful means to detect burn patients at risk for complicated recovery. Patients with four or more ACEs may have more limited psychosocial resources, more economic challenges, and suffer from psychological stress and mental illnesses at a higher rate than those with fewer ACEs. Targeted assistance using Trauma Informed Assessment techniques may help interpret their illness and level of needs in light of their behaviors and psychosocial circumstances subsequent to former trauma and capitalize on strengths to meet their needs and help burn patients successfully recover from their injuries more rapidly and effectively.

References

**Validation Studies of a Functional Genomic shRNA Screen Investigating Vemurafenib Resistance in BRAF V600E-Mutated Melanoma**

**Tyler Foley BS, Michael Henry PhD**

**BACKGROUND:** Mutations in BRAF, which constitutively activate the mitogen activated protein kinase (MAPK) pathway, are present in 50% of cutaneous melanomas. Vemurafenib, a small-molecule inhibitor of the most common BRAF mutation (BRAF V600E), elicited a 63% reduction in risk of death and 74% reduction in risk of disease progression in a phase III clinical trial of patients with metastatic melanoma. However, acquired resistance to single-agent BRAF inhibitor therapy is common, arising after 6-7 months of progression free survival. The mechanisms underlying this acquired resistance to BRAF inhibition have yet to be fully elucidated. Through a combined effort of labs in the Holden Comprehensive Cancer Center’s Cancer Metastasis Group, a functional genomic shRNA screen was conducted to identify loss-of-function mutations causing vemurafenib resistance in BRAF V600E-mutated A375 melanoma cells. Of the “hits” identified in the screen, the following 5 genes were chosen for validation studies to further investigate their roles in acquired resistance to BRAF inhibition: NF1, CUL3, SUV420H1, TAOK1, and ACE2. NF1 and CUL3 had been previously implicated in similar shRNA or CRISPR-Cas9 screens, while SUV420H1, TAOK1, and ACE2 would be novel mutational markers in the development of resistance to vemurafenib.

**AIMS:** To validate the results of a functional genomic screen of vemurafenib resistance in A375 melanoma cells by determining if cell lines with shRNA-induced knockdown of selected target genes will develop drug resistance more rapidly than control cells expressing a non-targeting (NT) shRNA.

**METHODS:** A375 cells were transduced with lentiviral vectors expressing shRNA targeting selected genes. For each gene, 2-4 shRNA constructs were utilized. Knockdown of mRNA and protein level of target genes for each cell line was assessed using RT-qPCR and Western Blotting. To determine the short-term response of cell lines to vemurafenib, 72-hour cell viability assays were performed in triplicate at a range of concentrations from 1 nM to 10 µM. Knockout cells were then cultured in the presence of 3 µM vemurafenib and cumulative population doubling was calculated every 5 days to assess emergence of acquired drug resistance.

**RESULTS:** RT-qPCR studies confirmed knockdown of RNA expression level in 4 out of 5 targeted genes. Due to low baseline expression of ACE2, relative expression levels could not be established for this target. Western blotting indicated knockdown of target gene protein levels for shNF1, shCUL3, and shTAOK1 cell lines, while visible bands could not be detected for shACE2 and shSUV420H1 lines. After 72 hours of vemurafenib treatment, only shCUL3 cell lines produced a shift in IC50 value compared to shNT control cells. shNT cells showed an IC50 of 158 nM, while the IC50 values of shCUL3-1 and shCUL3-2 were 589 nM and 313 nM, respectively. Only the shift in IC50 elicited by shCUL3-1 was statistically significant (p=0.0322). A long-term grow assay experiment comparing the doubling populations of each cell line in the presence of 3 µM vemurafenib confirmed that CUL3 knockdown conferred resistance to the drug, as both shCUL3 lines saw greater doubling populations (17.7 and 13.9) compared to shNT cells (4.4) after 30 days of treatment. Surprisingly, despite the fact that NF1, SUV420H1, and TAOK1 knockdown did not affect the IC50 of vemurafenib after 72 hours, at least 1 cell line for each gene developed resistance to vemurafenib before control cells in the long-term growth assay. Resistance appeared after a minimum of 10 days of vemurafenib exposure, explaining the lack of shift in IC50 after just 72 hours.

**CONCLUSION:** Only shRNA-induced knockout of CUL3 in A375 melanoma cells significantly increased the IC50 of vemurafenib after 72 hours. However, knockdown of CUL3, NF1, SUV420H1, and TAOK1 was capable of developing drug resistance before non-targeting control cells in long-term cell growth assays. These results serve to validate the functional genomic shRNA screen and identify potential therapeutic targets in the treatment of BRAF V600E-mutated melanoma. Moving forward, the mechanisms by which loss of function in these genes confers resistance to vemurafenib will be investigated.
Investigation into insulin signaling and the regulation of mitochondrial metabolism in skeletal muscle
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Abstract
Background: Diabetes Mellitus is caused by inadequate insulin signaling leading to elevated blood glucose levels. In skeletal muscle, insulin deficiency also causes an increase in protein degradation and decreased ATP production and mitochondrial oxidative capacity. Work from our lab showed that loss of the insulin receptor (IR) and insulin-like growth factor receptor (IGF1R) in mice increases protein degradation and decreases mitochondrial respiration in a similar way to diabetes. Recent work by Dr. O’Neill et al. showed that the skeletal muscle loss associated with IR/IGF1R deletion is mediated by FoxO transcription factors, but whether these control mitochondrial function is unknown. We hypothesize that insulin regulates mitochondrial-specific autophagy (aka mitophagy), and that in diabetes, dysregulated mitophagy contributes to skeletal muscle dysfunction leading to the deleterious effects of diabetes on exercise capacity and glucose regulation.

Purpose: Determine if insulin signaling and the closely related IGF-1 signaling regulates mitochondrial oxidative phosphorylation and mitophagy in cultured myotubes and whether this is dependent on FoxOs.

Methods:
A mouse line with skeletal muscle IR/IGF1R knockout (MIGIRKO) was created to investigate the role of insulin and IGF-1 signaling on mitochondrial metabolism. For a cellular model, C2C12 myotubes were then treated with or without 4 or 24 hours of insulin and with or without a mitochondrial proton gradient uncoupler (CCCP) to induce mitophagy. Mitochondrial function was evaluated using an Agilent Seahorse XF analyzer along with quantification of oxidative phosphorylation (OXPHOS) complexes and proteins specific for mitophagy (PINK1, BNIP3, and LC3A/B) via Western Blot.

Primary myoblast IR and IGF1R were then knocked out in vitro by treating IR/IGF1R lox/lox myotubes with adenoviral-Cre, as treatment, or Adenoviral-Luciferase as control. Myotubes were cultured and treated with or without CCCP. Protein isolation and quantification were performed using the same methods as the C2C12 cells.

Results:
Inducible knockout of IR and IGF1R in muscle of mice caused decreased oxidative capacity and reduced ATP production, mimicking the effects of type 1 diabetes on mitochondrial function. To recapitulate this effect in a cellular model, C2C12 myotubes were treated +/- insulin for 24 hours and +/- CCCP for 4 hours prior to Seahorse measurements. C2C12 myotubes had a significant reduction in mitochondrial respiration with CCCP treatment that was improved, though not completely, with insulin treatment. OXPHOS subunits were not significantly changed with either CCCP or insulin treatment. Insulin treatment decreased protein levels of mitophagy components, including PINK1 and BNIP3. Lastly, acute deletion of IR/IGF1R in lox/lox myotubes displayed a similar reduction in PINK1 protein as CCCP treatment did in the C2C12 myotubes.

Conclusion: Loss of IR/IGF1r signaling in muscle acutely decreases mitochondrial respiratory capacity and ATP production. In C2C12 myotubes, insulin significantly improves the mitochondrial respiration loss seen with CCCP treatment. These exciting data point to a critical role for insulin signaling in maintenance of muscle mitochondrial metabolism. Continued research is ongoing to determine the relationship between mitophagy and the role of transcription factors like FoxOs on mitochondrial function.
Two-way interactions between the nervous system and gut microbiota in a fruit fly model of epilepsy

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It is well established that bacteria residing on and in our bodies have significant effects on health. In the gut, the host organism provides heat and nutrients necessary for bacterial growth, while in turn, bacteria synthesize nutrients for use by the host; prevent the colonization of pathogenic bacteria; synthesize bioactive compounds; and influence immune system function. Interestingly, recent studies have further demonstrated that gut bacteria play critical roles in development and physiology of the host nervous system. However, the molecular and cellular mechanisms underlying the functional interactions between the brain and gut microbiota still remain elusive. Here we addressed this issue using a fruit fly model of epilepsy, *Shudderer* (*Shu*). *Shu* is a dominant mutant for the *Drosophila* voltage-gated sodium (Naᵥ) channel gene, exhibiting neuronal hyperexcitability and severe seizure-like behavioral defects, including spontaneous tremoring and heat-induced convulsions. Microarray analysis indicated that *Shu* mutants show an upregulation of genes involved in the innate immune response, raising the exciting possibility that the gut microbiota may be involved in *Shu* phenotypes. Intriguingly, rearing *Shu* and wild-type (WT) flies in food containing antibiotics not only eliminated the gut microbiota, but the treatment also significantly suppressed *Shu* behavioral phenotypes while having no observable effect on WT behaviors. These findings encouraged us to investigate whether *Shu* mutants exhibited dysregulation of their gut microbiota. Using a method of gut dissection and plating on MRS agar, we show that *Shu* mutants possess a significantly different gut microbiota compared to WT flies. Our findings indicate that a *Drosophila* Naᵥ channel mutation leads to alteration in gut microbiota, and that gut microbiota can regulate the seizure severity of the mutant. Overall this study has established a foundation for future studies of two-way interactions between the nervous system and gut microbiota using a fly model of epilepsy.
Mechanisms of Leptin-Angiotensin Cross-Talk for the Control of Resting Metabolism
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Background: Obesity is a primary risk factor for the development of cardiovascular, metabolic, and cancerous diseases, and it is a major burden on our healthcare system. Recent studies at the NIH indicate that the primary mechanism preventing sustainable weight loss in obese humans involves the adaptation (suppression) of resting metabolic rate (RMR). Recent studies from our group have implicated the renin-angiotensin system (RAS) within the arcuate nucleus (ARC) of the hypothalamus in the control of sympathetic nerve activity (SNA) and thereby RMR. Specifically, the angiotensin II type 1A (AT\textsubscript{1A}) receptor on neurons which express agouti-related peptide (AgRP) is necessary for metabolic control by leptin. Specifically, genetic disruption of AT\textsubscript{1A} in leptin receptor (LepR)-expressing cells (AT\textsubscript{1A}\textsuperscript{LepR\textsuperscript{KO}} mice) or AgRP-expressing cells (AT\textsubscript{1A}\textsuperscript{AGRP\textsuperscript{KO}} mice) abolishes SNA and RMR responses to leptin, while leaving proximal LepR signaling (ie – phosphorylation of STAT3) intact. Despite these insights, it remains unclear how leptin, acting through its LepR, activates AT\textsubscript{1A} signaling in AgRP neurons.

Objective: The goal of the present project is to understand the neurocircuitry and biochemistry which mediates the cross-talk between leptin and AT\textsubscript{1A} receptors located on AgRP neurons.

Hypothesis: We hypothesize that neurons of the arcuate nucleus respond to LepR signaling by increasing synthesis of angiotensinogen (AGT), the precursor for angiotensin II. This would result in increased local paracrine activation of AT\textsubscript{1A} on AgRP neurons, driving increased SNA and RMR.

Methods: To determine whether LepR may act within individual cells to regulate AGT expression, fluorescent in situ hybridization (FISH / RNAscope) methods were used to co-localize AGT with LepR, and with markers of glia (glomerular fibrillary acidic protein, GFAP), or relevant neuronal subtypes (AgRP; or those expressing proopiomelanocortin, POMC). To determine whether leptin drives AGT gene expression in neurons, immortalized mouse hypothalamic neuronal cell lines that express the gene profile of AgRP neurons (N47 cells) were treated with varying doses of leptin. cDNA was then synthesized from extracted RNA by reverse transcription polymerase chain reaction (rtPCR). The amount of AGT mRNA in treated cells was then determined using quantitative polymerase chain reaction (qPCR). To examine the physiological significance of AGT induction by LepR activation, AGT was selectively ablated in LepR-expressing cells by cre-lox technology in mice on a C57BL/6J background (LepR-Cre x AGT\textsuperscript{floX/floX} to yield AGT\textsuperscript{LepR\textsuperscript{KO}} mice). These AGT\textsuperscript{LepR\textsuperscript{KO}} mice and littermate controls underwent comprehensive metabolic phenotyping, including assessments of body mass and composition, food intake, and digestive efficiency.

Results: AGT mRNA co-localized with mRNA for LepR, GFAP, AgRP and POMC within the ARC, supporting a possible role for LepR in the regulation of AGT in both glia and neuronal cell types. Analyses of leptin-treated N47 cell cultures (n=10) indicated that leptin treatment did not significantly increase AGT mRNA. AGT\textsuperscript{LepR\textsuperscript{KO}} mice exhibited normal growth rates, body composition, food intake, and digestive efficiency when maintained on a standard chow diet (Teklad 7913) or when shifted to a 45% high fat diet for five weeks. Interestingly, previous studies indicate that AT\textsubscript{1A} colocalizes with LepR and AgRP within the ARC, and preliminary experiments demonstrate that leptin treatment of cultured cells increased mRNA for the AT\textsubscript{1A} receptor, and acute injection of leptin into wildtype C57BL/6J mice caused an increase in AT\textsubscript{1A} mRNA within the arcuate nucleus.

Conclusions & Discussion: Here we document that AGT co-localizes with LepR within the ARC, but that leptin fails to stimulate AGT expression in vitro and in vivo. Further, genetic disruption of AGT in LepR-expressing cells has no obvious major consequence upon energy homeostasis. In contrast, we previously demonstrated that AT\textsubscript{1A} also co-localizes with LepR, and here demonstrate that leptin stimulates AT\textsubscript{1A} mRNA both in vitro and in vivo. We conclude that leptin signaling does not appear to modulate availability of the AGT precursor, but rather it activates AT\textsubscript{1A} signaling within AgRP neurons through increasing the expression of the AT\textsubscript{1A} receptor, which should result in increased cellular sensitivity to angiotensin. Importantly, these data do not rule out additional possible roles for LepR modulation of the expression or activity of other critical components of the RAS within the ARC, including renin, the pro-renin receptor, angiotensin converting enzymes 1 or 2, neprilysin, aminopeptidases A or N, or other angiotensin receptors (AT\textsubscript{1B}, AT\textsubscript{2}, Mas, or LNPEP).

Clinical Significance: Increasing data demonstrates a critical role for RMR control in the pathogenesis and maintenance of obesity, and the dominant role of the brain in the control of RMR. Understanding the neurobiology of RMR control is therefore required to advance novel therapeutic approaches to the prevention and reversal of obesity.
Normal Saline Versus Lactated Ringer’s: A Randomized Control Trial Evaluating Quality of Recovery in Emergency Department Patients

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**Background:** Intravenous (IV) fluid administration is the most common procedure performed in the emergency department (ED), with 26% of ED visits involving fluid administration. Normal saline (0.9% sodium chloride, NS) and lactated ringer’s (LR), are the most common choices for first-line fluid therapy. Prior evidence suggests that NS use results in higher rates of hyperkalemia, hyperchloremia, and metabolic acidosis in critically ill patient. These studies indicate that LR may be associated with better clinical outcomes, but the clinical significance of these findings are uncertain.

**Objective:** The objective of this study was to test the hypothesis that intravenous LR administration will lead to better subjective quality of recovery than NS in non-critical, volume-depleted patients.

**Methods:** This study was a single-center, participant-blinded, randomized clinical trial comparing the quality of recovery after a single intravenous bolus of LR solution vs. NS. Volume-depleted adults (≥ 18 y) were recruited to participate in the study if they were determined to require fluid resuscitation, and likely to be discharged home. Patients were randomized to receive two liters of LR or NS by IV bolus. Quality of recovery was measured by the Quality of Recovery-40 (QOR-40) survey administered to the patient while in the ED and re-administered 24 hours later by phone. The QoR-40 is a validated survey tool used to measure patient-reported recovery across five independent domains: Comfort, Emotion, Physical Independence, Patient Support, and Pain. Scores range from 40-200 with higher scores indicating greater patient comfort and satisfaction. The primary outcome of this study was the QoR-40 scores at 24 hours, and secondary outcomes included scores of each of the five individual domains of the QoR-40. Between group differences in post QoR-40 scores were evaluated using the Mann-Whitney U test with significance defined as p<0.05.

**Results:** We are reporting preliminary results of the first 93 participants (NS =49; LR=47) of our total anticipated sample of 156 participants, with 58 of them (62%, NS=32; LR=25) completing follow-up surveys. QoR scores improved between the pre-enrollment scores and the 24 h scores (NS=21.5 vs. LR=17.9, p=0.403). For the primary outcome, the QoR scores were not different between NS and LR groups (162.1 vs. 170.3, p=0.251). While the pre-enrollment scores differed between groups (140.6 vs. 151.4, p=0.043), adjusting for pre-survey scores using linear regression continued to show no relationship between group allocation and post-survey scores (p=0.900). Examining individual domain scores, post-survey physical comfort and pain domains also showed no significant difference between the groups (NS=47.5 vs. LR=50.4, p=0.219; 28.7[NS] vs. 29.4[LR], p=0.627, respectively).

**Conclusions:** While volume depleted ED patients showed improved recovery scores 24 h after their ED visits, there was no difference in recovery between NS and LR groups based on a 2L bolus in the ED. Furthermore, the independent domains of physical comfort and pain, two dimensions of the QoR-40 score most likely to be affected by IV fluids, showed similar recovery scores. Based on the preliminary data, normal Saline was determined to be as effective as Lactated Ringer’s at treating non-critical, volume-depleted patients. It is important to note these results are only preliminary and a final conclusion will be determined once all of the data is collected.
Bovine Aortic Arch Shortens Available Clamping Distance for Extended End-to-End Repair of Infant Coarctation of the Aorta

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Background:
Bovine aortic arch is an anatomical variant in which the innominate and left common carotid arteries share a common origin. It is generally considered to be benign. The current literature estimates its prevalence may be as high as 30%. Coarctation of the aorta is a congenital heart defect characterized by narrowing of the aortic arch, usually near the insertion of the ductus arteriosus. Untreated coarctation of the aorta can lead to hypertension, poor perfusion of the lower extremities, and left ventricular hypertrophy and failure. Resection with extended end-to-end anastomosis from a left thoracotomy remains the standard of care in the absence of arch hypoplasia. Extended end-to-end anastomosis requires the placement of a proximal clamp just distal to the innominate artery and a distal clamp on the descending aorta. The distance between the proximal clamp and the coarctation represents the amount of vessel the surgeon has available for reconstructing the aorta with this technique. The goal of this study is to determine if bovine aortic arch anatomy affects the clamping distance.

Methods:
34 chest CTAs and 15 chest CTs performed at the University of Iowa Stead Family Children’s Hospital between 2012 and 2017 were obtained. Only scans from patients <1 year of age were included (mean age=41 days, 29% female, 37% bovine arch). Scans from patients with a history of previous aortic surgery (including aortoplexy), aberrant subclavian, right-sided aortic arch, double outlet right ventricle, hypoplastic left heart syndrome, D-TGA, truncus arteriosus, scoliosis, and Marfan syndrome were excluded. Scans were uploaded into CareStream software and a multiplanar reconstruction was performed. The distance between the distal edge of the innominate artery (or bovine trunk, in bovine arch patients) and the proximal edge of the left subclavian artery was measured. This measurement is denoted as the clamping distance (CD). The distance between the distal edge of the innominate artery/bovine trunk and the middle of the left subclavian artery was also measured as an alternate clamping distance (CD2). Clamping distances were standardized to the patient’s weight at the time of scan to give a clamping index (CI).

Results:
The clamping index in bovine arch patients was significantly smaller than in normal arch patients. This held true when clamping index was calculated with both CD and CD2 measurements. The average clamping index in normal arch patients using CD measurements was 1.54 mm while the average in bovine patients was 0.77 mm (p<0.05). The average clamping index in normal arch patients using CD2 measurements was 1.93 cm while the average in bovine patients was 1.16 cm (p<0.05).

Discussion:
Bovine aortic arch, where the innominate and left common carotid arteries share a common origin, is usually considered to be a clinically insignificant variant. However, this study suggests that the clamping distance for extended end-to-end anastomosis in aortic coarctation repair is significantly smaller in patients with bovine arches than in patients with normal arches. Since shorter clamping distances decrease the length of vessel available for this repair, the outcome may be poorer in patients with concomitant bovine arch and coarctation of the aorta.
A Prospective Cohort of Injuries in Youth Flag and Tackle Football

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Background: Injury rates in youth football are poorly described. Previous studies have reported injury rates between 2.3% and 38.2% per player year, but have suffered from low numbers of exposures and/or methodological limitations.

Objective: This prospective cohort study measured the rates, types and recovery time of injuries among youth tackle and flag football players.

Design/Methods: We partnered with three youth football leagues in Eastern Iowa (two tackle leagues and one flag league). Coaches used an online injury reporting form to take attendance at each game and practice, to document when a player was injured, and to identify the date that an injured player returned to play. We defined an exposure as participation in any practice or game and recorded all time-loss injuries. A severe injury was defined as any injury that resulted in a concussion, fracture, or ligament tear.

Results: Over the 2-year reporting period, there were 108,627 exposures and 316 injuries (2.91 injuries per 1000 exposures). 295 of the injuries occurred in tackle leagues and 21 occurred in the flag league. The relative risk of injury was 1.67 (1.07, 2.60; p = 0.0227) and the relative risk of severe injury was 4.84 (1.57, 15.23; p = 0.0069) in the tackle leagues compared to the flag league. Tackle football players experienced 0.59 concussions per 1000 exposures compared to 0.26 concussions per 1000 exposures in the flag leagues, but this was not statistically significantly different (0.71, 7.23; p = 0.1680). The median time to return to play after injury in the flag league was only 1 day versus 6 days in the tackle leagues.

Conclusion(s): Injury rates in youth football are similar to the rates previously reported in high school and college football. Tackle football players are more likely to be injured than flag football players and are more likely to suffer a severe injury. However, the rates of concussion between flag and tackle football were not statistically significantly different, even in this very large prospective cohort.
**Impact of Copy Number Variants and Genetic Defects on the Outcome of Neonates with Congenital Heart Disease**  
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**BACKGROUND:** Congenital heart disease (CHD) remains the most common birth defect, affecting 1% of newborns yearly. The underlying etiology of CHD remains poorly understood, yet CHD likely results from interactions between genetic and non-genetic factors. Chromosome microarray testing suggests that copy number variants (CNVs), structural variations of the genome, contribute to 5-15% of CHD cases. CNVs have been correlated with adverse outcomes in children with CHD harboring genetic defects. With a majority of CHD patients surviving into adulthood, the focus of research has transitioned to improving post-surgical outcomes and the quality of life of patients. Due to the rising emphasis on the use of quality metrics to evaluate congenital heart programs, understanding if CNVs affect clinical outcomes has increasing importance.

**PURPOSE:** This study aims to evaluate the effect CNVs and other genetic defects have on perioperative morbidity and mortality in neonates undergoing congenital heart surgery.

**METHODS:** A single institution, retrospective study was performed to evaluate the effect CNVs have on clinical outcome in neonates who underwent congenital heart surgery. The patients were assigned Society of Thoracic Surgeons - European Association for Cardio-Thoracic Surgery Congenital Heart Surgery Mortality Categories to allow clinical outcomes to be assessed in relation to the complexity of the operation performed. Two sample t-tests or ANOVA tests and Chi-square or Fisher’s Exact test were utilized for statistical evaluation of continuous and categorical data sets, respectively. Logistic regression was used to evaluate the association of CNVs with complications following surgery to adjust for multiple confounding risk factors.

**RESULTS:** 118 CHD patients underwent cardiac surgery and genetic testing within the neonatal period at the University of Iowa Stead Family Children’s hospital between 2008 and 2015. Of the 118 patients, 63 had normal genetic arrays and 55 had abnormal genetic arrays. No statistically significant differences in mortality, length of stay, length of intubation or the prevalence of renal, gastrointestinal, or neurological dysfunction between the groups were identified. Patients were further subdivided into high, moderate, and low surgical complexity case groups. No statistically significant differences in outcomes were found after accounting for case complexity.

**CONCLUSION:** CNVs do not appear to affect clinical outcome following congenital heart surgery in the neonatal period, regardless of case complexity. Adverse events following congenital heart surgery appear to be more greatly associated with the complexity of the surgery rather than the presence of genetic abnormalities. This should be considered when counseling patients prior to surgical repair.
Enhanced Salivary Stone Characterization through Utilization of a Novel Computed Tomography Protocol

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At the conclusion of this presentation, the participants should be able to evaluate salivary stone density using attenuation on computed tomography (CT) to assist in treatment decision-making and research protocols.

Objectives:
Define radiographic characteristics in comparison of submandibular and parotid intraductal calculi using a novel CT scan windowing protocol designed to assist in pre-operative planning and future research studies.

Study Design:
Retrospective imaging analysis at an academic tertiary care hospital

Methods:
Using the ICD-9 code (527.5), 193 patients diagnosed with sialolithiasis at a large academic medical institution between 03/2013 and 03/2017 were identified. Records review identified 116 patients with adequate imaging studies for analysis by standard abdominal window settings and novel ‘salivary stone window’ settings. Stone size and location were recorded and a selective free-hand region of interest tool was used to determine stone density measured in Hounsfield units (HU), and stone heterogeneity index (SHI): defined standard deviation of a HU.

Results:
116 patients (60 men and 56 women, age: 50.25 ±15.23 years) with 147 total stones met criteria for the study. Submandibular stones were significantly larger, denser, and had higher SHI compared to the parotid stones (p < 0.05). A nonlinear regression analysis of all patients suggests a logarithmic relationship between stone size and stone density (Density[H.U.] = 125.3 * ln(Volume[mm³]) – 181.1 ; R² = 0.779).

Conclusions:
Sialolith size is logarithmically related to stone density. Submandibular stones are larger, denser, and have greater heterogeneity compared to parotid stones. This novel CT salivary stone window protocol provides data to assist in treatment planning and provides standardized analysis for future research studies.
Characterization of cardiac specific Transcription Factor EB overexpression in a murine model
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Background
Autophagy is a dynamic cellular recycling pathway, in which defective organelles and proteins are engulfed and isolated into autophagosomes, and degraded following autophagosomal fusion with the lysosome. The products of autophagy can be re-used for anabolic cellular processes and energy generation particularly during nutrient starvation. Impaired autophagic flux has been observed in several cardiovascular pathologies, including ischemia/reperfusion injury, hypertension, and heart failure. Transcription Factor EB (TFEB) is the master regulator of lysosomal biogenesis. Furthermore, cell culture studies have shown that a stable overexpression (OE) of TFEB can upregulate autophagy. Therefore, the purpose of this study is to generate and characterize stable, cardiac specific TFEB OE in a murine model to further study its putative therapeutic potential in the context of cardiovascular disease.

Methods
A Tetracycline On (TET On) system was used to achieve a doxycycline inducible, cardiac specific OE of TFEB. This requires two transgenic components: 1) a reverse tetracycline-controlled transactivator (rtTA) transgene coupled to a cardiac specific promoter (α-MHC) and 2) a TFEB transgene coupled to a Tetracycline Response Element (TRE). In the presence of doxycycline, cardiac specific rtTA is activated and binds to the TRE element, promoting TFEB transcription. Transgenic and wildtype (WT) mice were treated for 30 hours with doxycycline (1g/kg) or normal chow (2920X). Echocardiograms were obtained to quantify cardiac function. The mouse hearts were subsequently harvested, weighed, and sampled for use in western blot analysis, transmission electron microscopy (TEM) and mitochondrial respiration using permeabilized fibers.

Results
Mice that harbored both the TFEB and rtTA transgenes overexpressed TFEB on both doxycycline and wildtype feed, suggesting a leaky transgene. TFEB OE mice revealed decreased cardiac function compared to WT mice, as evidenced by reduced ejection fraction (EF), end systolic volume and end diastolic volume (ESV and EDV) (p<0.05). TFEB OE mice showed evidence of cardiac hypertrophy, increased heart weight to body mass ratio (p<0.05), and heart weight to tibia length ratio (p<0.05). Western blot analysis revealed induction of vATPase, LC3-1, Beclin-1, p62, p-mTOR\textsuperscript{S2448}, and p-S6\textsuperscript{S235/236} protein content in TFEB OE mice relative to WT mice (p<0.05). No significant difference was found between OE and WT mice with respect to mitochondrial subunit expression, although PGC1α, a co-activator of mitochondrial biogenesis, was induced (p<0.05). Results from Transmission Electron Microscopy revealed disorganized cristae. However, there was no significant difference in mitochondrial respiration rates between WT and TFEB OE mice.

Conclusion
TFEB OE results in cardiac dilation, hypertrophy, and cardiac dysfunction. At the cellular level, TFEB OE results in increased lysosomes, increased autophagy markers, and upregulated mTOR signaling. Although PGC1α was induced, TFEB OE has no significant impact on mitochondrial function at 30 hours. Future work will involve switching to a Tetracycline Off (TET Off) system to mitigate leaky expression of the TFEB transgene, measuring autophagic flux via a chloroquine assay, and inhibiting mTOR signaling with rapamycin to determine if the cardiac phenotype (hypertrophy and heart failure) is mTOR dependent.
Assessing the effects of ripasudil, a novel Rho kinase inhibitor, on human corneal endothelial cell health.

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Abstract

BACKGROUND: Ripasudil hydrochloride hydrate (ripasudil; Glanatec® ophthalmic solution 0.4%; Kowa Company, Japan) is a selective inhibitor of Rho-associated coiled-coil protein kinase (ROCK) currently approved in Japan for ophthalmic use. ROCK is a kinase that plays a central role in the assembly of actin-myosin filaments, cell cycle progression, and apoptosis. Previous studies of ROCK inhibitors have shown promise that this class of drugs may provide protective and regenerative effects on corneal endothelial cells (CECs) by increasing cell migration in wound healing, improving cell adherence, promoting proliferation, and inhibiting apoptosis. As the donor corneal endothelium experiences a significant amount of cell attrition during keratoplasty procedures, endothelial protective drugs may improve the integrity and health of donor corneas in the postoperative period and ultimately improve transplant outcomes.

PURPOSE: To determine if ripasudil can improve endothelial cell health in donor corneas by quantifying changes in mitochondrial respiration and cell vitality.

METHODS: Corneoscleral tissues used in this study were obtained from cadaveric sources according to Iowa Lions Eye Bank and Eye Bank Association of America standards. All tissues were suitable for transplantation and research consent was obtained. The endothelial cell-Descemet membrane (EDM) tissue complex was peeled and isolated for use in mitochondrial function and apoptosis assays. To examine the short-term influence of ripasudil on mitochondrial activity (one-hour exposure), EDM tissues were exposed to standard assay media or assay media supplemented with 10 µM ripasudil. To examine the long-term influence of ripasudil (24-hour exposure), paired corneas were placed in standard Optisol GS or Optisol GS supplemented with 10 µM ripasudil. Untreated and treated EDM tissues were assayed for mitochondrial activity (oxygen consumption rate [OCR; pmole/min/cell]) using extracellular flux analysis of oxygen and compared. The protective effects of ripasudil were assessed by exposing EDM tissues to 1µM staurosporine for 4 hours. EDM tissues were assayed for apoptosis and necrosis and the percentage of injured cells compared between treatments.

RESULTS: Mitochondrial respiration metrics did not differ between ripasudil-treated and untreated tissues after exposure for one hour (P>0.25) or 24 hours (P>0.19). Following exposure to staurosporine, the percentage of necrotic CECs did not differ (P=0.18) between ripasudil-treated (4.3%, SEM 0.7%) and untreated (6.8%, SEM 1.8%) tissues. In contrast, the percentage of apoptotic cells was lower (P=0.02) in ripasudil-treated tissues (2.8%, SEM 0.7%) compared to untreated controls (3.8%, SEM 0.9%) following exposure to staurosporine.

CONCLUSIONS: Mitochondrial respiration in CECs is not altered following acute or long-term exposures to ripasudil. However, CECs appear to gain some anti-apoptotic protective effect from a 24-hour treatment with ripasudil. These data suggest that ripasudil does not appear to pose any toxic effects on CECs and may help improve the integrity of the corneal endothelium.
Dendritic cells are bone-marrow derived cells of the innate immune system with antigen presenting capacity and the ability to initiate an inflammatory cascade by generating protective cytokines. Recent studies have proposed that migration is guided independent of adhesive factors and is controlled by dynamic internal changes that provide for the protrusive flow of cytoskeletal elements, primarily through changes in actin, myosin, and microtubule organization.\(^1\) Thus, there is a necessity for greater elucidation and understanding of how the cytoskeleton generates forces and what enzymatic and signaling factors are involved in the coordinated intracellular changes that polarize cells and signal the formation of the leading edge in cytoskeletal organization within the cell, ultimately allowing for a holistic migration process. This analysis requires a combination of biophysics, cellular and molecular biology, and imaging techniques that we hope to utilize to study these unique processes in real-time. Moreover, we aimed to understand these locomotive dynamics in the context of mechanical and chemical cues within tissues. A general aim of the project was to monitor dendritic cells in various physiological environments with live-action imaging in order to better clarify mechanisms by which dendritic cells can coordinate migration and cell motility in time and space.

Nucleotide-binding domain leucine-rich-repeat-containing receptors (NLRs) are a class of pattern recognition receptor that respond to host perturbation from either infectious agents or cellular stress. NLRP10 is the only NLR lacking the putative ligand-binding leucine-rich-repeat domain, and is thought to be a negative regulator of other NLR members. Adaptive immunity has been shown to be impaired in the absence of Nlrp10.

To prepare the BMDCs, primary dendritic cells were generated from murine bone marrow by culturing the cells and using GM-CSF solution. At day 7 of culture, the cells were transferred to a tissue-culture dish. To induce maturation of DCs, LPS solution was added to the dish for 24 hours of incubation at 37°C, 5% CO\(_2\), 95% H\(_2\)O. Construction of a migration chamber was completed a few days ahead of time. After incubation of the LPS stimulated cells, a collagen gel matrix containing cells was cast using a pre-determined pipetting scheme. A chemotactic gradient of CCL19 was then added to both populations, WT and NLRP10, and time-lapse DIC video microscopy was initiated. Analysis of the imaging data was conducted with ImageJ software.

In this system, wild-type (WT), but not Nlrp10\(^{-/-}\) BMDCs, could successfully traverse the matrix towards a CCL19 chemokine gradient. Migratory WT DCs exhibit leading edge coordination and cell polarity as key defining features. However, Nlrp10 BMDCs exhibit features that are drastically different than WT. NLRP10 DCs exhibit multiple, long protruding lamellipodia, failed leading edge coordination, failed cell polarity, and an appearance of embedment in collagen. However, the trapped Nlrp10 BMDCs could both sense the chemokine gradient and continuously move dendrites suggesting that basic chemotactic machinery was intact.

Brainstem projections to Anterior Insula may promote arousal

The neural circuits that control arousal are some of the most important and clinically translatable circuits in the entire human brain. Understanding the wakefulness circuitry of the human brain would allow the development of novel therapeutics for sleep disorders, including narcolepsy, insomnia, and coma. However, existing literature pinpointing the central nuclei of arousal is contradictory, ranging from the “Ascending Reticular Activating System” to the Parabrachial Nucleus (PBN)\(^2\), Basal Forebrain\(^3\) and Locus Ceruleus\(^4\). Other recent research has shown the upper pontine tegmentum is frequently damaged in coma patients, and that this area is correlated on fMRI with the anterior insula\(^5\).

To identify the neuronal basis of this correlation, we performed retrograde tracing experiments using Cholera Toxin Subunit B (CTb) in 8 week old wild-type mice. As expected, this retrograde tracing showed that there is significant contralateral cortical connectivity, as well as projections from the thalamus. However, there was only weak labeling from the PBN.

We hypothesized that the insula must also project back to the PBN, as a feedback mechanism. We injected CTb in the medial portion of the PBN to identify these connections. We found a surprising amount of projections from the insula to the PBN, although these results were complicated by the spread of the CTb into the auditory nuclei ventral to the PBN.

Finally, we hypothesized that because the PBN is critical for arousal\(^6\), and that most neurons in the parabrachial nucleus are glutamatergic\(^7\), lesioning the glutamatergic neurons of the PBN would produce coma. We injected AAV2-Flex-Cas3 bilaterally into the PBN of a VGlut2-Cre mouse. These mice remained alert and grossly normal for the following week, indicating that other structures are also critical for arousal.

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Relationship Between Evaluation for Pulmonary Embolism and Provider Fatigue in the Emergency Department

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BACKGROUND: Because of its pervasiveness and potential ill effects on patient health outcomes, fatigue is often referred to as the ‘Achilles’ heel’ of medicine. Previous attempts to quantify how fatigue affects clinical practice have mostly focused on surveys and objective cognitive performance. While these may be important to limiting fatigue’s effects, little is known on how fatigue directly affects a clinician’s decision making at the bedside.

OBJECTIVE: Our primary objective in this study was to understand how fatigue affects adherence to a well-established clinical guideline. We hypothesized that physicians fatigued either by lengthy shifts or nightshifts would be less likely to follow evidence-based clinical guidelines.

METHODS: Our study utilized the University of Iowa medical records, EPIC, focusing on patients who had a computed tomography (CT) angiography ordered for a suspected pulmonary embolism (PE) in the Emergency Department. Patients were retrospectively risk-stratified using data contained in the medical record according to the simplified revised Geneva score to find patients with a low risk for PE. Adherence was based on whether a D-dimer test was ordered for this patient group. Additionally, time of CT order and length of shift were recorded as a surrogate measure of fatigue. Relative risks of adherence to PE evaluation guidelines was recorded between nightshifts compared to dayshifts, as well as late into a physician’s shift as compared to earlier in their shift.

RESULTS: Results indicated a relative risk of adhering to guidelines at 1.01 (95%CI 0.88-1.17) for physicians late into their shift compared to early, and 0.93 (95%CI 0.72-1.19) for physicians working at night compared to the day, both of which lacked statistical significance.

CONCLUSIONS: These results suggest that fatigue produced by lengthy shift work and nightshifts do not significantly affect a clinician’s adherence to evidence based guidelines for the evaluation of PE, though there were several limitations. We suggest that future research in fatigue should look at other clinical guidelines, draw from a large pool of physicians, and utilize other measures of fatigue. Though this study did not find a significant connection between fatigue and clinical decision-making, we believe it takes an important first step in describing fatigue in a real clinical setting.
The Role of Extracellular Histones in Cancer Metastasis

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BACKGROUND: Histones are a family of positively charged proteins used in DNA packaging in eukaryotic cell nuclei. Histones are released extracellularly during cell apoptosis, necrosis, and neutrophil immune responses known as NETosis. Outside of the cell, histones act as damage associated molecular patterns (DAMPs) and activate Toll-like receptors (TLRs). Studies have shown that histone-induced activation of TLR4 increases invasion of cancer cells in vitro. However, the role of extracellular histones in other stages of cancer metastasis (intravasation, microthrombi formation, anoikis inhibition, adhesion, extravasation, and proliferation) have not been defined. The purpose of this study was to examine the role of extracellular histones in the adhesion and proliferation stages of cancer metastasis.

HYPOTHESIS: Extracellular histones promote cancer metastasis by increasing endothelial cell adhesiveness and tumor cell proliferation.

METHODS: To investigate the role of histones in endothelial cell adhesion, BALB/c mice were injected with purified calf thymus histones at a concentration of 50 mg/kg via tail vein. Mice were sacrificed two hours post-injection. Lung, heart, liver, spleen, and kidney tissue was harvested and fixed in a 4% PFA solution and cryopreserved in sucrose solution before being embedded in OCT and sectioned. Immunohistochemical staining was performed to visualize the actin cytoskeleton, cell nucleus, and H3 histone subunits. To study the effect of extracellular histones on endothelial cell adhesiveness, EA.hy926 endothelial-epithelial hybrid cells were treated with purified calf thymus histones (CTH) in Opti-MEM at concentrations 0, 25, 50, 75, and 100 µg/mL for 6 hours. Following treatment, cells were harvested using 0.05% trypsin and a cell scraper. Cells were washed with DPBS and frozen at -80°C overnight. RNA was purified from cell lysates using Qiagen RNeasy RNA extraction kit. cDNA was synthesized using purified RNA, and qPCR was performed to determine E-selectin and ICAM-1 mRNA expression. To examine the effect of extracellular histones on tumor cell proliferation, HT29 human adenocarcinoma cells were seeded 3000 cells/well in 96 well plates. Cells grew for 24 hours before being treated with CTH in Opti-MEM at concentrations of 0, 0.8, 1.6, 3.1, 6.3, 12.5, and 25 µg/mL. Cells were also treated with Opti-MEM + 10% FBS as a positive control. Cells were cocultured with treatments for 0, 24, 48, 72, and 96 hours. An MTS cell proliferation assay was performed at each timepoint. Percent proliferation relative to the average 0-hour timepoint measurement was calculated for each treatment group.

RESULTS: Tissue from CTH-injected mice showed histone localization at the endothelium of hepatic blood vessels. Treatment of EA.hy926 cells with CTH resulted in a 30-fold increase in E-selectin mRNA expression and a 7-fold increase in ICAM-1 mRNA expression in the 50 µg/mL treatment group. CTH treated HT29 cells showed significant (p < 0.05), dose-dependent increases in proliferation after 96 hours of cotreatment.

CONCLUSIONS: Extracellular histones localize to endothelium when injected intravenously in mice. In vitro, treatment of EA.hy926 endothelial-epithelial hybrid cells with CTH results in increased expression of E-selectin and ICAM-1 mRNA. This suggests that circulating extracellular histones cause increased endothelial adhesiveness. Additionally, HT29 adenocarcinoma cells display significantly increased proliferation when treated with CTH. Taken together, these results indicate that extracellular histones may play a consequential role in cancer progression and metastasis.
Title: Clinical Frailty Scores Predict Re-Admission with Fall Following Traumatic Injury

Victor Hatcher; Kathleen Romanowski, MD

Introduction: Fall related injuries are significant sources of morbidity and mortality in the elderly. Unfortunately age alone is not a sufficient predictor of physiological fitness in the elderly population. Many scales have been developed to measure patient frailty, but most cannot be used to study retrospective data. The Canadian Study of Health and Aging clinical frailty scale (CSHA CFS) is a simple 7-point clinical opinion scale which has been validated to predict mortality and institutionalization in elderly internal medicine patients. Using the CSHA CFS, we hypothesize that patients admitting with higher frailty scores will re-present with a greater incidence of falls post-admission.

Methods: After obtaining IRB approval, a five year (2010-2015) retrospective chart review was performed of all patients ≥50 years of age admitted to the University of Iowa Hospitals and Clinics after a trauma or burn injury. Data will be collected from the institutions trauma and burn registry and electronic medical record will include: Demographics (age, gender, race, co-morbidities); Admissions data (Admission date, Discharge date, Length of Stay, Number of admissions, Complications, Therapy Notes); and Injury data (ISS). Frailty scores were assessed from admission data and calculated using the Canadian Study of Health and Aging Clinical Frailty Scale. Values expressed as mean± SD.

Results: A total of 759 patients with a mean age of 70.25± 13.5 years, 358 men (47.2%) and 401 women (52.8%) were analyzed. The mean CSHA CFS among all participants was 4.0± 1.14. Thirty patients (3.95%) included in the study population died of their injuries. There was not statistical difference in CSHA CFS between those who survived and those who died of their injuries. Mean injury severity score of the population was 9.65± 7.76. There was not a statistically significant difference between the frail patients (CSHA CFS 5-7) and the non-frail patients (CSHA CFS 1-4) with regards to ISS. One hundred and eight (14.2%) patients in this study population had previously presented with falls. Patients who had previously presented with falls had a mean CSHA CFS of 4.639± 0.97 while those who had not previously presented with a fall had a mean score of 3.897± 1.13 (p<0.001). Patients who were frail (CSHA CFS of 5-7) had a mean of 1.3± 0.75 falls in the year following admission compared to their less frail counterparts (0.91± 0.80 falls, p<0.001). There was no statistical difference in the mean length of stay (LOS) between the frail and non-frail patients. Upon discharge, non-frail patients were statistically more likely to be released home with no assistance; while those with higher pre-admission CHSAA CFS scores were more likely to be discharged to skilled nursing facilities (p<0.001). Frail patients were statistically more likely to be on anticoagulant therapy than their counterparts (p<0.00001). While not statistically significant results approach significance when comparing mortality and anticoagulation therapy use in the non-frail (CSHA CFS 1-4) patient population. The same can be said for the mortality and anticoagulation use in the in the frail (CSHA 5-7) cohort.

Conclusions: Frailty scores on admission allow for an improved assessment of pre-injury physiologic condition in burn and trauma patients 50 years and older. Poor pre-injury physiologic fitness, as assessed by frailty scores, is a predictor fall readmissions post-trauma/burn, post-hospitalization discharge locations, and anticoagulation therapy use, but does not appear to influence mortality or length of stay. Going forward we must use the results of this study to further investigate the relationship between falls, frailty and anticoagulant use to determine the point at which risk of fall in a frail patient outweighs the risk the stroke, especially for patients who are on atrial fibrillation anticoagulation.
BESS Score as an Injury Predictor

John Henstrom, BS, Andrew Peterson, MD

Abstract

Background: Injury rates in NCAA football have been found to be as high as 8.1 injuries per 1000 exposures (games and practices per player). Our research was intended to advance the ability to predict injuries so they can ultimately be prevented. The Balance Error Scoring System (BESS) test is an integral part of the Standardized Concussion Assessment Tool (SCAT). A baseline BESS is established for each player at the beginning of the season and then used as part of a standard concussion protocol when an event transpires. The BESS score is indicative of postural stability. Athletes reporting previous lower extremity injury (-ies) and/or concussion(s) have lasting postural stability deficits and are statistically more likely to be re-injured. Various other balance tests have been studied in conjunction with injury rates, but none have been found to be predictive of injury. This study aimed to determine if the results from the BESS score that all players receive at intake is correlated with the rate of injury post assessment.

Hypothesis: The Null hypothesis is that there is no association between baseline BESS score and rate of injury among Division 1 college football players.

Methods: Working with the football trainers we collected the charted BESS scores from each player from 2011-2016. Using SIMS (Sports Injury Monitoring System) we viewed each injury sustained by a player. An injury was defined as an event that required the player to either come out of the game/practice and receive treatment or miss the game/practice/day entirely. We categorized each injury based on the time missed and whether it was in-season. Retrospective chart review was performed on the EPIC database for all the players with time-loss injuries as a control between the reporting systems. The total numbers were gathered and analyzed on SAS software.

Results: The average BESS score of the 180 players sustaining at least one injury during their career at Iowa was 14.93 (range: 3-33) vs 13.19 (range: 9-19) for the 16 players who did not sustain an injury within the time limits. The overall Pearson correlation coefficient was found to be only 0.025 between BESS score and injury in general. Even when testing lower extremity musculoskeletal injuries, the Pearson correlation was only 0.068. Furthermore, there was not significant correlation between BESS score and specific types of injury or time (days, practices, or games) missed.

Conclusions: Data compiled and analyzed during this retrospective study does not indicate that the BESS score at intake is predictive of injury risk in NCAA division 1 college football players. When comparing injury rates overall or stratifying the severity of the injury, the BESS scores did not show significant differences between the groups.
King Devick Test as a Monitor of Anesthetic Recovery – A Validation Study
Stijn Hentzen, BS, Denisa Haret, MD, Caitlin Ward, Andrew Peterson, MD, MSPH

**Background:** Complete recovery from anesthesia is vital to avoid developmental impediments, especially in pediatric patients. It is important for providers to quickly recognize a patient’s poor recovery from anesthesia so that they can be treated and returned to a homeostatic balance before any physiological damage to the patient occurs. Previous studies have evaluated the utility of psychomotor tests to measure anesthetic recovery; however, most of these tests are time consuming and are rarely used in the post-anesthesia recovery area. The Trieger Dot Test (TDT) is one of these tests and is a well validated measure of anesthetic recovery. The King Devick Test (KDT) has emerged as a scientifically valid test for a multitude of neurological medical conditions. The test uses a system of Rapid Number Naming which assesses the subject’s ability to read aloud a series of numbers and determines their ability to discern levels of contrast. This test is able to detect impairments of eye movements, language, attention, and overall neurological function and is validated down to an age of 5 years.

**Hypothesis:** There is no measured difference the return to baseline between the King Devick Test and the Trieger Dot Test in determining sedation recovery in non-neurological pediatric patients.

**Methods:** Subjects were recruited from the University of Iowa pediatric sedation clinic. The recruited subjects were children and adolescents between the ages of 7-17. No patients with a neurologic diagnosis were included in the study. Consent of parent/guardian and ascent of child was obtained prior to testing. Each patient performed both the KDT and TDT pre-anesthesia to establish a baseline. These tests were repeated once when the patient woke up and again 20 minutes later. These tests did not interfere with clinical care, and the discharging sedation physician was blinded to the results. After discharge, the family repeated both tests on the subject at home 24 hours post-anesthesia.

**Results:** A total of 41 patients were recruited for the study. Extraneous deviations for the TDT were compared to a total score for the KDT which summed the time and errors from all three cards. Baseline was established as baseline +/- 1/5(median change in score after sedation) in order to account for natural random variation. The results of the paired t-tests for KDT show that there is a highly significant difference between scores at all three time points (p < 0.001). The p-values for the same tests for TDT were also all significant at the 5% level, however the difference between deviations at the second test after sedation is much less significant (p = 0.04). The results of McNemar’s test show agreement between the two tests at the first test post sedation (p = 0.31), but a significant difference between the tests at the second test (p = 0.04).

**Conclusion:** McNemar’s test shows that at the second post test the TDT significantly classified more patients at baseline than the KDT. This corresponds with the results of the paired t-tests, in that more patients were close to baseline at the second post test for the TDT, than for the KDT. Overall, we can conclude that there is evidence that the KDT is more sensitive to impairment than the TDT, and if it were used in place of the TDT it would take more time for children to return to baseline after sedation.
Thalamic coma? The neuroanatomical correlates of impaired arousal following diencephalic lesions in humans
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Background: Arousal, or wakefulness, is a critical feature of consciousness, yet the neural circuitry that maintains it is poorly understood. Early models of an ascending reticular activating system (ARAS) originating from the reticular formation have been revised to include discrete brainstem regions that are preferentially involved in maintaining wakefulness, but the ascending projections from these brainstem regions to the cortex are not well understood. Classically, these projections were thought to synapse in the thalamus en route to the cerebral cortex. Recent work in experimental animals has challenged this long-held assumption, positing that the thalamus is not a critical structure in the arousal pathway. Here, we systematically evaluate this question in humans for the first time using lesion mapping to address whether the thalamus is a critical node in the arousal circuit.

Hypothesis: Severe impairments in arousal (coma, stupor) are not seen following focal lesions limited to the thalamus, but may be seen when thalamic lesions extend into the hypothalamus and brainstem.

Methods: This was a retrospective study of patients with a focal ischemic stroke of the thalamus +/- extension into the brainstem. Patients were identified by searching electronic medical records of two large academic hospitals. The level of arousal of each patient during the first 12 hours after the onset of symptoms was rated on a 6-point scale based on neurological exam findings (coma, 1; stupor, 2; obtunded, 3; somnolent, 4; lethargic, 5; awake, 6). The location of the stroke was identified from magnetic resonance imaging (MRI) acquired for clinical purposes. Lesion location was reproduced onto a 3D template brain and the accuracy of each lesion was reviewed by two neurologists blinded to clinical outcome. Voxel-based lesion-symptom mapping (VLSM) was used to identify voxels that, when lesioned, were associated with greater impairment in level of arousal (p < 0.05, correcting for false discovery rate).

Results: Across 33 patients included in the analysis, four lesions caused coma/stupor and each of these were bilateral thalamic infarcts that extended into the midbrain and pons. Thalamic lesions that did not involve the brainstem did not cause any severe impairment of arousal. VLSM revealed that regions in the mesencephalic tegmentum, left posterior hypothalamus, and posterior ventromedial thalamus near the bilateral parafascicular and centromedian nuclei are associated with impaired arousal (P <0.05, corrected for false discovery rate).

Conclusions: This study provides the first systematic evaluation of thalamic lesions and the associated thalamic role in arousal and level of consciousness. In this sample, all thalamic lesions that severely impair arousal extend into the brainstem, whereas lesions limited to the thalamus are associated with less severe impairments in arousal. These results are consistent with previous studies in experimental animals that challenge the long-held view that the thalamus is a critical node in the arousal pathway.
Rapid Identification of *Pseudomonas aeruginosa* by Species-Specific Endonuclease Activity

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Abstract

**BACKGROUND:** Deficiencies in current clinical diagnostic methods for bacterial infections result in costly delays in the proper diagnosis of many infections. Infected patients typically spend days on broad-spectrum antibiotics prior to the identification of the offending pathogen, a situation that generates greater antibiotic resistance among bacterial pathogens. Healthcare systems pay for days of extra hospitalization and patients are more likely to develop serious complications or succumb in the interim due to suboptimal therapy. Nosocomial infections from *Pseudomonas aeruginosa* (*P. aeruginosa*) can be particularly problematic because of their difficulty to treat and their distinctive antibiotic susceptibility profiles. Additionally, the emergence of antibiotic resistance in some strains of *P. aeruginosa* has earned it the CDC designation of “serious threat” that requires better identification and treatment. Previous work has established the ability to use secreted endonuclease activity on synthetic fluorescent oligonucleotide probes as a highly sensitive means of detecting bacteria. Previous preliminary experiments with a knockout library of *P. aeruginosa* indicated that an enzyme we refer to as “ChomperX” could be predominantly responsible for the activation of a quenched fluorescent oligonucleotide probe (“Probe A”) in *P. aeruginosa* culture supernatants. If ChomperX can be confirmed as a robust biomarker of *P. aeruginosa*, clinical diagnostic methods could be generated that use this sensitive oligonucleotide-based method to rule out the possibility of a *P. aeruginosa* infection on the order of minutes to hours, not days as is the current standard.

**PURPOSE:** We investigated if “ChomperX” accounts for the predominant nuclease digestion of “Probe A” in supernatants of *P. aeruginosa* liquid cultures. To answer this question, we created two strains of *P. aeruginosa* - one with the gene for ChomperX overexpressed, and one with the gene deleted. We hypothesized that the overexpression construct would show a substantial increase in Probe A activation, whereas the deletion construct would show activity much closer to that of a negative control.

**METHODS:** We generated a ChomperX in-frame deletion mutant wherein most of the coding sequence was removed from the chromosome genome leaving the first 3 codons of the gene fused to the c-terminal 3 codons. A targeting vector encoding gentamycin-resistance was conjugated from *E. coli* Sm10 into *P. aeruginosa*. PCR-based analysis confirmed that the ChomperX gene was deleted as intended. For the overexpression construct, *P. aeruginosa* strain PA14 was transformed with a pJN105 plasmid expressing the poly-histidine-tagged ChomperX gene. To detect activity of the ChomperX protein on Probe A, supernatants of bacterial cultures were combined with the probe in reaction buffer and incubated for 1 hour. The resulting fluorescence was measured in triplicate with a 96 well plate reader.

**RESULTS:** Overexpression of ChomperX yielded a fluorescent signal 5 times stronger than wild type *P. aeruginosa* and 10 times stronger than background in the reaction conditions measured. Deletion of ChomperX from the genome of *P. aeruginosa* yielded an above-background fluorescent signal only 1/7th that of the wild type *P. aeruginosa*.

**CONCLUSIONS:** The results are consistent with our hypothesis; *P. aeruginosa* was not able to strongly digest Probe A when ChomperX was deleted from the genome, whereas wild type *P. aeruginosa* (with functional ChomperX) was able to do so, exhibiting a 140% increase in fluorescent signal above background. When ChomperX was overexpressed, the fluorescent signal was 5 times stronger than wild type and 10 times stronger than background. The evidence suggests that ChomperX is the enzyme that is predominantly responsible for digesting Probe A in *P. aeruginosa*. Future work will focus on development of a highly sensitive clinical diagnostic assay that detects *P. aeruginosa* via ChomperX activity. Such a clinical assay has the potential to decrease time to diagnosis to just a few hours for *P. aeruginosa* infections. With a faster diagnosis, the right antibiotic regimen can be started sooner, leading to better patient outcomes, reduced cost to the hospital, and reducing the generation of antibiotic resistance.
Role of Individual Motivation and Role on Team Identity in Assertive Community Treatment Teams.

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Abstract
KEYWORDS: Assertive community treatment, Team performance, Team identity, Motivation.

INTRODUCTION:
Assertive Community Treatment (ACT) is a team-based treatment model that aims to provide 24/7/365 support to people with serious mental illness in major areas of life such as work, social relationships, and living independently. To provide comprehensive care, ACT team members come from many distinct and diverse disciplines ranging from social work to substance abuse counseling. With a high requirement for teamwork, there is value in being able to predict the quality of care a team will provide when selecting team members. It would then be ideal to have a measure we can evaluate early in a team’s formation that can predict long term success. We thus chose individual team member motivation to join the team, which can be measured from the start of a team design process, to predict team identity, defined as cognitive, evaluative, and emotional attachment to a group, which has been shown to reflect a team’s long term success.

OBJECTIVES:
We aim to determine whether an individual team member’s motivation to join the ACT team and their role on the team is correlated with their sense of team identity.

METHODS:
From a survey of 374 team members from 26 ACT teams, we conducted factor analysis to reduce the amount of data we were working with. For motivation, we reduced 10 questions to 3 underlying factors which we characterized as need, value or fit - paralleling our definition of team identity as cognitive, evaluative or emotional attachment to a group. For identity, we reduced the 9 questions to a single, “overall identity,” since reducing to more variables produced significant overlap. We then used multivariate regression analysis to test our hypotheses, controlling for individual team role, the multilevel data structure and potential dependency by including a random team effect, a role fixed effect. After, we conducted post estimation analysis to confirm the validity of our assumptions.

RESULTS:
Individuals who had the role of team leads or who joined teams based on “fit” were associated with greater team identity, whereas individuals who joined teams based on “need” was associated with less team identity. There was insignificant association with being a psychiatrist or joining the team based on values on the individual’s resultant team identity.

CONCLUSION:
As teams become more ubiquitous in healthcare and corporations have more choice over the composition of their teams, management should consider how motivation and role might impact their teams’ performance. Applying such a strategy could allow management to better predict which teams will succeed, and position teams for success.
Hemorrhage Associated With External Ventricle Drain Placement In Aneurysmal Subarachnoid Hemorrhage Patients On Dual Anti Platelet Therapy: A Retrospective Analysis

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2-Department of Neurosurgery, University of Tokyo, Tokyo, Japan

Object: Stenting and flow diversion of ruptured intracranial aneurysms requires the use of dual antiplatelet therapy (DAPT) to prevent in stent thrombosis. These patients may be at increased risk for post surgical hemorrhagic complications. In this study, we sought to investigate whether DAPT prophylaxis is a risk factor for hemorrhagic complications associated with placement of external ventricular drains (EVD) in patients with aneurysmal sub-arachnoid hemorrhage (aSAH).

Methods: Patients presenting to our institution with aSAH requiring placement of an EVD for obstructive hydrocephalus between July of 2009 and November of 2016 were included. Rates of radiographically identified hemorrhage associated with EVD placement were compared between patients who were on DAPT (Aspirin and Clopidogrel) for use of a stent or flow diverter, and patients who underwent microsurgical clipping or coiling and were not on DAPT by way of a backward stepwise multivariate analysis. Hemorrhages were judged to be clinically significant if they were temporally associated with neurologic declines, required further surgical intervention, or caused seizures.

Results: 443 patients were admitted for management of aSAH. 298 Required placement of an EVD. 120 (40%) were treated with stent assisted coiling or flow diverters and required DAPT, while 178 (60%) were treated with coiling without stents or microsurgical clipping and were not on DAPT. 42 (14%) cases of new hemorrhage along the EVD catheter were identified radiographically. 32 of these hemorrhages occurred in patients on DAPT, while 10 occurred in patients not on DAPT. After multivariate analysis, DAPT was significantly associated with radiographic hemorrhage [OR: 4.92, 95% CI: 2.45-9.91, p=.0001]. 5 hemorrhages (5 of 10 [50%]) were classified as symptomatic in those patients not receiving DAPT, while 10 hemorrhages (10 of 32 [31%]) were classified as symptomatic in those patients on DAPT (p=.4508).

Conclusions: Our clinical series confirms that patients with aSAH who are candidates for stent assisted coiling or flow diversion are at higher risk for radiographic hemorrhage associated with EVD placement. Despite this risk, the hemorrhages do not appear to be clinically significant. These data suggest that stent assisted coiling and flow diversion for aSAH are viable options in an era of evolving endovascular therapeutics.
Central line capability and critical care telemedicine reduce the odds of transferring a sepsis patient: A mixed methods study

Steven Ilko, BA; Azeemuddin Ahmed MD, MBA;; J. Priyanka Vakkalanka, ScM; Karissa Harland, PhD, MPH; Steven Q. Simpson, MD; Nicholas Mohr, MD, MS

BACKGROUND
Severe sepsis is a complex, resource intensive and high mortality condition. Though high-volume sepsis centers generally have improved sepsis outcomes, rural patients who undergo inter-hospital transfer from low-volume centers continue to do worse than their urban counterparts. These findings support a need for improved systems of care for regional quality improvement in the treatment of sepsis.

AIMS/HYPOTHESIS
The objective of this study was to identify hospital-specific factors associated with inter-hospital transfer and sepsis survival. We hypothesized that decreased inter-hospital transfer was associated with institutions that have a physician in-house 24-hours a day, intensive care units, the use of critical care telemedicine, the ability to place and maintain central venous catheters (CVC), and formal sepsis protocols/care plans/order sets.

METHODS
This study was a cross-sectional telephone survey of Iowa Emergency Department (ED) administrative leaders and a de-identified retrospective cohort of adults seen in Iowa EDs for severe sepsis and septic shock between January 2005 and December 2013. Logistic regression was performed to identify predictors of inter-hospital transfer in both univariate and multivariable models and presented in the form of unadjusted and adjusted odds ratios.

RESULTS
One-hundred fourteen institutions provided data (response rate= 98%), corresponding to a total of 150,845 visits for severe sepsis/septic shock at these facilities over the 7-year period. The univariate model suggested that many factors were associated with decreased odds of inter-hospital transfer. After adjusting for annual ED volume-septile and for Center for Medicare and Medicaid Services (CMS) hospital classification, the multivariable models suggested that only having the capability to place central venous catheters and having a subscription to a critical care telemedicine service were independently associated with lower odds of inter-hospital transfer (aOR: 0.69, 95%CI: 0.54-0.86; aOR: 0.69, 95%CI: 0.54-0.88 respectively). Furthermore, a facility’s participation in a sepsis-specific quality improvement initiative was independently associated with 62% higher odds of inter-hospital transfer (95%CI: 1.10-2.39).

CONCLUSION
Although many factors were associated with sepsis-specific inter-hospital transfer, the ability to place CVCs and the availability of CC-telemedicine were the only 2 factors independently associated with transfer behaviors. These factors suggest that sepsis capability indices could be developed to stratify hospitals on their ability to care for sepsis patients, as a first step in developing regional sepsis networks.
Respiratory Inhibition Upon Electrical Stimulation of the Amygdala in Mice
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Department of Neurosurgery¹ – UIHC, Department of Psychiatry² – UIHC

BACKGROUND: Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in individuals with medically refractory epilepsy. SUDEP cases in epilepsy monitoring units have suggested that death results from seizure induced peri-ictal disruption of breathing. Our previous work in humans has shown that seizure propagation to the amygdala produces apnea and oxygen desaturation, an effect reproducible with electrical stimulation of the amygdala. Strikingly, these patients were completely unaware that they had stopped breathing and displayed no struggle to breath (dyspnea). Thus, due to inhibition of breathing and a loss of dyspnea and awareness of apnea, this data suggest that seizure activity within the amygdala may lead to SUDEP, especially if combined with severe postictal unresponsiveness while face down in bed.

These observations above suggest the existence of a functional connection between the amygdala and neurons that control respiratory motor output in humans. There are multiple possible brainstem effector sites downstream of the amygdala that may mediate the observed apnea. These include neurons that generate respiratory rhythm, such as those in the pre-Botzinger complex and neurons sensitive to CO₂ and pH in the medullary raphe nuclei and retrotrapezoid nucleus.

HYPOTHESES:
1. Electrical stimulation of the amygdala in mice will induce apnea and oxygen desaturation
2. Apnea observed during amygdala stimulation is due to inhibition of the respiratory rhythm generator and therefore apnea will still be observed with isoflurane inhibition of CO₂-sensitive neurons

METHODS: C57BL/6 mice age 12-14 weeks were used to assess breathing during amygdala stimulation under two different types of anesthesia, ketamine/xylazine (k/x) and 1% isoflurane. Breathing was assessed via plethysmography or via an end tidal CO₂ monitor which sampled the animals’ expirations to record respiratory rate and instantaneous CO₂. Pulse oximetry and heart rate were assessed. For each mouse, a stainless steel bipolar electrode was inserted into one of the target brain regions: basolateral amygdala (BLA, relative to bregma in mm: anteroposterior -1.4, lateral 3.1, ventral 4.9 from skull surface), central amygdala (CeA, AP -1.4, ML 2.6, DV 4.9), hippocampus (AP -1.4, ML 1.0, DV 1.0), and motor cortex (AP 0 to +0.5, ML 1-2, DV 0.07). The stimulation paradigm consisted of 0.2 ms duration, bipolar, 5 mA square current pulses delivered at 50Hz for 15 s using an isolated pulse stimulator with at least 2 minutes between each trial. Multiple sites were stimulated in each mouse for comparative purposes.

RESULTS: Stimulation of the amygdala (both BLA and CeA) induced apnea similar to what is observed in humans, with both k/x and isoflurane. CeA stimulations resulted in more pronounced reduction in respiratory rate and apnea than BLA stimulations. Isoflurane reduced the respiratory rate more significantly than k/x anesthesia and often produced more pronounced apnea effects with amygdala stimulation.

CONCLUSIONS: This study strengthens the proposed mechanism of amygdala activation as a component of SUDEP. Isoflurane has been previously shown to inhibit the activity of CO₂-sensitive neurons, which normally stimulate the respiratory CPGs to increase respiration when hypercapnic. Since amygdala stimulation under isoflurane still induced apnea, this strongly suggests that the amygdala is functioning more directly to inhibit the CPGs rather than indirectly working by inhibiting the CO₂-sensitive neurons.
Title: Amino Acid Solvation Energy Calculations Perform 20x Faster on GPUs Compared to CPUs

Authors: Hernan Bernabe, Andrew Kalenkiewicz*, Michael Schneiders**

*Poster author & presenter, **Mentor/Principal Investigator

Background: Fast and accurate methods for computing biomolecular thermodynamics are increasingly playing a critical role in drug design and therapeutic discovery. Alchemical simulations have historically provided inspiration for structural modifications that may increase the binding affinity of synthetic leads to their biological target. However, properties of larger biochemical systems such as protein-protein binding affinities have been difficult to assess due to the tradeoff between speed and accuracy (since the speed of such simulations decreases when there are larger numbers of atoms).

Purpose: Our aim was to implement a computationally faster version of a previously described alchemical simulation method—named Monte Carlo orthogonal state random walk (MC-OSRW). We sought to achieve this by running a portion of the algorithm on NVIDIA GPUs. The overarching goal is to build a fast and accurate method for estimating protein-protein binding affinities.

Methods: The algorithm was implemented in Java as part of the Force Field X package. We utilized the CUDA-accelerated molecular dynamics method from the OpenMM package in order to delegate the most computationally intensive processes in our algorithm to NVIDIA GPUs. We tested our algorithm by using it to compute the solvation free energies of amino acid side chains and comparing the results to the non-GPU MC-OSRW algorithm.

Results:

We were able to accurately reproduce the solvation energies for the majority of the amino acids tested. Moreover, our algorithm gave a 20x speedup compared to the non-GPU MC-OSRW method.

Conclusion:

Our MC-OSRW GPU algorithm is a fast and accurate method for estimating amino acid solvation energies in silico. Further work will involve ironing out the minor inconsistencies encountered for a subset of the amino acids tested, as well as implementing our algorithm on larger systems.

Figure 1: CPU MC-OSRW (non-GPU) speed vs. GPU MC-OSRW speed (in nanoseconds per day)
**Title:** Evaluation of Central Sensitization and Psychological Factors in Patients with Chronic Achilles Tendinopathy

**Introduction:** Achilles tendinopathy (AT) is generally a difficult condition to treat as the pain mechanisms are not fully understood. A wide range of conservative and surgical treatments options are available, but as much as 40% of patients fail conventional methods and report chronic pain. New research is emerging that suggests central sensitization plays a role in continued chronic pain even after intervention. Additionally, psychological factors of kinesiophobia and catastrophizing are thought to play a role in impeding improvement with current therapeutic treatments for AT.

**Purpose/Hypotheses:** The purpose of this study is to evaluate the presence and persistence of central sensitization and kinesiophobia/catastrophizing before and after an anesthetic injection in people with chronic AT pain. We hypothesize that AT patients 1) will exhibit altered psychological factors compared to controls, 2) will exhibit signs of central sensitization compared to healthy controls, and 3) removal of peripheral pain stimuli, via an anesthetic injection, will decrease signs of central sensitization and altered psychological factors.

**Methods:** Participants with chronic AT and no prior surgical interventions, or additional autoimmune/inflammatory disorders, were recruited. Twenty patients (75% women), 48.8 ±2.2 years old, were matched with controls of similar age (±5 years), gender, and BMI (±3 kg/m2). Participants with AT were given a 0.5% ropivacaine injection to the Achilles in between two sets of repeated testing. All participants were asked to complete psychological questionnaires via Tamps Scale of Kinesiophobia (TSK) and the Pain Catastrophizing Scale (PCS) following standardized physical activities at two-time points. Participants also underwent pain pressure threshold (PPT) and temporal summation (TS) testing. Lastly, as a follow up, participants repeated the PCS questionnaire one-week later. Two-way mixed effects ANOVAs were used to compare groups (fixed effect) and time points (repeated effect) for TSK, PCS, PPT and TS.

**Results:** Preliminary results indicate that participants with AT had higher TSK (P<0.001) and PCS (P=0.001) than controls. Both groups had a small, yet consistent, decrease in TSK with the second set of testing (P=0.026). There was an interaction between group and time for the PCS with the AT group having a large decrease in PCS after the anesthetic injection (P=0.002). There were no differences between groups or time for PPT or TS (P>0.05).

**Conclusion:** Our preliminary findings suggest that patients with AT have heightened fear of movement related injury and catastrophizing of injury. This study indicates that patients with AT may benefit from education targeting kinesiophobia/catastrophizing. There does not seem to be a significant sign of central sensitization playing a role in pressure/pain. However, on an individual level there were some participants that exhibited significant changes, further research is necessary to better assess this question.
T Cell Characterization in Yellow Fever, Zika Virus, and Mumps Infection

Wahida Khan, BS, Jon Houtman, PhD, Jinhua Xiang, MD, Jack Stapleton, MD

BACKGROUND

Yellow Fever Virus (YFV), unlike Zika Virus (ZKV) and Mumps Virus (MV), has been shown to reduce T cell signaling post-infection. This preliminary study examined how infections with YFV, ZKV, and MV affect IL-2 release of T cells and viability and growth. We also examined how conventional techniques of T cell maintenance through anti-CD3/CD28 and IL-2 stimulation affect T cell behavior in contrast to unstimulated T cells in the context of a viral infection.

HYPOTHESES

1. CD3/CD28 and IL-2 stimulation is not required for maintaining T cell number.
2. YFV will lower IL-2 release from T cells, whereas ZKV and MV will not.
3. T cell activation and IL-2 may reduce YFV infection in T cells

METHODS

Peripheral blood mononuclear cells (PBMCs) were extracted from healthy blood donors by Ficoll-gradient centrifugation. PBMCs were infected overnight with YFV, ZKV, and MV at concentrations of 10⁶ PFU/million cells. For the IL-2 treatment group, PBMCs were incubated with IL-2 and anti CD3/CD28 for 24 hours before infection. Cells were washed and resuspended in fresh media with or without IL-2. Cell samples were collected 24 hours later then daily for a total of five days. All samples were tested for total and viable cell counts, and IL-2 release and viral RNA were measured in culture supernatants.

RESULTS AND DISCUSSION

Cell viability in non-stimulated cells averaged 90%, indicating that CD3/CD28 and IL-2 stimulation were not required for maintaining T cell number for short-term experiments. Surprisingly, none of the virus infections appeared to be cytopathic in non-stimulated PBMCs. However, in stimulated PBMCs, all three viral infections decreased cell viability with ZKV appearing to be the most cytopathic. All viral infections increased IL-2 release into culture supernatants compared to uninfected control cells. Thus, although YFV impairs IL-2 release following anti-CD3, it does not lower IL-2 release from non-TCR-stimulated, YFV infected T cells. PBMCs activated with anti-CD3/CD28 and maintained in IL-2 had higher YFV RNA produced than control cells. In contrast, the activated PBMCs had lower levels of ZKV RNA produced compared to control cells.

CONCLUSION

Our data suggest that anti-CD3/CD28 and IL-2 stimulation is not necessary for maintaining T cell viability for experiments less than 5 days duration. Despite data showing that YFV infection reduces TCR-mediated IL-2 release post infection, non-stimulated PBMCs produced IL-2 similar to controls. Further, activation and IL-2 enhanced viral cytopathic effects indicating that IL-2 is not protective for T cells. Further studies using PBMC subpopulations, to determine which cell type is preferentially infected and additional donors will be studied to follow up on these results.
Biomarkers of emphysema susceptibility and blood flow heterogeneity associated emphysema progression

Tyler Klenske, Krishna Iyer, MD, PhD, Eric A. Hoffman PhD, Akar Jani

Background: It has been established that, in the case of regional inflammatory injury, the normal response of the lung to constrict local vasculature and shunt blood to better-ventilated regions is counterproductive to the injury repair process. There is evidence in the literature suggesting that, in humans, hypoxic pulmonary vasoconstriction (HPV) is blocked in the presence of inflammation. Animal data suggest pulmonary vascular abnormality precedes emphysema development and alveolar destruction with tobacco smoke exposure. In a sheep model, Easley et al. demonstrated the regional failure of HPV in a lung region flooded with endotoxin but not with saline. Hoffman et al. showed both intact HPV in normal lung regions and loss of HPV in inflamed lung regions of the same subject. Thus it is reasonable to expect that the lung’s HPV response is regional and is normally blocked in areas of smoking related lung inflammation. We have demonstrated that smokers with normal pulmonary function tests, but small visually detectible signs of localized central acinar emphysema (CAE), have an increase in heterogeneity of pulmonary parenchymal perfusion mean transit times (MTT). We have further demonstrated that dual energy CT (DECT)-based measures of pulmonary parenchymal perfusion blood volume (PBV) heterogeneity (coefficient of variation (CV) increased in smokers susceptible to emphysema, are reduced with a single dose of sildenafil. Magnitudes of PBV heterogeneity (PBV-CV) were mirrored by upstream pulmonary arterial cross-sectional areas standardized to the cross-sectional area of the associated sub-segmental airway segment.

Hypothesis: Peripheral pulmonary vascular function is closely tied to smoking-associated emphysema, and further imaging-based vascular bio-markers are linked to the progression of the emphysematous disease process.

Methods: A review of measurements from 44 subjects over three visits was carried out in which emphysema progression was followed. Each subject was a current smoker and was examined with DECT for perfused blood volume (PBV) as a surrogate for pulmonary perfusion. Emphysema affected volume (voxels <-950HU) was measured as a percentage of total lung parenchymal volume and followed over the three visits. In the same subjects, arterial cross-sectional areas (CSA) were measured in the right (RB10) and left (LB10) sub-segmental bronchial branches, corresponding to lower lobes, over the three visits.

Results: When grouped by PBV-CV, an upward trend in emphysema affected volume was seen to be associated with increased PBV-CV. A positive correlation was demonstrated between airway-standardized arterial size and rate of change in the emphysema index.

Conclusion: These preliminary results add support to our body of work and indicate that there is a link between PBV-CV in the lung and emphysema progression. Future studies look to further elucidate this link and take advantage of it through clinical interventions in combination with smoking cessation.
Surgical site infection (SSI) prevention bundle compliance monitoring for abdominal, laparoscopic, and robotic hysterectomies
Susannah Koch, BS and Noelle Bowdler, MD

Introduction: One in nine women in the United States will undergo a hysterectomy in her lifetime. Surgical site infections (SSIs) are the most frequently encountered complications of hysterectomies. In an effort to reduce the incidence of SSIs after hysterectomies at an academic tertiary care center, a SSI prevention bundle was implemented in August, 2015. The bundle includes practices to maintain patient normothermia, antiseptic prophylaxis, parenteral antimicrobial prophylaxis, hair clipping, surgical safety checklists, door openings and wound care. Implementation involved changing ordersets to include instructions on patient warming, hair clipping, and antimicrobial prophylaxis as well as discussions with physicians, perioperative nursing staff, and anesthesia staff. This study explores compliance with various components of the bundle.

Objective: The goal of this study was to determine compliance with the SSI prevention bundle in the department of Obstetrics & Gynecology at the University of Iowa Hospitals and Clinics. By determining the extent to which these practices were being used, we could better determine their benefit and utility in preventing surgical site infections in this population.

Methods: For four weeks in April 2017, one student observed a total of 31 abdominal hysterectomies performed in the Main OR over 17 days. Compliance with all components of the bundle was observed and recorded on a checklist. Percentages were computed for compliance for each bundle component. The rate of post-operative surgical site infections was collected for the same period of time, plus thirty days from the last procedure observed. This data was collected through the Tableau service monitored by the UIHC epidemiology service.

Results: 17 of 27 patients observed in DOSA (Day of Surgery Admissions) received active warming pre-operatively when not indicated (temperature > 36.5 C) and 1 of 3 patients received active warming when indicated. Only 6 of 31 (19.4%) Operating Rooms had a temperature and set-point of >76 C at the time of patient arrival. 7 of 31 (22.6%) of procedures had warming before induction of anesthesia. 17 of 17 patients had their warming device disposed of immediately following surgery. During 8 of 31 (26%) cases, re-dosing of antibiotics was not discussed at any point during the surgery. Hair Clipping was performed in 9 cases, 4 of which occurred in the operating theatre. Door openings during the first hour after patient arrival ranged from 10 to 37 (mean 23.2, SD 5.09).

Conclusions: There was inconsistent compliance with aspects of a bundle intended to reduce SSIs after hysterectomy. Specific components were identified for interventions to improve compliance. This study illustrates difficulties that can occur with implementation of a bundle of interventions when a large number of personnel in various disciplines (physicians, nursing staff, and anesthesia staff) are involved.
Utilizing surgeon intuition to strengthen preoperative surgical risk assessment

James Kohler, BS, Natalie Glass, PhD, Nicolas O. Noiseux, MD, John J. Callaghan, MD, and Benjamin J. Miller, MD, MS
University of Iowa Department of Orthopaedics and Rehabilitation

Introduction: Many clinical factors (such as age, sex, smoking, BMI, and medical comorbidities) are known to increase an individual patient’s risk of perioperative complications and hospital readmission. Recently, several novel risk calculators have been created to predict the risk of postoperative complications for specific procedures. In general, these calculators rely primarily on objective measures and do not quantitatively account for the treating surgeon’s risk assessment. Our goal was to determine if surgeon intuition (an estimate of the percent likelihood of minor and major medical and surgical complications and 30-day readmission) could provide an additional source of data in the preoperative setting that may enhance the prediction of complications after surgery. Specifically, we questioned 1) if there are surgeon predictive values that distinguish between low, intermediate, and high likelihood of complications, 2) if the values maintain discrimination when applied to other observers, and 3) the relative importance of including a measure of the surgeon’s assessment in a multivariate regression model.

Methods: We targeted the operative practices of three subspecialized orthopaedic surgeons (one oncology and two total joint arthroplasty) over a 6-month period (February 1 to July 31, 2015). We administered surveys to attending surgeons and assisting residents or nurse practitioners prior to each operation. Surgeons were asked to predict each patient’s likelihood, on a scale from <1-100, for experiencing minor medical complications, major medical complications, minor surgical complications, major surgical complications, and readmission in the first 30 days after surgery. Following the procedure, we analyzed each patient’s discharge summary, postoperative clinic notes, and documented telephone conversations within our electronic medical record to determine any adverse events and readmissions experienced in the 30 days following surgery. We used simple descriptive statistics to determine levels of association between predictor variables and complications, utilizing Chi square or Fisher’s exact testing for categorical variables and simple t-test for continuous variables. Lastly, we incorporated the surgeon prediction variable into a multivariate logistic regression model.

Results: A total of 417 surveys in 270 patients were available for analysis. We found that defining the predicted likelihood of minor medical complications as <10% (low risk), 10-40% (intermediate risk), and >40% (high risk), provided discrimination of postoperative complications for a single observer in the first three months of data recording. Next, we applied these values to the second three months of data collection for the same observer, and to the data provided by the other attending surgeons and residents (Table 1). We then incorporated the surgeon prediction variable into a multivariate model controlling for age, sex, body mass index, ASA class, diabetes, smoking status, and Charlson comorbidity score. We found the only two variables predictive of minor medical complications were ASA class (Odds Ratio [OR] = 3.21, 95% Confidence Interval [CI] 1.70-6.05 comparing ASA ≥2 to <2) and surgeon prediction (OR = 2.43, 95% CI 1.46-4.02 comparing high risk to low risk). We attempted to define a cutoff for the other outcome variables, but did not find a similar level of discrimination (Table 2).

Discussion and Conclusion: We found that a quantitative surgeon preoperative risk assessment was able to accurately discriminate between low-, medium-, and high-risk groups of minor medical complications. Although we did not find a similar association between major complications and readmissions, it may be explained by the low frequency of these outcomes in our limited data set. Further work along this line may allow for better patient selection, more effective counseling regarding complications following surgery, and increased accuracy of preoperative risk calculators.

Table 1. Number of minor medical complications in each category of risk stratification

<table>
<thead>
<tr>
<th>Category</th>
<th>Low (0-10)</th>
<th>Intermediate (11-40)</th>
<th>High (&gt;40)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>2/41 (4.9%)</td>
<td>3/12 (25.0%)</td>
<td>6/7 (85.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Second timepoint</td>
<td>5/27 (18.5%)</td>
<td>4/14 (28.6%)</td>
<td>8/16 (50.0%)</td>
<td>0.111</td>
</tr>
<tr>
<td>Other attendings</td>
<td>12/79 (15.2%)</td>
<td>8/22 (36.4%)</td>
<td>3/5 (60.0%)</td>
<td>0.0084</td>
</tr>
<tr>
<td>Residents</td>
<td>25/151 (16.6%)</td>
<td>20/37 (54.1%)</td>
<td>3/6 (50.0%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 2. Performance of low- and high-risk values for readmission and complications

<table>
<thead>
<tr>
<th>Outcome</th>
<th>% complications</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-Day Readmission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prediction &lt;5</td>
<td>6/189 (3.2%)</td>
<td>2/34 (5.6%)</td>
</tr>
<tr>
<td>6/223=3.6%</td>
<td></td>
<td></td>
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<tr>
<td>Major medical</td>
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<td></td>
</tr>
<tr>
<td>Prediction &lt;5</td>
<td>3/192 (1.6%)</td>
<td>3/31 (9.7%)</td>
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<td>3/223=1.4%</td>
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<tr>
<td>Minor medical</td>
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<td>Prediction &lt;10</td>
<td>19/147 (12.9%)</td>
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<td>5/223=22.9%</td>
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<tr>
<td>Prediction &lt;10</td>
<td>7/179 (3.9%)</td>
<td>2/44 (4.6%)</td>
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Atrial Antitachycardia Pacing in Complex Congenital Heart Disease: A Case Series

Collin C. Kramer, M2 (presenting author), Jennifer Maldonado, BS, RTR, Mark Olson, PA-C, Jean C. Gingerich, BSN, Luis Ochoa, MD, Ian H. Law, MD (mentor). The University of Iowa Stead Family Children’s Hospital Department of Pediatrics, Division of Pediatric Cardiology and the University of Iowa Carver College of Medicine, Iowa City, IA.

Abstract

Background: Among the congenital heart disease (CHD) population, intra-atrial reentrant tachycardia (IART) is a common sequela resulting from anatomical anomalies and surgical scars which significantly increases morbidity and mortality. Atrial anti-tachycardia pacing (ATP) delivered by atrial antitachycardia devices (ATD) has been used to treat IART in the CHD population. However, there is limited data on ATP safety and efficacy as well as comparisons amongst different CHD morphologies.

Purpose: To describe and compare the clinical history and ATP efficacy in three patients with unique forms of complex CHD.

Methods: A single-center review of three patients with ATDs was carried out at the University of Iowa. One patient with each of the following CHD morphologies was selected for comparison: systemic left ventricle, systemic right ventricle and single ventricle. Data collected included ATP success rates, medications, direct current (DC) cardioversions and any complications related to ATDs.

Results: The patient with a systemic left ventricle had an ATD implanted for approximately 9.5 years, with 695 of 956 (73%) episodes successfully converted. Unsuccessfully treated episodes were generally asymptomatic and self-terminating in this patient. The patient with a systemic right ventricle had an ATD implanted for approximately 16 years with 333 of 348 (96%) episodes being successfully converted. The patient with a single ventricle had an ATD implanted for approximately 12.5 years with 404 of 416 (97%) episodes being successfully converted. The patients with systemic right and left ventricles were able to forgo DC cardioversion after receiving their ATDs. However, due to medical non-compliance as well as multiple episodes of IART which presented with 1:1 conduction or low rates, the single ventricle patient still required DC cardioversions status post ATD implantation.

Conclusions: Our findings demonstrate that while ATP can be effective in a wide variety of CHD, experiences can vary based on individual arrhythmia substrates, morphologies and medical compliance. Additionally, challenges remain in IART detection in patients with especially complex CHD morphologies.
Perspectives of Patients and Parents on Medical Alert Tattoos in the Context of Type I Diabetes

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\textsuperscript{1}University of Iowa Carver College of Medicine, \textsuperscript{2}University of Iowa Department of Pediatrics, \textsuperscript{3}University of Iowa Department of Orthopedics & Rehabilitation

Background
Medical alert jewelry is frequently advised for patients with chronic life threatening health conditions. Type 1 Diabetes Mellitus (T1D) is a common condition for which medical alert jewelry is recommended. Medical alert jewelry is a useful identifier to promote optimal care in emergency situations, and first responders are trained to look for such jewelry. More recently, a trend has arisen such that patients are electing to get a permanent medical alert tattoo (MAT) in place of wearing jewelry. MAT’s are permanent, cannot be lost, and are able to worn in situations in which jewelry is not allowed or is impractical, such as competitive sports. To date, no one has investigated perspectives of parents and patients with chronic life threatening health conditions warranting the recommendation of medical alert jewelry surrounding MAT’s.

Purpose/Aims
The purpose of this study is to further our understanding of how pediatric patients with T1D, a chronic life threatening health condition, as well as their parents, view the option of a tattoo as a means of medical alert identification. The data can be used to better understand how medical alert tattoos can be integrated into the recommendations for these patients.

Methods
Surveys were designed with input obtained from the University of Iowa Public Policy Center. IRB approval was granted. Paper surveys were distributed to patients, between the ages of 12 and 22 years, with Insulin-dependent T1D as well as their accompanying parent(s) when coming in for a routine visit at the University of Iowa Pediatric Diabetes Clinic. Verbal consent was obtained from parents and patients over 18, and verbal assent was also obtained from patients under the age of 18. Participants completed the survey while waiting in their clinic room. Surveys were collected by the pediatric endocrinology clinic upon closing the visit. Patient and parent responses were analyzed for differences in perspectives related to medical alert tattoos using t-tests. Statistical analysis was performed using SPSS. Census data was utilized to classify zip codes as urban or rural for demographics.

Results
Fifty-one patients (56% male) with T1D completed surveys. 66% of patients reported that they currently have MA jewelry but only 24% reported that they wear their jewelry. 55% of patients had heard of the use of Medical Alert Tattoos (MAT’s). Of patient respondents, 85% Agreed or Strongly Agreed that MAT’s can be lifesaving, and 81% Agreed or Strongly Agreed that a MAT is a good idea for athletes. 49 parents (age range 32-63 years, 75% female) of children with T1D completed surveys. 52% of parents had heard of the use of MAT’s, and 67% of parents Agreed or Strongly Agreed that they would support their child in getting a MAT. Significant differences between the patients and their parents were noted in responses to questions relating to a standard for required features of a medical alert tattoo, the potential to be prevented from getting a job due to having a tattoo, as well as the utility for athletes who cannot wear jewelry when competing. Parent responses were further divided based on income, age, and rural vs urban, with no significant findings observed in these regression analyses. Trends were noted in parents who had tattoos themselves and support for their child pursuing a medical alert tattoo.

Conclusions
We found that the majority of patients with T1D and their parents were aware of the use of MAT’s and viewed them favorably as potentially life-saving. Further research and discussion is needed in populations of children with chronic life threatening health conditions beyond that of T1D, to consider appropriate standards for MAT’s, and to review standards of care at the first responder level.
Role of Early Splinting, Positioning, and Edema Control of Burn Affected Joints in the Prevention of Burn Scar Contracture

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Background: Burn traumas result in more than 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 challenges during and after their recovery. Large burn injuries and those involving joints can be particularly physically debilitating. For these, daily rehabilitation is required to restore function and strength. Despite this, burn scar contracture (BSC) occurs in up to 38% of survivors, severely limiting their range of motion and complicating their re-entry to family life, society, and work incredible difficult.

The standard treatment protocols for BSC prophylaxis include splinting, positioning and edema control. However, there is little evidence-based medicine supporting these interventions post injury, and controversy exists regarding optimal timing of these maneuvers. This study seeks to determine factors contributing to the formation of BSC and to determine the efficacy of early splinting, positioning, and edema control in the prevention of BSC during the initial admission for burn injury.

Hypothesis: The use of splinting, positioning, and edema control of burn-affected limbs and joints does not prevent the formation of BSC in the acute phase of burn injury.

Methods: The records of 364 patients which were prospectively entered into the Burn Patient Acuity Demographics, Scar Contractures, and Rehabilitation Treatment Time Related to Patient Outcomes (ACT) study from 2010-2013 were reviewed. 307 patient records were analyzed after 57 patients were removed due to expiration or request for removal from study. Data abstracted included demographics, burn data, surgical procedures, level of care and treatment, rehabilitation hours (rehab hrs), BSC prevention (hours splinted, positioned and with edema control), pain control, therapy compliance and strength measurements. The main outcome variable was BSC at time of discharge. BSC was classified as none, mild (<30%), moderate (30-60%), or severe (>60%) categories based on normal joint passive range of motion.

Univariate and multinomial logistic regression (MNLR) was used to determine the effect of BSC prevention methods on the incidence of BSC at hospital discharge. All univariate and multivariate statistical analysis were performed with Stata 15.0 (StataCorp LLC, College Station, Texas, USA). Significance was determined at the p<0.05 level.

Results: Of the 3,578 joints analyzed, 42% had loss of range of motion in 251 of patients. On univariate analysis, statistically significant (p<.05) contributing factors to BSC development were increased age, increased TBSA burned, increased percentage of skin grafted, increased length of stay (LOS), use of venous thromboembolism prophylaxis, increased hours splinted, escharotomy/fasciotomy, increased days of edema treatment, increased days of bedrest, and increased days in the ICU. Protective factors included being non-white (p=.043), increased rehab compliance (p<.001), and increased rehabilitation hours (p=.05). Total hours splinted and positioned was not significant (p=.179). Only female gender, non-white, days in the ICU, total rehab hrs, younger age, percentage of skin grafted, and TBSA remained independently related to severe contracture or mild contracture at discharge on MNLR.

Conclusions: BSC occurs in a significant number of survivors during the initial phase of their burn injury. Increased amounts of direct rehab were the only preventive measure in this multicenter study associated with decreased formation of BSC. While early splinting, positioning and edema control do not seem to be associated with BSC prevention, this study was not designed to address these techniques in a systematic fashion. Future studies are needed to refine treatment for BSC.
The role of nuclear TRAF3 as a regulator of B cell survival and activation

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Background: Tumor necrosis factor receptor-associated factor 3 (TRAF3) is commonly lost or mutated in B cell malignancies including multiple myeloma and B cell lymphoma, suggesting a role for TRAF3 as a tumor suppressor. B cell-specific deletion of TRAF3 in a mouse model results in increased B cell numbers due to increased B cell survival. The long-term goal of our lab is to identify the mechanisms by which TRAF3 regulates B cell survival.

Previous studies of TRAF3 have focused on its regulatory roles in the cytoplasm and at the plasma membrane. TRAF3 was shown to inhibit B cell activating factor receptor (BAFF-R), CD40, Toll-like receptors (TLRs), and interleukin 6 receptor (IL-6R) signaling pathways in the cytoplasm. Our lab has recently shown that TRAF3 traffics to the nucleus and regulates the stability of cAMP Response Element-Binding (CREB) protein, a transcription factor important for promoting expression of pro-survival genes in B cells. The purpose of this study is to expand upon our initial studies to identify additional roles of nuclear TRAF3 in the regulation of B cell survival.

Hypothesis: Nuclear TRAF3 regulates the BAFF-R, CD40, TLRs and IL-6R signaling pathways independently of its cytosolic functions.

Methods: We used a plasmid encoding a TRAF3 mutant lacking the nuclear localization signal (NLS), which causes TRAF3 to be largely excluded from the B cell nucleus. We utilized the CH12 mouse lymphoma cell line stably transfected with an inducible nuclear localization sequence mutant of TRAF3 (ΔNLS-TRAF3). We used three CH12 control cell lines: wildtype (WT), TRAF3⁻/⁻, and TRAF3⁻/⁻ with WT-TRAF3 transfected (WT-TRAF3) for comparison. We performed nuclear fractionation followed by Western blotting to measure nuclear and cytosolic signaling proteins. We then chose to focus on NF-κB expression due to its known role in BAFF-R and CD40 signaling. TRAF3 inhibits non-canonical NF-κB (NF-κB2) signaling downstream of BAFF-R and CD40 activation in the cytoplasm, but it is unknown if this inhibition also has a nuclear component.

Additionally, we also wanted to develop a stably transfected ΔNLS-TRAF3 A20 mouse lymphoma cell line to elucidate the role of nuclear TRAF3 in IL-6R signaling. We confirmed our transfection success with flow cytometry.

Results: We were able to isolate nuclear and cytosolic fractions from CH12 cells. Resting ΔNLS-TRAF3 CH12 cells had increased levels of the activated form of NF-κB2, p52, in the nuclear fraction compared to WT-TRAF3 CH12 cells. The A20 transfection experiments are ongoing.

Conclusions: The ΔNLS-TRAF3 CH12 cells showed increased basal levels of activated NF-κB2 in the nucleus suggesting that nuclear TRAF3 may play a role in stabilizing p52 protein in the B cell nucleus. Additional experiments are needed to identify whether nuclear TRAF3 has a role after activation of the BAFF-R and CD40 signaling pathways. Through these studies, we will understand more about the complex roles of TRAF3 as a regulator of B cell survival.
Access to Technology and Interest in Telemedicine Among UIHC Home-Based Dialysis Patients

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1University of Iowa Carver College of Medicine, 2University of Iowa Stead Family Children’s Hospital, 3The Signal Center for Health Innovation

BACKGROUND: Telemedicine is the use of electronic communication for exchanging medical information in order to improve patient health. Telemedicine is a high-potential healthcare model for improving patient outcomes, cost-effectiveness, and satisfaction via remote communication. As such, telemedicine technologies are becoming increasingly prevalent in the management of patients with chronic conditions. Current evidence suggests that telemedicine is an effective vehicle for the management of end-stage kidney disease (ESKD) patients who utilize peritoneal or other forms of home-based dialysis, and is associated with a variety of benefits.

Despite its benefits, telemedicine and remote home monitoring is still not a widely adopted delivery model. In 2012, the pediatric dialysis team at the University of Iowa Hospitals and Clinics (UIHC) conducted a successful pilot study involving one pediatric PD patient to assess the benefits and feasibility of patient monitoring via a remote technology program. However, relatively little is known about the optimal design, barriers to patient involvement, and overall patient interest in a standardized telemedicine program for home-based dialysis patients. This research seeks to assess technological readiness for and patient interest in such a program.

HYPOTHESIS: Patient interest in and technological readiness for a telemedicine model involving the management of home-based end-stage kidney disease (ESKD) patients can be assessed.

METHODS: Current home-based adult dialysis patients (n=26) and caregiver representatives of pediatric patients (n=11) were provided paper surveys at their monthly appointment at UIHC throughout July 2017. Nurses at each dialysis center distributed the surveys to patients at the beginning of the appointment. Surveys were available in 3 different forms: Adult Patient, Adult Caregiver, and Pediatric Caregiver. The survey was divided into 5 sections: Patient Demographics, Caregiver Demographics, Household Information, Experience with Technology, and Perspectives & Preferences. REDCap software was used for data entry. REDCap and Excel software were used jointly to perform descriptive analyses of 21 subjects.

RESULTS: Twenty-one surveys were returned. The surveys were representative of 14 households – 8 adult patients and their caregivers, and 6 pediatric caregivers. One adult caregiver declined to complete a survey. Upon analysis, adult caregiver responses were identical to those of their corresponding patient, therefore their responses were analyzed separately. Overall, 36% of households were interested in using a synchronous telemedicine program to replace some monthly appointments, 36% were unsure, and 29% were not interested. Patients or caregivers who were more likely to be interested in using a telemedicine program were pediatric households, patients or caregivers with patients living more than an hour from UIHC, and those who cited that they had to make outside accommodations in order to attend appointments. Additionally, the majority of patients owned at least one technology that would be necessary to support telemedicine programs. However, across the patient population, there was no common technology owned by all patients.

CONCLUSION: Preliminary data suggests that there is interest in utilizing a telemedicine program, especially among the pediatric home-based dialysis population. Patients also have the necessary technologies to begin such a program. However, there is no common technology that all patients have access to, which should be considered when designing the technological platform.
Evaluating the effectiveness of Shared Plans of Care (SPoC) for children and youth with special health care needs (CYSHCN) within the Iowa Pediatric Integrated Health (PIH) program

Liza Mann, Thomas Scholz, MD, Jean Willard, MPH, Martha Hanley, MA, Jennifer Cook, MPH, Deanna Wahl, Patrick Ten Eyck, PhD (ICTS)

Background
Child Health Specialty Clinics (CHSC) is a community-based public health agency that serves Iowa children and youth with special health care needs (CYSHCN) and their families through a network of regional centers and clinics throughout Iowa. Approximately 106,000 CYSHCN live in Iowa. However, less than half of CYSHCN receive integrated care. One of the many barriers to care for CYSHCN is a lack of an accessible, secure platform on which healthcare information is stored and can be accessed by providers as well as family. CHSC has recently implemented a Shared Plan of Care (SPoC) model to provide a single platform for families that defines goals for their child and includes up to date medical and other service provider information.

Purpose of Study
The current pilot study being conducted is a retrospective case-control chart review to investigate the efficacy of SPoC as a tool for care coordination.

Methods
Efficacy was studied by assessing healthcare utilization six months before and six months after SPoC implementation for Iowa children and youth who are eligible for Medicaid and have a mental or behavioral health diagnosis with and without a SPoC. Measures considered include 1.) Emergency Department visits, 2.) hospitalizations, 3.) primary care visits.

Results
There were 19 children with a SPoC and 8 children without a SPoC included in the study. The average age for children with a SPoC was 13.11 years and 10.72 without a SPoC (p=0.027). Upon comparing children with a SPoC vs. children without a SPoC, there were fewer instances of primary care visits in the children with a SPoC. (MR=0.69, p=0.41). There was not a significant number of hospitalizations (0 for case, 3 for control) or Emergency Department (1 for case, 2 for control) to conduct meaningful analysis.

Conclusions
There is research supporting the use of care coordination for children and youth with special health care needs. Recent studies have focused on specific tools to effectively coordinate care as well as proper ways to evaluate programs. Although SPoC is becoming a widely used tool, larger studies are needed to determine if significant improvements are sustained. This pilot study demonstrates that outcomes can be compared across like populations with different interventions, but that it is often difficult to assess outcomes with a small cohort and dataset. Further data collection and analysis is needed.
Impact of Primary Care Provider to Dermatology store-and-forward (SAF) Teledermatology on Outpatient Dermatologic Care: A Retrospective Review of eConsults in an Integrated Electronic Health Record System

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BACKGROUND:
Store and forward teledermatology and emerging telehealth platforms continue to change the healthcare landscape and reduce unnecessary in-person visits. The value of teledermatology in addressing patient needs in the primary care setting in a timely manner remains relatively unknown. The objective of this study is to evaluate the dermatology eConsults from primary care providers to dermatologists at the University of Iowa within an integrated electronic health record system.

AIM:
We reviewed and analyzed eConsult services from primary care provider to dermatology in an integrated electronic health record system to assess the impact on outpatient diagnosis, management, and to determine whether subsequent in person dermatology care was sought by the patient.

METHODS:
We performed a retrospective review of store-and-forward teledermatology EPIC eConsults submitted by 94 primary care providers from 7 clinics to University of Iowa Dermatology between 5/20/2015 and 9/22/16. We assessed patient demographic information, proportion of eConsults accepted, concordance between primary care provider and dermatology diagnosis and management plans, time to eConsult completion, proportion of eConsults managed without in person dermatology care, and number of patients ultimately referred to dermatology for in person care.

RESULTS:
The study included 400 eConsults (61% female) with a median age of 46 and 80% identified as white Caucasian. eConsults were answered by two dermatologists at the University of Iowa with an average time to consult completion of 12.6 hours compared to a 42.71 day wait time for an in-person dermatology consult. 78% of eConsults were accepted and of the 22% of rejected cases, 67% were deemed too complex for teledermatology alone and recommended in-person evaluation. eConsults encompassed both inflammatory dermatologic conditions and solitary neoplasms. 36% of patients originally managed by eConsult sought in person dermatology care, and 39% of those patients received the same care plan initially provided via eConsult. 81% of accepted eConsults were resolved by teledermatology and the patient did not seek additional care with University of Iowa Dermatology.

LIMITATIONS:
By nature of retrospective chart review, there is limited patient follow up and information is limited to data available in the electronic medical record.

CONCLUSION:
eConsults can be an efficient and effective modality for delivering dermatologic care to patients in primary care settings and reduce the time to dermatologic care and need for in person visits. Store and forward teledermatology can be a useful tool to help improve access to dermatology care in this community. Focusing on the education provided to primary care and the patients also would be future measure to further improve the teledermatology care.
Teledermatology in Rural Tanzania: A Prototype for Dermatology Service in Underserved Areas

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Background: Globalization of healthcare and universal access to information technology have dramatically expanded the potential role of telemedicine in the provision of specialist health services in remote areas. Dermatology in particular lends itself to this role both by virtue of the fact that it is a field in which diagnoses are based largely on superficial morphologic features that can be easily photographed and because these photographically acquired dermatologic data are electronically transferrable. Sub-Saharan Africa, with a physician:patient ratio as low as 1:10,000 and a dearth of specialist care, is an ideal venue for investigation of this option.

Objectives:
(i) To establish the required elements of a teledermatology platform that would enable remote diagnosis and treatment of dermatologic conditions in Zinga, Tanzania
(ii) To institute a sustainable infrastructure at the remote site that could be maintained by local healthcare providers
(iii) To identify the potential impact of teledermatology on diagnosis and treatment in a limited-resource setting in rural Tanzania
(iv) To study the spectrum of dermatologic diseases in the region
(v) To assess patient satisfaction with the teledermatology program and to identify potential implementation barriers

Methods:
(i) Dermatologic data were acquired by digital photography and relayed to an existing teledermatology platform for data entry, diagnostic interpretation and therapeutic recommendation
(ii) Local medical staff were educated on proper techniques for data acquisition (including a pertinent detailed patient history and digital photography of dermatologic lesions), data entry, data transfer, and access of remote diagnostic and therapeutic opinions
(iii) Preliminary data were analyzed to identify the potential value of implementing a teledermatology program in the area as measured by concordance between primary care physician and dermatologist
(iv) A preliminary spectrum of dermatologic diseases in the region was documented
(v) A patient satisfaction survey was performed

Results: A teledermatology platform was implemented and utilized to provide dermatologic care to a total of 21 patients over an 8-week study period between May and July 2017. Diagnoses of Tanzanian primary care providers were fully concordant or partially concordant with those of University of Iowa Dermatologists in 28.6% and 38.1% of cases, respectively. In contrast, treatment plans were fully concordant in only 1 of the 21 cases, and partially concordant in 23.8%. In 52.4% of cases, therapy was postponed until the remote opinion had been received. The mean time from consult request to response completion was 40.5 hours (range 0.5 – 124.5 hours). A disease spectrum of dermatologic conditions was photographically documented. Over the 8-week study a total of 35 dermatologic conditions were observed. Patient survey acquisition rate was low (9 out of 21 patients), attributable to cultural issues and sub-optimal communication due to the language barrier. Of the 9 patients surveyed, 7 felt that teledermatology was an acceptable way to receive healthcare, 7 felt that they were receiving the same quality of care as face-to-face contact, and all said that they felt comfortable having their skin concerns treated using photographs sent to a specialist.

Conclusions: The implementation of a sustainable teledermatology program in a rural setting in sub-Saharan Africa is feasible. Teledermatology has a meaningful impact on diagnosis and a profound impact on treatment of dermatologic conditions in such an environment. The extremely low concordance rate between the initial therapy proposed by the primary care physician and that recommended remotely by the consulting dermatologist validates the addition of an educational component to the teledermatology program. Further investigation is warranted into more widespread application of this technology to provide optimal dermatologic care in underserved areas.
Normal Saline Versus Lactated Ringer’s: A Randomized Control Trial Evaluating Healthcare Utilization Following Discharge in Emergency Department Patients

Natalie Martin; Andrew Fiederich; Kari Harland, PhD, MPH; Priyanka Vakkalanka, ScM; Morgan Bobb; Nicholas Mohr, MD, MS; Brett Faine, PharmD, MS

Background: Intravenous fluid administration is the most common procedure performed in the emergency department (ED), and crystalloid fluids, such as normal saline (NS) and lactated ringer’s (LR), are the most common fluids. Prior evidence suggests that NS may be associated with significant adverse effects in critically ill patients, but little evidence guides choice of fluid in those without critical illness.

Purpose of the Study: The purpose of this study was to determine whether NS or LR for ED fluid resuscitation reduces healthcare utilization seven days after ED discharge in non-critical, volume-depleted patients.

Methods: This randomized, double-blind trial was conducted in a 60,000-visit, Midwestern, university-based ED. Adults (≥18 y) who presented to the ED with symptoms of volume depletion, being administered intravenous fluids, and were likely to be discharged were screened for eligibility. Patients who were pregnant, non-English speaking, undergoing chemotherapy, or prisoners were excluded. Participants were randomized using opaque, sequentially-numbered envelopes, and the participant and research assistant evaluating outcomes were blinded to the treatment group. Seven days after discharge, participants reported (1) ED return visits, (2) prescription use, and (3) other health care for the same complaint. Responses were evaluated with a chi-square test using two-tailed tests with p<0.05, determined to meet the threshold of statistical significance.

Results: This preliminary analysis reports the findings of the first 96 patients enrolled (anticipated total sample 156 participants). The response rate was 57% (n=55) and was similar between groups. One participant was omitted due to ongoing hospital admission at 7 days. Five participants (10%) in the NS group had returned to the ED within 7 days, compared to one (2%) in the LR group (p=0.067). Both groups had four patients follow up with another provider (p=0.791). In the NS group, 13 patients filled their ED prescription, compared to 10 in the LR group (p=0.540).

Discussion: Our preliminary results show physicians’ fluid selection is not associated with ongoing healthcare utilization. Notably, however, ED return rate appears higher in the NS group at preliminary analysis, suggesting either that there is a small effect or that the two groups were imbalanced at randomization. Completion of the trial is necessary to further assess the significance of the findings.
Should Play Dough Be On Your Non-Toxic List?
High Salt Content of Home-Made Play Dough Products

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²Iowa Poison Control Center, Sioux City, IA
³Drake University College of Pharmacy, Des Moines, IA

Background:
Commercially made Play-Doh® is considered non-toxic and has a sodium chloride content listed as 5.52%. However, there are many recipes available for home-made play dough on the internet and social media. Some recipes call for glitter to make “galactic” playdough, while others add powdered drink mix or spices to make it smell good. Many of these recipes call for large quantities of salt which can change this seemingly non-toxic exposure into a toxic one. Due to the potential increased risk of toxicity with home-made play dough, we wanted to more accurately quantify the salt concentration in these products.

Purpose of the Study:
Assess the salt content and safety of homemade Play Dough products and review our current clinical practice and send-in criteria for ingestion of Play Dough at the Iowa Poison Control Center.

Method:
Nine popular play dough recipes were obtained from the internet. Ingredients were purchased from local grocery stores. Each recipe was made in triplicate, with one person making one product for each of the nine recipes; each person’s nine products are referred to as a “batch.” Exact weight of each component was recorded before being added to the mixing bowl; exact weight of the final product was also recorded. Salt content based on weight of the components was calculated. To more directly measure the sodium content for each of the 27 products made, 25 grams from each product was homogenized with 100 mL distilled water and the supernatant was analyzed with a sodium-specific ion electrode connected to a pH meter in mV mode. Commercially made white Play-Doh® was used as the standard for the sodium ion measurements.

Results:
The average calculated salt content (weight / weight) of the nine recipes ranged from 16.8% (SD 0.78%) to 34% (SD 3.7%). The final average weights of the three products for each recipe had small standard deviations, with the standard deviations ranging from 1.7% (avg. wt. 557.8 g +/- 9.4 g) to 7.4% (avg. wt. 1519 g +/- 113 g) of the three products’ average weight. Direct sodium measurement was complicated by impurities in the supernatant, but still showed good correlation between calculated salt content and sodium ion measurement, with correlations ranging from 0.85 (batch 3) to 0.92 (batch 1).

Discussion:
The lowest average calculated salt content (16.8%) is more than three times the amount of NaCl reported in commercially produced Play-Doh® (5.52%), while the highest (34%) is more than 6 times that amount. Direct sodium ion measurement confirmed the significantly higher sodium content of home-made play dough compared to commercially produced Play-Doh®. Using an ingestion of 8 mEq (470mg) NaCl/kg as a threshold for referring a person to medical attention, the amount of home-made play dough that would need to be ingested to reach this threshold would be between 1.38 g/kg (34% NaCl) and 2.86 g/kg (16.8% NaCl).

Conclusion:
Poison center specialists need to ask callers to identify the maker of the play dough that is ingested given the very high salt content of home-made products. So what was your play dough exposure made of and what was the recipe? Did you ask?
Validation of the Low Profile Modification of the Losee Pivot Shift Test in the Assessment of the ACL Deficient Knee

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Department of Sports Medicine/Orthopedics, UIHC

Background: The Lachman’s test has become the standard test for detecting anterior cruciate ligament (ACL) disruption with the more specific valgus based pivot shift tests known to be difficult to elicit and therefore having a low sensitivity. The valgus based examination often inadvertently elicits patient pain and protective muscular guarding which can then inhibit the tibia from subluxing anteriorly. Additionally, the patient’s anticipation of iatrogenic induced pain often masks a positive test result and prevents a reliable evaluation. However, the low profile modification of the classic Losee pivot shift test does not necessitate the use of applied valgus forces, and is proposed to provide a more accurate clinical examination of the ACL deficient knee.

Purpose: To first demonstrate that the unique kinematics of the pivot shift test done without valgus force can be characterized and the inter-observer reliability of the test can be established in its purest form in a cadaver model, and second to establish through multiple observers the sensitivity of the low profile modification in the clinical setting.

Methods: Cadaver Study: Using the Computerized OrthoPilot Navigational System, with detectors applied to the femur and tibia, twenty fresh, frozen cadaver knees with and without an intact ACL were taken through an entrance and exit pivot shift range of motion procedure by four independent operators. The position of sagittal plane flexion and extension as well as the rotational relationship of the tibia and femur were recorded when the pivot shift subluxation occurred. Clinical Study: A retrospective study was performed on 226 patients who were diagnosed based on imaging with an ACL deficient knee between May 2010 and June 2017. The results of the low profile pivot shift test and the Lachman’s test were recorded for each. Statistical analyses were performed to determine sensitivity for the low profile pivot shift and Lachman exams.

Results: The exit pivot was found to have an average maximum anterior tibial translation of 7.82 mm in the ACL deficient knee specimens and 1.44 mm in the ACL sufficient knee specimens (p<0.01). The ACL deficient knees exhibited an average maximum internal tibial rotation of 12.44 degrees compared to 11.13 degrees in the ACL sufficient knee specimens during the exit pivot (p=0.02). The average knee flexion where the maximum anterior tibial translation occurred was 9.23 degrees in ACL deficient knees and 9.12 degrees in ACL sufficient knees during the exit pivot. The average knee flexion where the maximum internal tibial rotation occurred was 28.05 degrees in ACL deficient knees and 30.18 degrees in ACL sufficient knees during the exit pivot. The sensitivity of the low profile pivot shift test was determined to be 91.85% (95% confidence interval 86.91-95.37) and the sensitivity of the Lachman’s test was determined to be 81.22% (95% confidence interval 74.75-86.63).

Conclusion: The non-valgus producing low profile modification of the Losee pivot shift test provides a uniquely improved sensitive and specific exam of the ACL deficient knee. In a cadaver, we demonstrated that the unique kinematics of the pivot shift test done without valgus force occurs at 9 degrees of flexion and is classically done as an exit pivot from flexion to extension. We have demonstrated that, while it is an esoteric test, it can quickly be learned so that the learning curve is shallow. The inter- and intra-observer reliability of the exam is high. In the clinical study when done by an experienced examiner, the low profile pivot shift test has been shown to not only be much more sensitive at 91.85% than the valgus-based pivot shift test, but even more sensitive than the Lachman’s test at 81.22%.
Do Prostaglandins regulate non-muscle myosin II?
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**Background:** Prostaglandins (PGs) are lipid signaling molecules that regulate many physiological processes including pain, inflammation, reproduction, and cancer. PGs are produced by the action of cyclooxygenase (COX) enzymes, which are the targets of nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin and ibuprofen. COX enzymes produce a PG intermediate, PGH2, which is converted to bioactive prostanoids by PG-type specific synthases. While PG synthesis and the physiological outcome of PG signaling are well understood, the cellular mechanisms of how PGs mediate their outcomes remains poorly understood. This research uses Drosophila as a model to study the cellular functions of PG signaling.

One cellular function of PGs is to regulate the actin cytoskeleton. PGs promote both actin filament formation and depolymerization, depending on the cell-type and specific prostaglandin. The molecular mechanisms by which PGs regulate actin dynamics are largely unknown. To overcome this knowledge gap, this research uses Drosophila as a model to study PG regulation of one actin regulator, non-muscle myosin II. While there is no prior direct evidence linking PG signaling to myosin regulation, both PGs and non-muscle myosin II mediate cellular contraction and cell migration. Importantly, such activities play critical roles in normal development, and cancer progression.

**Hypothesis:** Prostaglandins positively regulate developmental actin-remodeling processes through non-muscle myosin II, an actin-binding protein.

**Methods:** *In vitro* developmental assays utilized isolated Drosophila follicles of a specific stage to perform a pharmaco-genetic and genetic interactions studies. For the pharmaco-genetic interactions, a concentration of aspirin was identified that blocked development of 50% of wild-type follicles from developing in culture. Genetic perturbations in non-muscle myosin II were then tested for their ability to develop in the presence of aspirin. Enhancement or suppression of the follicles’ ability to develop in aspirin suggests PGs regulate the specific actin-binding protein during follicle development. For the genetic interactions, Drosophila that are heterozygous for mutations in both non-muscle myosin II and pxt (Drosophila COX-like enzyme) were generated. Isolated follicles were assessed for their ability to develop in culture. If PGs regulate non-muscle myosin II, then follicle development should be inhibited.

Immunofluorescence studies examined the expression and localization of active non-muscle myosin II in Drosophila follicles. Wild-type and pxt mutant follicles were stained with antibodies and fluorescent proteins to visualize filamentous actin, phosphorylated non-muscle myosin II regulatory light chain, and chromatin.

**Results:** *In vitro* follicle maturation assays revealed that genetic reduction in non-muscle myosin II did not significantly enhance or suppress the effects of aspirin. Genetic interaction experiments revealed that heterozygous mutations in both non-muscle myosin II and pxt did not significantly enhance or suppress the ability of follicles to develop in culture. Preliminary characterization of active non-muscle myosin in follicles demonstrated enrichment in areas of cortical filamentous actin.

**Conclusion:** The results indicate that PGs do not regulate non-muscle myosin II to mediate Drosophila follicle development.
Background & Significance:
Bullous pemphigoid (BP) is an autoimmune blistering disease affecting primarily the elderly. The pathogenic epitope is the NC16a region of collagen XVII (Col XVII; also called BP180). This transmembrane protein is a critical component of the hemidesmosome, linking basal keratinocytes to the underlying basement membrane. Binding of complement-fixing IgG autoantibodies results in recruitment and activation of inflammatory mediators, with subsequent blister formation. Multiple studies indicate a strong epidemiological link between preceding neurologic disease and the development BP. Thirty percent of all BP patients have underlying neurologic disease at the time of diagnosis. Of these, Parkinson’s disease (PD) and dementia are most strongly associated, where the incidence is 2-4 times the expected rate. Despite this well documented association, there remains a knowledge gap of how neurologic disease contributes to the loss of self-tolerance to Col XVII. It is hypothesized that neuronal cell death results in local inflammation and exposure of the immune system to intracellular neuronal Col XVII. Despite this theory, the precise mechanism remains unknown. Here, we analyze patient data to identify demographic and serologic features that may differentiate BP patients from those patients with both BP and neurologic disease.

Hypothesis:
BP patients with preceding neurologic disease will have identifiable features differentiating them from BP patients without neurologic disease.

Methods:
Patients were recruited from the University of Iowa Hospitals and Clinics and written informed consent was obtained prior to inclusion in this study. Samples were collected from patients with clinical and histological characteristics of BP. Patient serum from the initial visit was tested for BP180 IgG, BP230 IgG, NC16a IgE, and total IgE. Severity of disease was determined based on the bullous pemphigoid disease area index (BPDAI). During the visit, history was obtained to determine disease onset and whether the patient had any preceding neurologic diagnosis (Parkinson’s disease, dementia, stroke).

Results:
A total of 104 patients with BP were included in this study. Seventy-five patients had BP with no prior neurologic disease (BP-ND), nine patients had BP and a prior diagnosis of PD (BP+PD), eleven patients had BP and a prior diagnosis of dementia (BP+DEM), and nine patients had BP and a prior diagnosis of stroke (BP+STR). Of the examined variables, age at onset and age at diagnosis were significantly higher in the BP+PD (onset: 83.3 years; diagnosis: 83.4 years) and BP+DEM (onset: 83.3 years; diagnosis: 83.4 years) groups compared to BP-ND (onset: 72.2 years; diagnosis: 72.7 years) and BP+STR (onset: 66.7 years; diagnosis: 67.0 years) groups.

Discussion and Future Directions:
Both PD and dementia are progressive, irreversible neurologic diseases of the elderly. The significantly delayed BP presentation in patients with preceding PD and dementia could be explained by continuous stimulation of the immune system caused by gradual neurodegeneration, with eventual production of pathogenic autoantibodies. Ongoing and future experiments will utilize established animal models to test the theory that neuronal insult leads to Col XVII autoantibody production (preliminary data will be shown).
Retinal ganglion cell-specific ER stress underlies optic atrophy in two siblings with Wolfram syndrome

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ABSTRACT

BACKGROUND: Wolfram syndrome (WS) is an autosomal recessive multisystem disorder with variable clinical presentation depending on the specific mutations in the WFS1 gene. WFS1 is an endoplasmic reticulum (ER) bound transmembrane protein that regulates cytoplasmic calcium concentration. Dysfunction of this protein leads to the activation of ER stress responses and signaling cascades. Tissues with high secretory demand struggle to restore homeostasis under conditions of increased ER stress. Two such tissues, which ultimately undergo apoptotic cell death in WS, are retinal ganglion cells (RGC) and insulin producing pancreatic β-cells. Apoptosis of these tissues results in progressive optic atrophy and (non-immune insulin-dependent) diabetes mellitus respectively. Recent advancements in stem-cell biology make it possible to study the genotype-phenotype connection responsible for variable clinical presentations of inherited diseases like WS. Patient-specific induced pluripotent stem cells (iPSCs) derived from somatic tissues can be used to generate diverse cell types in-vitro that would otherwise be inaccessible by biopsy. Because the genetic makeup of these cells is identical to the patients’ they were derived from, this technique provides an opportunity to study the effects of specific mutations on specific tissues. Two siblings homozygous for an amino acid substitution (R558C) in the WFS1 coding sequence present clinically with optic atrophy and no diabetes. In this study we generated retinal ganglion cells (iPSC-RGC) and insulin producing pancreatic β-islet cells (iPSC-β) from patients harboring different WFS1 alleles in order to evaluate the mechanism underlying the isolated optic atrophy phenotype in these siblings. Cells derived from WFS1R558C/R558C patients were compared to control patients with no ocular history (WFS1WT/WT) or with severe mutations resulting in full-blown WS (WFS1Y291fs/W613X), which includes both optic atrophy and diabetes.

HYPOTHESIS: The R558C mutant allele encodes a hypomorph WFS1 protein that produces a RGC-specific ER stress phenotype responsible for isolated optic atrophy in homozygous patients.

METHODS: Homozygous R558C WFS1 variants were identified in two patients with non-syndromic recessive optic atrophy using exome sequencing. Dermal skin biopsies were obtained and fibroblasts were targeted for iPSC generation using Sendai viruses driving expression of OCT4, SOX2, KLF4 and c-MYC. Pluripotency was confirmed using rt-PCR, immunocytochemistry and the TaqMan Scorecard Assay. Differentiation of iPSCs to RGCs and pancreatic β-islet cells was performed according to previously described protocols. Custom TaqMan arrays were used to characterize expression of key marker genes using quantitative rt-PCR.

RESULTS: Consistent with the optic atrophy phenotype of all three WS patients, significant increases in ER stress relative to controls were observed in iPSC-RGCs derived from WFS1R558C/R558C (P = 0.0005) and WFS1Y291fs/W613X (P < 0.0001) patients. In iPSC-β cells a significant increase in ER stress was only observed in cells derived from the WFS1Y291fs/W613X (P = 0.0013) diabetic patient. No significant increase in ER stress over controls was observed in iPSC-β cells derived from WFS1R558C/R558C patients, which explains why these patients do not present clinically with diabetes.

DISCUSSION: The findings of this study are consistent with the clinical presentations of each WS patient. Upregulated ER stress responses were observed in iPSC-RGCs from all three patients with optic atrophy. Conversely, only iPSC-β cells from the patient with diabetes showed increased ER stress. These tissue-specific differences in ER stress suggest that the R558C mutant allele encodes a hypomorphic WFS1 protein, which is responsible for a RGC-specific phenotype. This manifests clinically as isolated optic atrophy in WFS1R558C/R558C patients. Understanding the genotype-phenotype connection of WFS1 mutations helps to explain the variable clinical presentation of this disease and could aid in the identification of pathogenic pathways that can be targeted for treatment.
Financial Cost Analysis of Combining Cardiac Catheterization and Electrophysiology Procedures in an Outpatient Setting

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Background: Pediatric patients with congenital heart disease require multiple procedures over their lifetime. The accumulation of these separate procedures becomes costly and time-consuming. Previous studies in fields outside of cardiology have shown combining care is an effective tool for saving both time and money.

Objective: This study aims to compare combined cardiac catheterization (cath) and electrophysiology (EP) outpatient procedures against separate cath and EP outpatient procedures to determine cost and time savings.

Methods: Outpatient combination procedures performed in the pediatric cardiac cath lab from 2013 – 2016 were matched to two or three similar single outpatient procedures from 2009 – 2016 for patients of similar age and cardiac anatomy. Procedure duration, recovery duration, length of stay, equipment charges, physician charges, all other hospital charges and total admission charges were analyzed for both study and control groups. The two groups were compared using an unpaired t-test. A p-value less than 0.05 was considered statistically significant. All costs were adjusted for inflation to the 2016 fiscal year.

Results: A total of 92 subjects, 32 study subjects and 60 control subjects, were included in this study. Average age, height, and weight of study subjects were similar to the control group (p-values 0.54, 0.94, and 0.91, respectively). Study group procedures were statistically significant for a shorter recovery duration (p-value 0.04) and length of stay (p-value 0.01). Study group procedure duration was shorter on average but statistically insignificant (p-value 0.20). Estimated total mean savings for patients undergoing combined procedures in the study group was $13,669.

Conclusions: Combining cath and EP outpatient procedures saves a significant amount of time while providing an economical advantage. Further large scale prospective studies are needed to assess the financial burden on patients undergoing separate procedures.
**Pessary Treatment of Pelvic Organ Prolapse: Self Care vs. Office-based Care**

**Background:** Pelvic organ prolapse (POP) is common and the prevalence is expected to increase in coming years with US populations increasing in age and obesity, key risk factors for POP. The most common POP treatments include surgery and pessary treatment. A pessary is a non-surgical device placed in the vagina for the purpose of supporting the uterus or vaginal walls. In past decades, pessary treatment was typically recommended for women who were poor surgical candidates, still planning childbearing, or planning temporary use prior to surgery. Today, pessary use is considered a long-term alternative to surgery. Unfortunately, current studies examining pessary use are limited, particularly those focused on long-term pessary use. Additionally, past studies and expert opinion widely held that most pessary care is office-based involving regular follow-up, typically every 2 to 3 months, for assisted removal and replacement of the device. Few studies mention the option of pessary self-care involving self-removal, usually daily or weekly, and then less frequent office visits (often annually). In contrast, University of Iowa Urogynecology clinic providers have found most patients are able to self-manage their pessaries. A change toward pessary self-management makes sense as more women are using pessary treatment as an alternative to surgery, rather than as temporary treatment until surgery can be performed.

**Aims:** To compare pessary outcomes in women with POP, including duration of use and complications, in patients using self-care versus office-based care. Additionally, to identify patient characteristics associated with the type of pessary care (self-care versus office-based care). We hypothesize that among women using pessary treatment for POP, pessary self-care is associated with longer duration of use and fewer pessary complications compared to more standard office-based care.

**Methods:** Procedure codes for pessary fitting(s) and diagnosis codes for pelvic organ prolapse from billing records were used to identify patients seen at the UIHC Urogynecology clinic from 2008-2017. Patients successfully fitted with a pessary for POP and who used the pessary for at least 3 months were included. Data abstracted from the electronic medical record included demographics, medical history, prolapse type and severity, and pessary treatment data. Patient characteristics and pessary outcomes were compared between self-care vs. office-based care groups using chi squared, Wilcoxon ranksum and student t tests as appropriate.

**Results:** In this interim analysis, 117 women were included who used a pessary for median 15.8 (range 3.1-123.1) months. Mean age was 66±12 years and body mass index 28±5 kg/m². 65% had severe POP (stage 3-4) at pessary fitting. 86 (73.5%) used self-care (SC) for pessary management and 31 (26.5%) used office-based care (OC). Compared to the OC group, the SC group were younger (62.2±11.2 vs. 78.0±10.0), reported fewer medications (median (range) 4.5 (0-16) vs. 8 (0-16)), were more likely sexually active (45.4% vs. 3.2%) and had less severe POP (57% vs. 89% stage 3-4) (p<0.01 for all). Duration of pessary use and continued use at last follow-up (51%) did not differ by type of pessary care. However, vaginal erosions (8 (26.5%) vs. 3 (3.5%); p<0.001) and frequency of office visits (median (range) 6.8 (2.5-14.7) vs. 3.0 (0.9-13.8) visits per year; p<0.0001) were more common in the OC vs. SC group.

**Conclusions:** Most women opted for self-management of their pessary over office-based care, and those who elected and were able to perform pessary self-care were younger women with less severe POP. Duration of pessary use and continued use at last follow-up were similar in self-care and office-based care groups. Women able to perform self-care of their pessaries had fewer complications (vaginal erosions) and required fewer office visits per year than women undergoing more traditional office-based care of their pessaries. These results related to self-care of pessaries, generally not available in the current pessary literature, will be useful for providers counseling women about treatment options for POP.
Identifying the TLQP-21 Receptor in Islet Beta Cells Using C3aR1 Knockout Mice

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Background: Type 2 diabetes mellitus is a common and chronic metabolic disorder that is characterized by glucose intolerance and sustained hyperglycemia due to insulin resistance and/or insufficient insulin secretion (insulinopenia). Decreased insulin secretion is caused by a deterioration of pancreatic islet β-cell function and mass. Thus, the need exists to develop pharmacological agents which enhance β-cell function and promote insulin secretion. Previous research has demonstrated that the pro-hormone VGF (non-acronymic; unrelated to VEGF), which is stored within insulin-containing secretory vesicles and is cleaved into bioactive peptides, enhances glucose-stimulated insulin secretion (GSIS) by the action of the C-terminal peptide TLQP-21. Recently, the complement C3a receptor 1 (C3aR1) was identified as a receptor for TLQP-21 on the surface of CHO cells and adipocytes. Previous pilot studies have suggested that the GSIS-promoting action of TLQP-21 is dependent on the expression of C3aR1 in mice; indicating that C3aR1 may be the TLQP-21 target within β-cells as well. Furthermore, C3a peptide itself has been shown to be an insulin secretagogue.

Aims: The purpose of this study was to determine if TLQP-21 enhances GSIS in pancreatic islets by activating C3aR1. It was hypothesized that the loss of C3aR1 expression in islets would result in the loss of TLQP-21 insulinotropic activity. The specific aims were: 1) Determine the role of C3aR1 in mediating the TLQP-21 response on glucose tolerance and insulin release in mouse models. 2) Determine the role of C3aR1 in mediating the insulinotropic response of isolated islets to TLQP-21.

Methods: The insulinotropic action of TLQP-21 was examined in wild type (WT) C57Bl/6NJ and C3aR1 knockout (KO) mice as follows (all genotypes were verified by PCR). 1) The ability of TLQP-21 to improve glucose tolerance was tested. Animals belonged to one of four groups receiving intraperitoneal injection of either vehicle saline or TLQP-21: 1) WT mice exposed to saline vehicle, 2) C3aR1 KO mice exposed to saline vehicle, 3) WT mice exposed to TLQP-21, and 4) C3aR1 KO mice exposed to TLQP-21. Following peptide (or saline) injection, mice received an intraperitoneal glucose injection and blood glucose levels were measured during a 2 h time course. Additionally, blood was sampled from the saphenous vein at specific time intervals and circulating insulin levels determined by ELISA. 2) Pancreatic islets were isolated from WT and C3aR1 KO animals and GSIS was examined using the BioRep perfusion system. Islets were divided into the following treatment groups 1) WT islets treated with DMSO, 2) C3aR1 KO islets treated with DMSO, 3) WT islets treated with TLQP-21, and 4) C3aR1 KO islets treated with TLQP-21. Islets were perfused with basal glucose (2.5mM) solutions for 32 minutes, followed by stimulatory glucose (11.2mM) solutions for 32 minutes. Glucose solutions contained either DMSO (control vehicle) or TLQP-21 peptide as indicated for the treatment groups. Perifusates containing secreted insulin were collected in 96-well plate format. Insulin secretion levels were assayed via ELISA.

Results: During the glucose tolerance test, WT animals injected with TLQP-21 had a significantly decreased blood glucose level at 90 minutes and were trending towards a decrease at 30, 60, and 120 minutes post-glucose challenge compared to WT animals injected with saline. TLQP-21-treated WT animals also exhibited a significantly decreased glycemic excursion as compared to vehicle control-treated animals (measured as the area under the curve of the blood glucose vs. time plot). Plasma insulin levels did not differ significantly between WT animals injected with TLQP-21 and WT animals injected with saline. In contrast, the C3aR1 KO animals injected with TLQP-21 did not show a difference in blood glucose levels compared to the KO animals injected with saline, nor did their glycemic excursions differ. This corresponded with no difference in plasma insulin levels collected from the two groups of KO mice. In vitro islet experiments demonstrated a trend toward increased peak insulin secretion in WT islets exposed to high glucose containing TLQP-21 compared to high glucose alone. No difference in insulin release was seen in C3aR1 KO mice exposed to TLQP-21 as compared to vehicle control.

Conclusion: This study demonstrates that C3aR1 expression is required for both the blood glucose-lowering effect of TLQP-21 in mice following a glucose challenge, and the increased glucose-stimulated insulin secretion (GSIS) in perfused islets treated with TLQP-21. Together, these data suggest that C3aR1 is the TLQP-21 target receptor operating within pancreatic islet β-cells. Future studies will corroborate that the improvement in glucose tolerance by TLQP-21 in WT mice is driven by increased release of insulin into the plasma.
Reversal of Antithrombotic Therapy Prior to Heart Transplant in Pediatric Patients

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Background

Every year, 350-400 children receive a heart transplant in the United States. Unfortunately, approximately 70 others die awaiting a heart transplant each year. Although still unacceptably high, this number represents an improvement in recent years through the use of ventricular assist devices (VAD) as a bridge to transplant. VADs, however, require anticoagulation to prevent clotting. The University of Iowa (UI) pediatric heart transplant program has primarily used warfarin as the maintenance anticoagulant when VADs are required. This must quickly be reversed before transplant. There is controversy on the ideal reversal strategy prior to surgery. Recently, due to a UI Blood Bank mandate, our program shifted from fresh frozen plasma (FFP) to vitamin K for warfarin reversal.

While many studies have assessed outcomes in pediatric VADs, none have established a standard warfarin reversal protocol.

Methods

This retrospective chart review of UI Children’s Hospital’s medical records found 5 patients who received FFP and 5 who received vitamin K for reversal prior to surgery. Data collected included blood products used to reverse warfarin prior to transplant, during the operation, and 48 hours post-operatively. Additionally, overall patient outcomes, operating room time, and post-surgical complications were recorded.

Results

During the operation, the vitamin K group received more FFP, packed red blood cells (RBC), platelets, cryoprecipitate, Factor VII, and vitamin K than the plasma group. Furthermore, post-operatively the vitamin K group received more RBC, platelets, and Factor VII, while the plasma group received more FFP. In addition, the patients who received pre-operative FFP consistently had INRs (a measure of anticoagulation) in the target range, unlike the vitamin K group who also saw longer OR times and longer hospital stays.

Discussion

Vitamin K alone does not appear to be equal to or superior than FFP for quick reversal of warfarin prior to surgery, although our numbers may be insufficient to see that trend. The vitamin K group used more products during surgery, but both the vitamin K and FFP groups used many products post-operatively. Our team believes a combination of FFP with vitamin K prior to surgery would allow the optimal reversal situation: boosting synthesis of clotting factors with vitamin K and more immediate reversal with FFP.
Cullin3 Regulates eNOS Activity via Ubiquitination-Mediated PP2A Degradation in Primary Mouse Aortic Endothelial Cells

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**Background:** Cullin3 (Cul3) is a critical subunit of the Cul3-Ring-Ligase (CRL3) ubiquitin ligase complex. Cul3 bridges the interaction between E3 ubiquitin ligase Rbx1 and BTB-domain-containing adaptors for many substrates. Global mutations in Cul3 (causing in-frame deletion of exon 9, Cul3Δ9) causes hypertension. The Sigmund lab has made the intriguing observation that expression of this hypertension-causing Cul3Δ9 mutation selectively in the endothelium (E-Cul3Δ9) causes endothelial dysfunction and severe nocturnal hypertension. Phosphorylation of eNOS (encoded by NOS3) is required for nitric oxide production and this process is negatively regulated by the serine/threonine protein phosphatase 2A (PP2A). Perturbations in PP2A/eNOS interactions contribute to vascular dysfunction in diabetes, obesity and hypertension. Because Cul3 regulates PP2A activity by targeting its subunits for degradation via ubiquitin-proteasome pathway, loss of Cul3 function in endothelial cells may lead to accumulation of PP2A and sustained dephosphorylation of eNOS (Ser1177) and impaired eNOS activity.

**Aim:** To determine if Cul3 regulates eNOS activity via ubiquitination-mediated PP2A degradation.

**Experimental Design:** Studies were performed in low passage primary mouse aortic endothelial cells (MAECs) isolated from aortas of mice expressing inducible Cul3Δ9 transgene and tdTomato reporter. Expression of transgenes was induced *in vitro* by infecting cells with AdCre-GFP, while control cells received an empty adenovirus vector. In previous experiments, co-localization of GFP and tdTomato signals was used to confirm the efficiency of transduction and activation of the Cul3Δ9 transgene.

**Results:** Two weeks following viral transduction, tdTomato expression was confirmed by Western blot. Cul3Δ9 is difficult to detect because we previously showed it forms unstable heterodimers with wild type Cul3 (Cul3WT), leading to proteasomal degradation of both proteins. Indeed, cells treated with AdCre-GFP showed a marked reduction in wild type Cul3 protein but not Cullin1, another member of the Cul3 family. Interestingly, a decrease in Cul3WT was associated with a reduction in phosphorylated eNOS (Ser1177) but not total eNOS, and with a marked decrease in nitric oxide production as indicated by the intracellular nitrate/nitrite levels. These decreases were rescued by a selective PP2A inhibitor Okadaic Acid (4 nM), but not protein phosphatase 1 inhibitor Tautomycetin (4 nM). Interestingly, the levels of the catalytic subunit of PP2A remained unchanged, suggesting that Cullin3 targets other PP2A subunits.

**Conclusions:** Together, these data indicate that Cullin3 regulates eNOS activity via PP2A in vascular endothelial cells, defining a novel pathway involving Cullin-3/PP2A/phospho-eNOS in the endothelium. These results suggest that mutations in Cullin-3 cause human hypertension in part through a vascular mechanism characterized by impaired endothelial function.
Magnetogenetic modulation of pancreatic beta cell excitability and insulin secretion

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Abstract:
Ion channels are ubiquitously expressed throughout the human body and play an integral role in many essential cell processes. Among other functions, they transduce extracellular stimuli into cellular responses, regulate growth and nutrient metabolism, facilitate cell communication, and control cell motility. However, the basic biological mechanisms linking ion channels to these cellular processes remain poorly characterized. This problem is compounded by the shortage of tools to precisely modulate ion channel activity in vitro and in vivo. For example, a current technology, optogenetics, lacks the ability to penetrate deep tissues to investigate ion channel function. A new technique, magnetogenetics, offers a potential solution to this problem. Magnetogenetics involves engineering a protein fused to a magnetic nanoparticle followed by magnetic stimulation of the nanoparticle to mechanically activate the protein. In this study, we transiently co-expressed the TRPV1 ion channel with an N-terminal anti-GFP camelid antibody and a chimeric ferritin protein fused to GFP – resulting in a TRPV1-Ferritin chimeric protein. The TRPV1 channel is a cation channel that conducts Na$^+$ and Ca$^{2+}$ into the cell. Ferritin is an iron storage protein that can act as an endogenous magnetic nanoparticle. We hypothesized magnetic stimulation would activate Ca$^{2+}$ import in cells expressing the TRPV1-Ferritin protein. As a proof of concept, we first tested the TRPV1-Ferritin construct in transiently transfected HEK cells. We observed reproducible intracellular Ca$^{2+}$ elevations in TRPV1-Ferritin expressing HEK cells upon magnetic stimulation with an estimated field strength of 5000 Gauss (0.5 Tesla) using a 1/16" x 1/16" x 1/8" thick neodymium magnet (F/Fo = 1.76 ± 0.08, n = 48). Next, we explored the utility of this approach to activate Ca$^{2+}$ transients in pancreatic islets and stimulate insulin secretion from pancreatic β-cells; as elevations in intracellular Ca$^{2+}$ in pancreatic beta cells stimulates insulin secretion into the bloodstream. We adenovirally co-transduced murine pancreatic islets with TRPV1-Ferritin and a genetically-encoded Ca$^{2+}$ indicator, GCaMP6s under the control of the β-cell specific rat insulin promoter (RIP-GCaMP6s). Magnetic stimulation of these murine pancreatic islets activates an intracellular Ca$^{2+}$-wave within β cells in the islet (F/Fo = 1.56 ± 0.03, n = 54). Finally, TRPV1-Ferritin transduced neonatal porcine pancreatic islets exhibit a 1.32-fold increase in insulin secretion upon magnetic stimulation as assessed in perfusion assays. These studies demonstrate the functionality of using magnetogenetics to manipulate ion channel activity in vitro to regulate pancreatic β-cell excitability and insulin secretion. Future work will be directed toward applying this system to in vivo models with the aim of creating a platform for magnetogenetic modulation of ion channel signaling and intracellular signaling in deep tissues.
**MgrA Regulates Attachment of *Staphylococcus aureus* to Mucin**

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**BACKGROUND:** Airway mucus defends the respiratory tract by trapping inhaled pathogens and facilitating their removal by ciliary transport. Mucins are hydrated and self-assemble in the airway surface liquid (ASL). In cystic fibrosis (CF), ASL is abnormally viscous and mucus detachment is impaired. Patients with CF develop chronic bacterial airway infections with *Staphylococcus aureus*, but it remains unclear how these bacteria establish attachment to airway mucin. Recently, the *S. aureus* global gene regulator *mgrA* has been shown to negatively regulate the expression of several of the bacteria’s large surface proteins, including Ebh and SraP. These regulatory mechanisms are thought to play a role in the bacteria’s ability to bind extracellular surfaces such as fibrinogen, and to other bacteria. **Our aim was to determine if *mgrA* and associated bacterial surface factors played a role in attachment to mucin, to further understand *S. aureus*’ pathogenesis in cystic fibrosis and other diseases.**

**METHODS:** We used a competition assay to measure the interaction of bacteria and mucus. Wild-type (WT) and mutant GFP *S. aureus* grown for 18 hours overnight in tryptic soy broth were diluted to an OD₆₀₀ of 0.6. Porcine Gastric Mucin Type III (Sigma-Aldrich) was diluted to various working concentrations in pH 7.2 phosphate buffered saline (PBS), and 100ul was added in replicates of six to wells of a 96-well plate. 50 ul of our OD 0.6 *S. aureus* was then added to each well, and the plate was incubated at 37°C for 1 hour. Wells were immediately aspirated post-incubation and subsequently washed with 150ul of PBS. After washing, the fluorescence of each well was recorded and wells with equal mucin concentrations were averaged together.

**RESULTS:** Strains lacking *mgrA* (∆mgrA) displayed over a 10-fold reduction in their ability to bind porcine mucin when compared to WT (p > 0.05). This inability to bind mucin was reduced when *mgrA* was partially complemented back into ∆mgrA. The reduction in mucin binding was not observed when WT *S. aureus* was tested against a strain deficient in clumping factor (∆clfA). Strains lacking combined expression of *mgrA*, *ebh*, and *sraP* restored binding to WT levels.

**CONCLUSIONS:** Deletion of *mgrA* greatly reduced the bacteria’s ability to bind mucin, which was shown to be ∆mgrA specific as partial complementing of the gene restored some binding capacity. This loss of mucin binding was not due to loss of a clumping phenotype, as clumping-factor deficient strains demonstrated no reduction in their ability to bind mucin. Instead, the reduction in mucin binding is most likely due to increased expression of *ebh* and *sraP*. In WT *S. aureus*, functional *mgrA* suppresses the expression of *ebh* and *sraP*, which encode large surface proteins on the bacteria. Deleting *mgrA* thus allows for dramatically increased expression of these proteins, which in turn sterically inhibits the bacteria from binding both self and to other surfaces such as mucin. This is supported by experiments showing that combined deletion of *mgrA* and the surface proteins it inhibits expression of (*ebh* and *sraP*) restores mucin binding to WT levels. Finally, using the irrelevant mucin substitute methyl cellulose, we observed no differences between strains, indicating that this is a mucin-specific result.
Radiation Therapy Combined with Pharmacological Ascorbate Increases PD-L1 Expression in Lung Cancer Cells and Induces an Abscopal Effect in a Murine Lung Cancer Model

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Introduction: Programmed cell death-1 (PD-1) is a membrane bound receptor found on the surface of T lymphocytes that inhibits auto-immunity when bound to its ligand, programmed cell death ligand-1 (PD-L1). Interaction between PD-1 and PD-L1 anergizes tumor specific T-cells and increases tumor resistance to cytotoxic T-cell mediated immunity. High doses of Vitamin C (pharmacological ascorbate) has recently been shown to enhance radiation and chemotherapy in a variety of tumor models. Pharmacological ascorbate selectively increases tumor cell hydrogen peroxide. Increases in cellular hydrogen peroxide activates and stimulates the translation of NF-kappa B and nuclear factor of activated T cell (NFAT). NFAT and NF-kappa B upregulate the expression of PD-L1. Tumor cell upregulation of PD-L1 by ascorbate may increase the sensitivity of tumors to anti-PD-1 therapy.

Hypothesis: We hypothesize that combining radiation therapy with pharmacological ascorbate will increase tumor cell hydrogen peroxide and thereby induce expression of PD-L1.

Methods: Lewis Lung Carcinoma (LLC), A549, and H292 cells were incubated at 1% and 4% oxygen tension. Cells were plated in 60 mm dishes at 1.0 x 10^5 cells per plate and treated with radiation (4 Gy and 9 Gy per fraction), ascorbate (5 pmol/cell), or interferon gamma (100 u/ml). Cells were harvested 48 hours post treatment with 2.0 x 10^5 cells collected per treatment group to normalize staining stoichiometry. Cells were stained with an anti-mouse anti-CD279 (PD-L1) PE labeled antibody. Samples were analyzed by FACS at the University of Iowa Flow Cytometry Facility.

Fifteen female C57BL/6J immune competent mice were inoculated with 5x10^4 LLC cells in right and left flanks. Tumors were allowed to grow to 3-4 mm. Three mice were assigned to each group including: 1) control animals injected with 450 µL 0.9% sterile saline i.p. daily; 2) mouse interferon gamma (IFNy) at 25,000 unit dose subcutaneously once; 3) pharmacological ascorbate at 4 g ascorbate/kg i.p. daily; 4) radiation (18 Gy/ in 2 fx) to the right flank tumor; 5) pharmacological ascorbate + radiation. Only tumors on the right flank were irradiated. Right and left tumor volumes were calculated daily starting first day of radiation. Animals were sacrificed once tumors reached 15mm in maximum diameter.

Results: PD-L1 expression increased in LLC, A549, and H292 cell lines following radiation. Ascorbate treated LLC cells increased PD-L1 expression by 1.5-fold, whereas radiation treated cells increased expression by 2-fold. Combined treatment proved to be too lethal to reach statistically significant numbers of cells for flow cytometry analysis (>10,000). No differences in tumor growth were noted in ascorbate, IFNy, and radiation treatment groups when compared to the control animals, whereas the tumor growth rate was slower in the combined ascorbate and radiation treatment. Control mice survived for an average of 12 +/- 2.94 days following treatment. Radiation treated mice survived for an average of 11.33 +/- 2.49 days. Ascorbate treated mice survived for an average of 11 +/- 2.45 days. IFNy treated mice survived for an average of 13 +/- 0.82 days following treatment. Radiation + Ascorbate treated mice survived an average of 29 +/- 7.49 days. Remarkably, animals in the Radiation + Ascorbate group saw reduced tumor growth rates in both irradiated and un-irradiated legs. These results support the speculation that an abscopal effect involving increased T-cell mediated tumor killing in the un-irradiated leg may have been increased by the combination of radiation plus ascorbate.

Conclusions: The in vitro studies showing upregulation of PD-L1 in LLC after treatment with ascorbate and radiation suggest that the combination of ascorbate, radiation and anti-PDL1 therapy may be a promising anti-cancer therapy. Animals treated with Radiation + Ascorbate had markedly reduced tumor growth in both flanks as well as improved survival when compared to the control, radiation, IFNy, and ascorbate mice suggesting a possible abscopal effect.
ULK1 Regulates Recovery From Skeletal Muscle Damage

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Abstract

BACKGROUND: Autophagy, a catabolic process that degrades long-lived proteins and organelles, is thought to be instrumental for the maintenance of normal muscle integrity and function. However, the molecular regulation of skeletal muscle autophagy is poorly understood. Here, we investigated the role of ULK1, the closest homolog of the first identified autophagy gene Atg1, in the maintenance of muscle fiber integrity and basal autophagy flux in skeletal muscle. Preliminary results in adult skeletal muscle electroporated with a DNA plasmid encoding a miRNA specifically targeting the Ulk1 gene (p-miR-Ulk1) revealed a high percentage of centrally nucleated fibers, indicative of fiber damage and/or regeneration, when compared to contralateral muscles electroporated with a DNA plasmid encoding a scramble miRNA (p-miR-Control). In addition, ULK1 deficient muscle presented high expression of MTOR, which has been shown to cause myofiber damage when chronically activated.

HYPOTHESIS: We hypothesized that ULK1 would be required for the maintenance of normal fiber integrity by regulating MTOR stability via autophagy.

METHODS: We determined the impact of muscle ULK1 deficiency on basal autophagy in adult muscle electroporated with p-miR-Ulk1 and p-miR-Control. We also examined MTOR protein levels in ULK1 muscle-specific knockout mice, and evaluated the effect of Ulk1 deletion on MTOR, RAPTOR and RICTOR protein stability using cycloheximide chase experiments in primary mouse myotubes.

RESULTS: ULK1 deficiency does not seem to impact overall basal autophagy flux in skeletal muscle. In addition, our preliminary results demonstrate that MTOR is not increased in skeletal muscle of ULK1 muscle-specific knockout mice, and that ULK1 deficiency does not affect MTOR protein degradation in primary mouse myotubes.

CONCLUSIONS: Taken together, these preliminary results are consistent with a potential role for ULK1 in the maintenance and/or recovery of myofiber integrity after mild muscle damage, which can be elicited by plasmid DNA electroporation in adult skeletal muscle. The increased MTOR protein levels observed in this condition might be reflective of delayed recovery of the muscle rather than the culprit of myofiber damage. Studies using other muscle damaging models are still required to further delineate a potential ULK1 role in the maintenance and/or recovery of myofiber integrity and to reveal if overall autophagy, selective autophagy (e.g., mitophagy) and/or other cellular processes are mechanistically involved.
Patients with Croup: Epinephrine Treatment and ED Observation Time
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**Background:** Croup is a common respiratory illness mostly affecting children under 6 years of age. Symptoms include: inspiratory stridor, barking cough, and hoarseness. Treatment with nebulized epinephrine (NE) usually relieves croup-related stridor; but, it may recur once the medication wears off. This potential return of symptoms led to recommendations that children receiving NE be observed in the ED with times ranging from 2 to 5 hours. After observation, patients with no audible stridor at rest may be discharged; those who have a return of stridor are usually given a second NE treatment and admitted to the hospital. This observation time is costly, hampers examination room turnover and ED throughput, and is an inconvenience and hardship for patients and their families. It is unclear if a prolonged observation period is necessary for all patients.

**Purpose of the study:**

**Aim 1.** Describe the current observation practices of providers after administering NE and determine whether this observation period has changed over the period of the study.

**Aim 2.** Identify subsets of croup patients treated with NE at risk for requiring a second NE treatment after initial resolution of stridor, and those who are low to no risk to need subsequent interventions.

**Methods:** A retrospective medical chart review was performed of patients who presented with croup to the University of Iowa ED from February 2007 through January 2017 and were identified as having received at least one NE treatment. Numerous variables pertaining to the patient’s demographics, history of their acute illness, physical exam, treatment, past medical history, social history, and family history were recorded. Patients were grouped based on having received one NE treatment vs. having been administered a second NE ≥30 minutes after the first. Those who received a second NE treatment <30 minutes after their first NE treatment (N=33) were considered non-responders and would not normally be observed in the ED for potential discharge; thus, they were excluded from the analysis. Patients who received only one dose of RE but were admitted were also excluded because there was another factor leading to their admission other than croup (N=39). Univariate, multivariate and multivariable logistic regression analysis was executed with SPSS Statistics software.

**Results:** During the study period, 1202 Croup patients presented to the UIHC ED. Of these, 398 (33%) received at least one NE treatment, and 146(37%) were admitted to the hospital. Observation times after NE administration increased significantly from 2007 (1.3± .84 hours) to 2016 (3.28± 1.68hours), p <0.01. After multivariable logistic regression analysis, the following variables were found to be associated with an increased likelihood of requiring a second NE treatment during the observation period: age <18 months (OR 2.36, 95% CI 1.17-4.77), retractions on initial physical exam (OR 2.90, 95% CI 1.44-5.85), initial pulse oximetry measure less than 94% (OR 2.49, 95% CI 0.98-6.33), corticosteroid administration ≥30 minutes after the initial NE (OR 2.38, 95% CI 1.00-5.64) and presentation time from 1-6pm (OR 2.82, 95% OR 1.19-6.67).

**Conclusion/Discussion:** Our results support the need to further study the practice of a prolonged observation period after NE administration for croup-related stridor. With additional analysis, we will develop a decision tree with regards to patients who are low risk and may be discharged after NE treatment and those who should be observed. This will then be utilized in a prospective study. This data will be essential in developing evidence-based clinical guidelines for the management of croup patients, and has the potential to improve ED throughput and patient satisfaction as well as decrease overall healthcare costs.
Population-based Evaluation of Factors Associated with Use of Intravesical Bacillus Calmette-Guérin for High Grade Non-Muscle Invasive Bladder Cancer
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Background
There are almost 80,000 new cases of bladder cancer every year in the United States. At diagnosis, the vast majority of bladder cancers are non-muscle invasive bladder cancer (NMIBC) which are treated with transurethral resection and then bladder sparing therapy. Since initial publication in 1999, guidelines from the American Urological Association (AUA) have strongly recommended that patients with high-risk NMIBC receive a six-week course of intravesical treatment with Bacillus Calmette-Guérin (BCG), an immunotherapy that has been shown to decrease recurrence and progression. However, studies have shown suboptimal compliance to guideline-based care. We set out to determine if this was due to failure to begin treatment or treatment intolerance. Using a population-based analysis, we wanted to elucidate the factors associated with receiving BCG for high grade NMIBC. In addition, we set out to determine if low utilization of BCG was due to failure to begin treatment or treatment intolerance.

Hypothesis
We hypothesize that compliance with BCG will be suboptimal and that marked variability will exist in patient receipt of BCG, and that inadequate receipt of intravesical BCG would be driven primarily by provider variability, rather than by patient-specific factors.

Methods
The evaluation used SEER-Medicare, a database from the National Cancer Institute which records information on individuals 65 and older with cancer. The study cohort consisted of 847 Iowans aged 66 years and older with high-grade urothelial NMIBC diagnosed between January 1, 1992 and December 31, 2009 who survived 2 years and were not treated with cystectomy or radiation. Patients were assessed for BCG treatment (yes/no) and number of BCG treatments received during 2-year follow-up. Variables associated with receipt of BCG were evaluated by odds ratios using univariate logistic regression analysis and those variables with p<0.05 were included in the multivariate model. Patient-specific treatment patterns were analyzed via graphical representation.

Results
During the 2-year follow-up period, only 65.3% of patients received at least one BCG treatment. Of those who did receive BCG, the most common frequency was six treatments (16.5% of patients) and 11.5% received incomplete induction therapy of less than 6 treatments. Compliance with optimal BCG usage (at least 6 BCG treatments) showed significant improvement from 44.7% to 55.8% between 1992-1997 and 1998-2002 (p=0.048), but there was no improvement in 2003-2009 with compliance of 56.0% (p=0.97). On univariate analysis, factors associated with receiving BCG were ages 75-79 years (vs. ages 66-69 years), being married, T1 or Tis classification (vs. Ta), and more recent diagnosis (p<0.05). Academic versus nonacademic treatment center, gender, and Charlson comorbidity did not affect BCG treatment likelihood. On multivariate analysis, both the T1 and Tis classifications remained significant, as did more recent diagnosis (p<0.05).

Conclusions
BCG treatment of high-grade NMIBC was suboptimal as 34.7% of patients did not receive any BCG treatment. Patient-specific characteristics (age and stage) and more recent treatment were associated with receiving BCG. A significant number of patients started on BCG treatment do not complete a full induction course. As evidence-based medicine has shown the effectiveness of BCG treatment, increasing the number of eligible patients receiving adjuvant BCG treatment through corrective measures requires improved dissemination and implementation of guideline-based care with a focus on both offering BCG and improving treatment tolerance.
Thiol-Redox Imbalance Results in an ER-stress induced autophagy which creates a resistance to MAPK Pathway Inhibitors in Melanoma
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Background: The incidence of melanoma is growing faster than any other cancer in the United States. Surgery (and surgery combined with external beam radiation) can be curative at early stages. However, metastatic melanoma is almost uniformly fatal. While clinical investigations of chemotherapeutic agents have seen little success over the past three decades, recent discoveries have led to the introduction of promising targeted (mitogen-activated protein kinase) MAPK pathway inhibitors (MAPKi) (e.g., inhibitors of the BRAF and MEK kinases [BRAFi and MEKi] in the MAPK pathway) and immunotherapies (e.g., CTLA-4 and PD-1 inhibitors); that are administered as single-agents; and in combinations with other drugs. While in some cases responses to these new therapies can be remarkable, response rates are more appropriately defined as low. Further, drug resistance (5-yr survival 17%), and adverse events remain as significant challenges to long-term quality of life for metastatic melanoma patients. The precise mechanisms of acquired drug adaptation are not completely understood; but include immune-response avoidance, altered/alternative oncogenic pathways, the presence of subpopulations of resistant-melanoma stem-like cells, and enhanced DNA repair. Although a detailed understanding of the mechanisms that drive acquisition of resistance to MAPK pathway inhibition remains elusive, evidence points toward drug-induced alterations in oxidative metabolism in melanoma cells, that appear to be correlated with disruption of the protein folding machinery in the endoplasmic reticulum, and concomitant increases in autophagic flux that conveys resistance. However, the therapeutic potential of simultaneously inhibiting autophagy and relieving ER stress for drug-resistant metastatic melanoma has not been explored.

Methods and Results: Our data show that in vitro (A375, 451LU BRAFi-sensitive melanoma cell lines), MAPK pathway inhibition (MAPKi) via continuous administration of vemurafenib (BRAFi) and cobimetinib (MEKi) perturbs mitochondrial functioning (Seahorse), disturbing the oxidative state of the cell (quantified by mean fluorescence intensity of mitosox) and consequently the ratio of oxidized to reduced glutathione (GSH). The disturbed GSH ratio disrupts the protein folding machinery in the endoplasmic reticulum (ER), which activates the unfolded protein response (UPR) to the accumulation of misfolded proteins (ER-stress) in the ER lumen. Our data further demonstrates that prolonged activation of the UPR initiates an adaptive response to MAPKi via activation of an autophagic response that conveys a resistant phenotype. Excitingly, our data further demonstrates that simultaneously relieving ER-stress (using FDA approved, 4-phenyl butyric acid; 4-PBA) and inhibiting autophagy (using FDA approved drug Plaquenil, hydroxychloroquine HCQ) inhibits adaptation to MAPKi in melanoma cell lines (A375) (clonogenic survival assay). Our studies further demonstrate that administration of the combination of 4-PBA and HCQ in with BRAFi inhibitor (vemurafenib) improved tumor response and overall survival of mice bearing metastatic melanoma xenograft tumors (BRAFi-resistant metastatic melanoma cell lines 451-LUBR). The combination therapy (4PBA+HCQ+Vem) led to complete remission in 20% and partial remission in 30% of mice as compared to controls (mice treated with vemurafenib alone or in combination with 4-PBA and HCQ individually).

Conclusion: The resistance acquired by melanoma cells is a result of metabolic changes that occur after exposure to MAPKi inhibitors that deplete cellular glutathione. This creates a redox imbalance in the cell and causes ER stress and subsequent autophagy which is protective. An understanding of this mechanism will pave the way for novel combination therapies which can help to overcome the MAPK resistance in metastatic melanoma and prevent patients who have metastatic disease from having a recurrence or progression of their melanoma.
Abstract

Background:
Screening mammography is used to screen for breast cancer. Abnormal readings lead to a variety of follow up tests including diagnostic mammography, ultrasound, and biopsy. These abnormal results and potential cancer diagnoses tend to cause distress and anxiety in returning patients.

Tomosynthesis was introduced in the United States without randomized controlled trials. Proponents of this method believe it is advantageous for cancer detection. The full effect of tomosynthesis is unknown in terms of recall rates for additional imaging, recommendations for biopsy, and rates of cancer diagnosis.

Hypothesis:
Tomosynthesis will decrease the rate of follow-up imaging (additional views and ultrasounds) and will increase the cancer detection rate, compared to digital screening mammography alone.

Methods:
This was a mixed methods study, using both quantitative and qualitative methods.

A dataset was created of women aged 40-89 years who underwent screening mammograms at UIHC between 2009 to 2017 (n=23,960). Based on the initial screening, women were categorized into two groups: 1) digital (n=19,097) or 2) tomosynthesis (n=4,863). The proportion of follow-up imaging, biopsy, or a cancer diagnosis was calculated. Positive predictive values were calculated for cancer diagnoses that resulted from any follow-up test, as well as cancer diagnoses that resulted from any biopsy.

For the qualitative component, four focus groups were conducted that included women who had abnormal mammograms with either: 1) benign biopsy results or 2) a cancer diagnosis. The groups were audio recorded, transcribed, and then analyzed according to the constant comparative method. General themes across the focus groups were assessed.

This study was approved by the University of Iowa Institutional Review Board.

Results:
The mean age in the digital group was 54.3 years, as compared with 52.6 years in the tomosynthesis group (p<.0001). The rate of follow-up imaging for the digital group was 15.99% (3,053/19,097) compared to 15.55% (756/4,863) for tomosynthesis (p=0.45). The biopsy rate was 1.70% for the digital group compared to 2.88% for the tomosynthesis group (p<0.0001). Cancer detection rates were 0.97% and 1.01% (p=.81), respectively. The positive predictive value for cancer detection for any follow-up test was 3.94% for digital mammography and 5.39% for tomosynthesis (p=.07). The positive predictive value for cancer detection following a biopsy was 28.40% for the digital group and 27.14% for the tomosynthesis group (p=0.78).

A majority of the benign focus group participants wanted to know probabilities of having an abnormal mammogram and felt anxious during the wait for follow-up results. The cancer diagnosis groups overwhelmingly communicated that they feel the ordering physician of the screening mammogram should be in contact with patients throughout the follow-up period. A majority of women in both categories of focus groups expressed confusion over mammogram screening frequency due to the conflicting practice-based guidelines of national medical groups.

Conclusion:
Tomosynthesis did not result in a decreased rate of follow-up imaging. However, tomosynthesis was associated with a statistically significant increased rate of biopsy, with no increase in the cancer detection rate. Further research is necessary to assess the clinical significance of tomosynthesis vs. standard digital mammography. Providing women with the probabilities of having an abnormal mammogram and clarification of which screening guidelines are being used might help allay patient anxiety.
Cognitive Workload in Single Pilots Using Human-Autonomy Teaming

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Background: Single Pilot Understanding through Distributed Simulation (SPUDS) is a NASA-sponsored study involving reduced crew operations in airliners. The aim of the study is to investigate novel tablet software, developed in conjunction between NASA and Rockwell-Collins, which provides automation of tasks normally conducted by the first officer. This would theoretically allow for a single pilot in the cockpit. Using Human-Autonomy Teaming (HAT), the pilot collaborates with the automated tablet system to complete tasks in non-normal scenarios. The automation includes smart checklists, voice interaction, and an Autonomous Constrained Flight Planner, a tool that assists pilots in choosing divert airports if necessary.

Aims: The aims of this study were to evaluate cognitive workload in pilots participating in SPUDS through subjective and objective measures.

Methods: SPUDS was conducted using 12 commercial airline pilots, all of whom had glass cockpit experience in passenger jets. The study was conducted in the OPL Boeing 737-800 simulator. The roles of dispatch and ATC were covered by CHAAT students. Pilots flew six scenarios involving non-normal events (wheel well fire, medical emergency, etc.). Scenarios were stratified into low (L1, L2), medium (M1, M2), and high difficulty (S1, S2). Three scenarios allowed the pilot to utilize HAT, while three did not. In the NoHAT scenarios, pilots were still able to use tablet functions like ACFP, but did not receive the benefit of electronic checklists or automated actions. Following each scenario, pilots completed questionnaires which included two established subjective measures of cognitive workload, the NASA Task Load Index (TLX), and the Situational Awareness Rating Technique (SART). To evaluate objective cognitive workload, 3-lead electrocardiogram was also collected for the duration of each scenario. ECG values were converted to transitive probability variance (TPV) values using the OPL algorithm, which were then used to calculate mean relative workload for each scenario.

Results: No statistically significant difference was observed in the TLX or SART scores between scenarios conducted in HAT and NoHAT conditions. Similarly, there was no significant difference in mean relative workload as measured by ECG between HAT and NoHAT scenarios.

Conclusions: Although there were no statistically significant differences in overall cognitive workload between HAT and NoHAT scenarios, further study is needed to fully evaluate the merits and challenges of the software. A very likely reason for the similar values is that cognitive workload was measured as an average for the duration of each scenario. Cognitive workload varies significantly on a moment-to-moment basis, and subtle variations during periods of high stress (i.e. alert first received) may be drowned out by the relatively low stress environment of normal flight. In addition, many of the scenarios may have been more benign than intended. None of the scenarios significantly impacted the flight capability of the aircraft, and most had been seen on multiple occasions by the pilot participants in their careers. Future studies would gain more meaningful information from either higher stress scenarios (loss of engine power, rapid decompression), or higher stress portions of flight. All scenarios in this study began and ended en route, while the most difficult portions of flight are often considered taxiing, takeoff, and landing. These are also times with more division of activity between the captain and first officer. Evaluation of a single pilot/automation environment in these higher-stress situations would be very informative.
Structurally-divergent ACE inhibitors differentially modify metabolism and weight gain

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Background: Hypertension is an important health condition, affecting 42% of adults and up to 71% of patients with metabolic comorbidities such as diabetes and obesity. There is growing evidence that suggests that the dysfunction of the renin-angiotensin system (RAS) is involved in metabolic disorders such as obesity and type II diabetes, and many groups are now exploring how the inhibition of the RAS may prevent or protect against obesity. A primary approach to treating hypertension is the inhibition of the RAS via the use of angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors such as Captopril (first-in-class drug / now rarely prescribed) and Lisinopril (now primarily prescribed) are used to treat hypertension. The compounds have structural differences that result in each compound having a distinct pharmacokinetic profile such as bioavailability and half-life. As a result, Lisinopril is used more commonly than Captopril due to its long half-life and convenient dosing schedule. Recently, our lab demonstrated that at therapeutically relevant doses, treatment of mice with Captopril but not Lisinopril increases energy expenditure in mice, thus causing reduced weight gain. This occurs despite no difference between the treatment groups in food ingestion, digestive efficiency, or calories absorbed. This indicates that Captopril induces a decrease in energy efficiency, highlighting that the host expends more energy per kcal absorbed than Lisinopril- or vehicle-treated animals. Since energy intake from digestive efficiency and food ingestion don’t differ between the groups, the increased energy expenditure may be the result of differences in resting metabolic rate (RMR). This leads us to hypothesize that the structural and pharmacokinetic differences between Captopril and Lisinopril differentially influence RMR, which is in turn responsible for the increased energy expenditure seen in Captopril- but not Lisinopril-treated mice. Additionally, RAS manipulation has robust effects to change gastrointestinal function and the composition of the gut microbiota, which leads us to the secondary hypothesis that the differential effects of Captopril and Lisinopril may be tied to their differential influence on the composition of the gut microbiota.

Methods: To explore our hypotheses, C57BL/6J mice were obtained from Jackson Labs at three weeks of age and maintained on a 2920k caloric chow diet. They were acclimated to the laboratory and then treated for one week with vehicle-, lisinopril-, or captopril-supplemented drinking water (160 mg/L and 500 mg/L, respectively, representing doses that effectively reverse hypertension). Body mass and composition was measured via NMR followed by measurement of RMR of each mouse under general anesthesia (ketamine+xyalazine) via direct calorimetry. Fecal bacteria were collected and underwent metagenomic sequencing to test for differential shifts in microbiome composition.

Results: As previously, mice treated with Captopril, but not Lisinopril, exhibited a significant decrease in body mass compared to vehicle-treated mice. Captopril-treated mice also exhibited a significant increase in RMR after adjusting for differences in body mass using univariate regression modeling (ANCOVA). This change in RMR corresponds with an increased energy expenditure of 5.5 kcal/week, which is sufficient to explain the observed weight loss exhibited by the Captopril-treated mice. In addition, Captopril caused unique shifts in the gut microbiota compared to vehicle and Lisinopril treatments.

Conclusion: ACE inhibitors are widely prescribed therapies for the treatment of hypertension, and we have shown that differences in structure influence RMR, likely due to differences in pharmacokinetics and possibly through effects on the gut microbiome. Clinical Relevance: Despite the primary role of RMR suppression in human obesity, there is a lack of FDA-approved anti-obesity drugs that work by stimulating RMR. These findings identify Captopril as a potentially useful RMR stimulator, and may explain why clinical trials of RAS inhibitors (which have almost exclusively utilized Lisinopril and Enalapril) have failed to detect beneficial effects on energy balance and weight gain. Future Directions: Ongoing experiments are examining the consequences of transplanting the gut microbiota from Vehicle-/Captopril-/Lisinopril-treated mice to naïve control mice, to establish Koch’s 3rd postulate; the transmissibility of the causative mechanism. Results will clarify whether the unique shift in the gut microbiota is responsible for the differential effects of Captopril upon RMR.
Evaluation of Focal Neurologic Lesions in Refractory Epilepsy Utilizing 7T Magnetic Resonance Imaging

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Introduction
Epilepsy is a common neurological disorder with increasingly recognized morbidity and mortality. The most common subset is temporal lobe epilepsy which in up to 30-35% of cases is medically refractory as defined by resistance to two separate appropriate medical regimens. In these cases, surgical resection of the epileptogenic focus was shown to completely resolve seizures in 64% of patients. Success is highly dependent on the ability to define and fully excise the entire seizure forming region. The most important prognostic factor for surgical success was identification of temporal pathology on MRI. For a significant percentage of patients with refractory epilepsy, no focal lesion can be identified either delaying or precluding surgical treatment. Successful identification of focal lesions can be influenced by the quality and spatial resolution of the imaging employed. Our aim was to develop a 7T MR epilepsy imaging protocol to maximize our ability to identify focal lesions and then apply this protocol to image patients with refractory epilepsy. Our hypothesis was that the enhanced spatial resolution and contrast available at 7T would allow us to identify and morphologically characterize focal epileptogenic lesions not detectable on standard 3T images.

Methods
A high resolution 7T imaging protocol was initially optimized on a GE 950 scanner using a 2Tx/32Rx head coil (NOVA Medical) in control subjects. The protocol consisted of T1- and T2-weighted images along with T2-weighted fluid attenuated inversion recovery (FLAIR) sequences. All images were acquired in 3D with a 0.6mm isotropic resolution. These sequences served as the primary images for screening subjects. Two additional sequences were acquired through the medial temporal lobe. These included tissue border enhancement (TBE) and susceptibility weighted angiography (SWAN) sequences. Participants were recruited from the University of Iowa multidisciplinary epilepsy surgical patient workflow. In total 7 patients were recruited and imaged. Interpretation of the 7T MR data was conducted independently by two fellowship trained neuroradiologists blinded to clinical data including clinical localization, EEG lateralization, previous 3T MRI, and when available FDG-PET. Studies were reviewed using a systematic approach with specific attention to likely lesions in temporal lobe epilepsy including hippocampal sclerosis.

Results
Overall, three of the cases were interpreted as positive having hippocampal sclerosis, three cases were indeterminate, and one was read as normal. Two of the positive studies demonstrated a loss of hippocampal volume, each unilateral, and all three studies revealed a combination of loss of normal architecture and T2/FLAIR signal abnormality in the hippocampi (two unilateral and one bilateral). Two of the cases had semiology consistent with temporal lobe epilepsy on the side of diagnosis. The remaining case had a mixed clinical picture suggestive of uncertain sided temporal lobe epilepsy. Each case correlated with EEG localization. Finally, in two of the three cases, FDG-PET had been previously obtained which revealed hypometabolic foci where in one case unilateral hippocampal sclerosis was called and in the other case where bilateral hippocampal sclerosis was called.

Discussion
The current study, even with a limited number of subjects yielded promising results as we were able to diagnose pathology in nearly half of our studies and were able to see multiple abnormalities not visible on 3T images even when using a dedicated seizure protocol. Of note, the differences were noted even with routine sequences without using sophisticated techniques such as hippocampal volumetry. We felt that the improved detection was possible on 7T imaging owing to the significantly increased spatial resolution and a better signal to noise ratio. The spatial resolution of our 7T images was much greater than that of our 3T images. This allowed much greater diagnostic certainty in characterizing the shape and volume of the hippocampus and other mesial temporal lobe structures. Upon retrospective review of previous 3T images, it is possible that volume loss was demonstrated but not to an appreciable level justifying a call.

Conclusion
High resolution 7T imaging has allowed us to identify multiple focal neurologic lesions not previously seen on clinical imaging. If we are able to obtain similar results after increasing our sample size, this would suggest that images collected at 7T are significantly better at detecting focal epileptogenic lesions than 3T images.
Guillermo Romano Ibarra – John F. Engelhardt laboratory

**Title:** Gene-modification of ferret airway epithelia allows interrogation of mucin-mediated pathology in cystic fibrosis

**Background, Rationale, and Introduction:** Cystic fibrosis (CF) is a recessive genetic disease that involves mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). Those born with homozygous CFTR mutations exhibit aberrant ion transport across many epithelial surfaces. A key clinical manifestation is impaired mucociliary clearance in the respiratory tract, which has been classically thought to promote colonization by biofilm-producing bacteria that leads to the characteristic chronic infections and neutrophil-driven inflammation. These unremitting infections and the accompanying immune response interact with remodeling of the airways to drive a vicious cycle that ultimately culminates in respiratory failure, which causes most of the associated morbidity and mortality in CF.

Classical interpretations that suggest that inflammation follows infection in CF, however, CF ferrets raised on synergistic antibiotics are protected from bacterial and fungal infections, yet they develop classic structural bronchiectasis, neutrophil-mediated inflammation, and mucus accumulation. These observations suggest that the CF airway is inherently inflammatory, possibly due to aberrant mucin regulation.

**Purpose:** The purpose of this work is to explore the role that mucins play in driving neutrophil-mediated inflammation in airway epithelia. Muc5AC and Muc5B are large heavily-glycosylated polymeric proteins that constitute the major components of the mucus gel layer. Murine studies have shown that Muc5AC and Muc5B are implicated in airway diseases including COPD, bronchitis, asthma, and pulmonary fibrosis. We hypothesize that Muc5AC and Muc5B differentially influence airway inflammation in a CFTR-dependent manner. To this end, we modified airway basal epithelial cells (multipotent surface stem cells) to differentially express mucin genes using RNA-guided nucleases. Differentiation of basal cells into airway epithelia allows evaluation of ion conductance, mucus production, and quantification of inflammatory cytokine production. Furthermore, by performing this on a G551D-CFTR background, we can modulate CFTR activity using the small-molecule potentiator VX770 to evaluate the role of CFTR in the muco-inflammatory response. This approach provides a deeper understanding of the etiologies leading to airway remodeling in CF patients and will contribute to the rational development of novel therapeutics.

**Methods:** Airway basal cells were transduced with lentiviral vectors encoding RNA-guided nucleases and subjected to chemo-selection for 4-5 days. For mucin knockouts, cells were transduced with Cas9 or Cpf1 nuclease-encoding viruses. To test overexpression, cells were infected with a viral vector encoding an enzymatically-dead nuclease fusion protein that increases transcription, dCas9-Vp64. We have shown that this alone increases gene expression ~5-500 fold depending on the number of RNA-guides used. Co-expression of dCas9-Vp64 with the modulator P65 can further increase expression up to 6000-fold. Additionally, co-delivery of Cpf1 and dCas9-Vp64 will allow us to simultaneously knockout and overexpress mucins in the same cell. For overexpression, five Cas9 PAM sites were selected targeting the 1000bp region upstream of the start site for each mucin. For knockouts, Cas9 or Cpf1 PAM sites were chosen targeting exons or introns at the 5’ end of the gene. gRNA were delivered into basal cells using lipofection prior to or at the time of air-liquid interface (ALI) differentiation. ALI cultures were subjected to immunofluorescent (IF) staining in preliminary experiments and can be interrogated for ion conductance or cytokine profile in the future.

**Results:** Airway basal cells were successfully transduced with gene-modifying components and chemo-selected as described above. Using transgenic mouse basal cells expressing Cas9 and fluorescent reporters, we demonstrate that gRNA transfection can mediate biallelic cleavage in 75% of the population with <1% demonstrating monoallelic cleavage of the fluorescent reporter. Studies using the mucin targeting tools created from this rotation to study mucin targets are in progress. Preliminary studies using reverse transfection of dCas9-Vp64 expressing ferret basal cells demonstrated little induction of mucin protein expression in differentiated epithelia, however, molecular confirmation that the gRNAs chosen were capable of inducing transcription have yet to be completed. More experiments are required to complete this work.

**Conclusions/Discussion:** Based on these preliminary findings, one hypothesis is that premature expression of mucins in basal cells may be cytotoxic due to improperly formed cellular machinery. Mucins are large, polymeric proteins that undergo extensive post-translational modification before being secreted by mucus-producing epithelial cells (goblet cells), which requires the unfolded protein response at baseline. Alternatively, the gRNA promoter target sites chosen were not functional. The studies targeting disruption of mucin expression will be easier to interpret as internal control reporters for transfection can be incorporated as endpoints. An alternative strategy of delivering gRNA directly onto nuclease transduced differentiated epithelia could provide a method of modulating mucin expression. Despite the fact that no clear mechanistic findings emerged from this study, these tools created may be useful for elucidating molecular components involved in CF lung disease.
Elucidating The Role of TRAF3 in B Cell Metabolism

in the context of GSK-3, A Metabolic Regulator

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ABSTRACT

BACKGROUND: Tumor necrosis factor receptor associated factor 3 (TRAF3) is a known adaptor protein that restrains homeostatic B cell survival, via multiple regulatory pathways. TRAF3, when bound to NF-Kappa-B inducing kinase (NIK), recruits the E3 ubiquitin ligase complex of TRAF2-cIAP, leading to the degradation of NIK and inhibition of NF-Kappa-B activity. Preliminary work conducted in the Bishop lab has shown that TRAF3−/− B cells display enhanced cell viability accompanied by augmented activity of the non-canonical (NF-Kappa-B2) pathway, as well as increased expression of key metabolic proteins, such as glycogen synthase kinase 3 (GSK-3), hexokinase 2 (HXK2), and glucose transporter 1 (GLUT1). To further elucidate the role of TRAF3 in B cell metabolism, my research focuses on GSK-3, a ubiquitously expressed protein kinase which serves as a metabolic checkpoint regulator in B cells. Previous research has shown that GSK-3 can alter NF-Kappa-B2 activation by phosphorylating an NF-Kappa-B precursor protein, which ultimately leads to NF-Kappa-B2 nuclear translocation. Thus, in TRAF3−/− B cells, increased NF-Kappa-B2 activation that leads to enhanced cell viability is explained not only by the loss of ubiquitination and degradation of NIK, but also via increase in GSK-3 protein levels. TRAF3 deficiency is implicated in greater than 15% of B cell lymphomas and 20% of multiple myeloma cases. Thus, understanding how a TRAF3-deficient phenotype leads to enhanced B cell viability in the context of GSK-3 is vital in developing novel therapeutic targets and effective therapeutics for improving the treatment and outcome of patients with B cell lymphomas.

HYPOTHESIS: We postulate that human B cell lines deficient in TRAF3 will display enhanced levels of GSK-3 and subsequently, illustrate heightened sensitivity to external metabolic pressures through glucose starvation and GSK-3 inhibition.

METHODS: Eight human B cell lines with varying TRAF3 expression levels were obtained and subsequently tested for GSK-3 levels through Western blot procedures. After initial testing, inhibitory experiments were conducted using CHIR-99021, a potent GSK-3 inhibitor. Cell viability assays were conducted to quantify effects.

RESULTS: Interim data demonstrate enhanced GSK-3 beta levels in TRAF3-deficient B lymphoma cell lines and suggest a heightened sensitivity to GSK-3 inhibition via CHIR-99021.

CONCLUSION: Future directions will seek to elucidate the role of GSK-3 in TRAF3-deficiency enhanced B cell viability through glucose inhibitory studies and additional GSK-3 inhibitors with the aim of finding a novel drug that effectively and selectively targets the viability of TRAF3-deficient B cells.
Neural Mechanisms of Delayed Auditory Feedback: Insights from fMRI
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Background:
Language is one of the quintessential characteristics that separate humans from other animals. However, the neural mechanisms that underlie speech production remain unclear. Improved understanding of these mechanisms will lead to improved treatment options and help those afflicted when speech is impaired as a result of structural lesions such as tumors or stroke or disorders such as stuttering or dysphonia.

Delayed Auditory Feedback (DAF) is feedback shifted in the temporal domain and is known to alter speech production. For example, DAF is used as a treatment for some people with stuttering. The mechanisms of how DAF is processed are poorly understood. Our long-term goal is to elucidate these neural mechanisms using a systematic multi-modal investigation with both electrocorticography (ECoG) and functional magnetic resonance imaging (fMRI) to define the neurophysiology of primary, secondary and higher auditory, prefrontal, premotor, and sensorimotor areas. fMRI is a powerful noninvasive tool that provides excellent whole-brain anatomic resolution while ECoG provides sub-millisecond temporal precision. This novel methodological combination can advance our understanding of DAF procession.

Hypothesis:
For this project, we utilized fMRI to test our hypothesis that both motor and auditory cortices will exhibit BOLD response modulation during DAF compared to normal auditory feedback (NAF).

Methods:
Eight subjects (four males, four females) undergoing neurosurgical treatment volunteered for this study. Pre-operative fMRI was done during four behavioral tasks: speaking with DAF, playback of subject’s own speech with DAF, speaking with NAF, and playback with NAF. During the speaking trials, an auditory cue was played to prompt the subjects to produce the phrase “You Know Nina” at their normal conversation pitch, rate, and rhythm following a visual cue (“Go” prompt on a white background). Short breaks were interleaved between successive utterances. Approximately half of the speaking trials were produced in the presence of DAF delivered by headphones and the remaining trials were produced during NAF and therefore considered control trials. Two subjects’ data were acquired using a blocked / “sparse” sampling paradigm wherein speech was produced during silent periods (i.e. without scanner noise) and BOLD data was subsequently acquired immediately afterwards. The behavioral tasks were therefore blocks of speaking, listening, and rest. The remaining subjects’ data were acquired using a “continuous” scanning paradigm where speaking, playback, and rest tasks were done in the presence of ongoing scanner noise. Imaging data were processed using SPM 12 and brain surfaces were rendered using BrainSuite. All images underwent standard preprocessing including; motion correction, coregistration, normalization, and smoothing. As part of the processing all voxel sizes were changed to 3 x 3 x 3mm.

Results:
Single-subject pilot results have been obtained in 3 subjects; group analysis is ongoing. We have identified strong increased BOLD activation in auditory cortex on superior temporal gyrus, ventral sensorimotor cortex, and anterior cingulate cortex in addition to activation in basal ganglia structures. Single subject analysis has not identified BOLD response modulation due to DAF during either speaking or playback.

Conclusion:
These preliminary data suggest that neural structures involved in speech production (auditory cortex, motor cortex, cingulate cortex) can be visualized at a single-subject level. Group analyses are needed to further elucidate the mechanisms involved in DAF processing.
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. It 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 in lysosomes. The E3-ubiquitin ligase Nedd4-2 catalyzes ubiquitination of intracellular lysine residues, which enhances ENaC endocytosis and degradation. Defects in Nedd4-2 mediated regulation cause Liddle’s syndrome, an inherited form of hypertension. The purpose of this study was to determine the role of lysine residues on each subunit, and to examine the extent to which they contribute to 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 amounts of Nedd4-2, FRT cells were transfected as described above. Short circuit amiloride-sensitive current was examined in an Ussing Chamber. Biochemical endocytosis and degradation assays were also performed in HEK 293T cells.

RESULTS: Increasing amounts of Nedd4-2 decrease both current through and cell surface expression of wild type ENaC. Simultaneous mutation of all cytoplasmic lysines in α-, β-, and γENaC abolished the effect of Nedd4-2. We also 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 and analyzed the effect of Nedd4-2 on current and cell surface expression. A single N-terminal lysine residue at positions 6, 8, 10, 12 and 13 in γENaC was sufficient to reproduce the effect that Nedd4-2 had on the wild type channel with respect to current. Moreover, a single lysine at position 6 was sufficient for Nedd4-2 to reduce cell surface expression and induce both ENaC degradation and endocytosis. Interestingly, a single lysine at position 26 in γENaC was not sufficient for Nedd4-2 to reduce current, which supports a critical functional role for lysines within the 6-13 cluster. To test this hypothesis further, we inserted lysines at three additional positions in wt γENaC (I7K, A9K and I11K) and examined the effect of Nedd4-2 on current. Nedd4-2 inhibited each of these mutants (I7K, A9K and I11K) similar to wt ENaC.

CONCLUSIONS: Lysines in each ENaC subunit contribute to Nedd4-2 regulation. When all cytoplasmic lysines in ENaC are mutated to arginine, regulation by Nedd4-2 is abolished. Individual lysines within a specific cluster in the N-terminus of γENaC (amino acids 6-13) are sufficient for Nedd4-2 to reduce ENaC current. Additionally, a single lysine at position 6 in γENaC is sufficient for Nedd4-2 to reduce ENaC cell surface expression, and to induce both ENaC degradation and endocytosis.
Title: The Lamin Paradox: Shared features between a muscular dystrophy patient and an Olympic sprinter

Authors: Issac R. Schwantes, Gary S. Coombs, Ashley C. Goll, Diane E. Cryderman, and Lori L. Wallrath

Background:
Iowan Jill Viles, a 41-year-old female, has Emery-Dreifuss muscular dystrophy. Canadian Priscilla Lopes-Schliep, a 35-year-old female Olympic sprinter who won the bronze medal in 2008, has lipodystrophy. The muscular physique of Jill and Priscilla could not be more different, yet they both have a mutation in the LMNA gene, encoding laminas. Lamins are intermediate proteins that make up a meshwork that lines the inside of the nuclear envelope. Lamins provide structure to the nucleus and play roles in genome organization and gene expression. Our research focuses on the mechanisms by which mutant lamins cause muscle disease.

Purpose:
Understanding the pathogenesis of disease is the first step in designing any efficacious treatment for patients. Mutations in LMNA cause a collection of diseases called laminopathies, which includes rare types of muscular dystrophy. Currently there are no treatments for laminopathies. A The goal of our research is to understand how mutations in LMNA cause muscle disease and identify potential targets.

Methods:
To understand the mechanisms of muscle pathogenesis we generated Drosophila (fruit fly) models of lamin-associated muscular dystrophy. Mutations analogous to those that cause human disease were introduced into the Drosophila (fruit fly) Lamin C gene. Mutations used in this study are those that cause Emery-Dreifuss muscular dystrophy (such as Jill’s) and lipodystrophy (such as Priscilla’s). Wild type and mutant Lamin C was expressed exclusively in muscle using the Gal4/UAS system. Immunohistochemistry was performed using antibodies that recognize Lamin C, DAPI to stain for DNA and phalloidin to stain the actin cytoskeleton.

Conclusions, Discussion and Future Directions:
Human disease-causing mutations in the LMNA gene were modeled into Drosophila Lamin C and expressed in larval muscle. Expression of mutant lamins associated with Emery-Dreifuss reduced larval motility and caused premature death. Surprisingly, expression of mutant lamins associated with lipodystrophy caused these same phenotypes. These results suggest that genetic background might play a significant role in the clinical phenotype and that Priscilla might possess a genetic suppressor in her genome. The genetics of Drosophila will allow us to test candidate genes and screen for genetic suppressors.

To investigate the effects of mutant lamins at the cellular level, muscles expressing wild type and mutant Lamin C were stained with antibodies to Lamin C. The results showed that wild type Lamin C localized to the nuclear periphery as anticipated. In contrast, mutant Lamin C formed perinuclear aggregates in the cytoplasm. These results suggest that the mutant Lamin C might not fold properly, which could be addressed by future biophysical studies. Taken together, these results suggest that avenues for therapy might include the expression of chaperones that assist in protein folding and/or treatment with drugs that increase autophagy to eliminate the cytoplasmic aggregates.
The Articulated Oral Airway as an Aid to Mask Ventilation, a Randomized Crossover, Non-Inferiority Study
Isaac Sheffield B.S. & Dr. Ron Abrons M.D.

Abstract

Background

Difficult mask ventilation (MV) is common in the obese population[1] and can result in patient morbidity and mortality.[2] The Articulating oral airway (AOA) is a novel device which actively displaces the tongue, allowing for a greater cross-sectional area for mask ventilation (MV) and as an improved conduit for flexible scope intubation. The AOA may function as well as a Guedel oral airway (GDA), with additional functionality. Therefore, in this study, we examined the non-inferiority of the AOA for MV in terms of expired tidal volumes.

As a novel device, no prior data on the AOA currently exists. The most relevant study in this area was performed by Koga, et al,[3] who compared the expiratory tidal volumes achieved (with 2-handed MV by a single provider using pressure control ventilation at 15cm H2O) with no oral airway, and via a GDA and Cuffed Oropharyngeal Airway (COPA). This study found expiratory tidal volumes with the COPA > GDA > no oral airway. With the comparison of the GDA and no oral airway completed, we can focus on the comparison between the GDA and AOA.

Aims

1. Compare expired tidal volumes during MV with an AOA to a similarly sized GDA in neuromuscularly blocked patients.
2. Compare inspired tidal volumes during MV with an AOA to a similarly sized GDA in neuromuscularly blocked patients.
3. Identify any unforeseen complication associated with AOA use, such as oropharyngeal trauma, device placement difficulties, etc.; compare to GDA use.

Methods

Identify and consent patient who meets inclusion criteria and is not excluded by exclusion criteria. If consent is obtained, the attending anesthesiologist obtains patient history and performs physical exam. Induce patient following appropriate methodology. Ensure entropy is 40-60. Upon induction, the research assistant opens the randomized envelope and places the indicated device. The CRNA is blinded as she or he performs MV. Record tidal volumes and presence of blood on the oral airway. Repeat steps with the second device. Perform post-operative exam within 45 minutes of the conclusion of the surgery. Note any oropharyngeal trauma.

Results

The study is ongoing, and data currently inconclusive. Thus far 12 patients have been consented and 9 have been enrolled in the study, we aim to enroll 58 patients. Preliminary data shows no difference between the AOA and the GDA in terms of oropharyngeal trauma. Further statistical analysis is required to identify the presence of statistically significant differences between the AOA and the GDA.

Conclusion

The AOA is a novel device intended to increase functionality at no cost to MV. The design of the device is such as to actively displace the tongue to allow for increased cross-sectional area for MA, as well as provide a conduit for flexible scope intubation. At the current rate, we plan to enroll the last patient by the end of the year, at which point we will perform statistical analysis.

Novel adenovirus serotypes for improved delivery to human airway epithelial cells
Elizabeth Shirazi, BS & Patrick Sinn, PhD

ABSTRACT

BACKGROUND: Stable expression of a functional copy of the cystic fibrosis transmembrane conductance receptor (CFTR) in the airways would result in an immense improvement in the quality of life of people with cystic fibrosis (CF). Over 25 years ago, a gene replacement strategy was shown to reverse the ion transport defect in vitro, suggesting that gene transfer is a therapeutic option for the disease. The promise of gene therapy has encountered unforeseen challenges and fundamental questions still remain. We and others have used adenoviral (Ad)-based vectors to deliver CFTR to the airways. Viral vectors based on adenovirus serotype 5 (Ad5) are, by far, the most commonly used Ad based vector. In this study we evaluated Ad vectors derived from novel serotypes for the ability to transduce human airway epithelial cells (HAE). In collaboration with Dr. Anja Ehrhardt from Witten/Herdecke University in Germany, we screened 16 novel recombinant adenoviral serotypes and compared their transduction efficiency to Ad5.

HYPOTHESIS: We hypothesize that Ad5 is not the optimal Ad serotype for transducing human airway epithelial cells. Our goal is to identify an adenoviral serotype that will transduce HAE with greater efficiency than Ad5. Identification of a more efficient serotype will allow for a reduced minimal therapeutically effective dose, which in turn would decrease the severity of a vector-induced immune response.

METHODS:

Experimental protocol #1. Adenovirus serotypes expressing the reporter genes GFP and nanoluciferase were delivered either to the apical or basolateral surface of HAE at a multiplicity of infection of 50 for four hours. Forty-eight hours later, the GFP activity was imaged and nanoluciferase activity was quantified.

Experimental protocol #2. Adenovirus serotypes 5 and 69 (the leading candidate identified in protocol #1) were delivered to the basolateral surface of HAE at a multiplicity of infection of 50 for four hours. The GFP and nanoluciferase activity was quantified every twenty-four hours for a five-day period.

RESULTS: Following the screening of 17 adenoviral serotypes, we made the following observations: 1) No individual serotype transduced the apical surface better than the basolateral surface. 2) As measured by both GFP and nanoluciferase assays, transduction of HAE was more efficient using serotypes 4, 14, 20, and 69 as compared to Ad5. 3) Interestingly, Ad serotype 69 transduction of the HAE apical surface was more efficient than the basolateral transduction of Ad5 (although Ad69 basolateral transduction was most efficient).

CONCLUSION: Ad vectors are an exciting potential tool for delivering therapeutic genes. In this study, we identified 4 candidate Ad vectors with enhanced tropism for airway epithelial cells. These novel tools may potentially be used for pulmonary delivery of CFTR for gene therapy or CRISPR/Cas9 for gene editing applications.
Effects of Axial Loading on Post-traumatic Osteoarthritic Cartilage Regeneration

Michael E. Slattery BA; Marc J. Brouillette PhD; Dongrim Seol PhD; Mitchell C. Coleman PhD; James A. Martin PhD

**Background:** Post-traumatic osteoarthritis (PTOA) is a disease characterized by joint pain, loss of function, and loss of form due to articular cartilage degeneration after an injury to the joint. The risk of PTOA after serious joint injuries is as high as 70%. Recent studies have identified a potential treatment that may prevent or delay the onset of PTOA. This treatment utilizes an interpenetrating polymer network (IPN) hydrogel (HG) which provides a conduit for the migration of native chondrogenic progenitor cells (CPCs) from the surrounding cartilage matrix. *In vitro* experiments showed, given appropriate stimuli, CPCs are capable of populating the IPN gel and regenerating native articular cartilage. However, in a recent animal model study, it became apparent that immediate weight bearing with motion after implantation may have disrupted the HG scaffolding’s architecture or impaired CPC migration. It was not clear if preventing weight bearing or joint motion or both would improve these results.

**Purpose of study:** The purpose of this study was to determine if immediate axial loading of HG-filled defects is detrimental to cartilage regeneration and if delaying loading is beneficial.

**Method:** Sixteen osteochondral explants were harvested from a bovine femoral condyle. A defect to simulate PTOA was made measuring 4 mm in diameter and ~2 mm in thickness in each explant. These defects were then filled with a HG scaffold composed of fibrin, hyaluronic acid, and stromal cell-derived factor 1 alpha (SDF-1α), a potent CPC chemoattractant. Physiologic joint loading was simulated via cyclic axial compression (CAC) of the defect and surrounding cartilage to 10% strain at 0.5 Hz for 900 cycles. These explants were then separated into four groups: no CAC, CAC starting on the day of defect fill (time 0), CAC starting on day 7 after filling, and CAC starting on day 14 after filling. CAC was performed 3 times/week until 6 weeks after filling. Two weeks after filling, transforming growth factor beta 3 (TGF-β3), was introduced to media to induce chondrogenic differentiation in half of the explants. Osteochondral specimens were analyzed by safranin-O/fast green histology, a gold-standard method to evaluate the quality of the repair tissue. The samples were fixed in 10% neutral buffered formalin and decalcified in 5% formic acid. Tissues were then cryoembedded and 10 μM-thick sections were cut, mounted and stained.

**Results:** Upon examination of preliminary histology, axial loading delay did not appear to have a significant effect on the structural integrity of the HG. Loading starting day 0 after defect fill shows greatest HG integrity and integration with native cartilage (59.5% of HG remained in day 0 group compared to 53.7% and 43.5% for day 7 and day 14 groups, respectively). Chondrogenic differentiation appears to have occurred in all three axial loading delay groups that were treated with TGF-β3 when compared to groups not treated with TGF-β3. Loading groups treated with TGF-β3, on average, had 4x the number of chondrocyte-like cells compared to loading groups without TGF-β3 treatment.

**Conclusions:** Though the group sizes were small there were clear indications that immediate axial compression did not retard cartilage regeneration and may even have been stimulatory. This suggests that immediate weight bearing in the animal model was not problematic. Rather, it appears more likely that the regenerative process was disrupted by weight bearing with joint motion, which imposes shear stresses at cartilage surfaces. These findings suggest that lowering shear stress by joint immobilization or other means would be beneficial and a focus for future study.
Inappropriate use of vancomycin for GBS prophylaxis in patients who report a penicillin allergy

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BACKGROUND: Vancomycin is only recommended for Group B Streptococcus (GBS) prophylaxis for penicillin-allergic women at high risk for anaphylaxis if their GBS isolate is resistant to clindamycin or if results from antimicrobial susceptibility testing are unknown at time of delivery. This study investigated the adherence to national guidelines for GBS prophylaxis at our institution.

METHODS: A retrospective chart analysis of GBS-colonized, penicillin-allergic women was performed for patients who gave birth at the University of Iowa Hospitals and Clinics between December 2010 and February 2017. Patient demographics, allergies and reaction types, antibiotic administration, labor course, and neonatal outcomes were recorded in a secure online database. Data was analyzed using chi-squared and t-test statistics.

RESULTS: Of 211 penicillin-allergic women given antibiotics for GBS prophylaxis, 44% (94/211) were given vancomycin and 30% (64/211) were determined to have received vancomycin inappropriately. Regarding secondary outcome analysis, 29% (27/92) of the neonates of vancomycin-receiving mothers were given antibiotics within the first 72 hours of life, compared to 24% (17/70) of women given clindamycin prophylaxis (p=0.47). Length of stay for infants of mothers who received vancomycin was 4-5 days in 18% and >5 days in 17% compared to 16% and 9%, respectively, for infants of mothers who received clindamycin prophylaxis.

CONCLUSION: At our institution, penicillin-allergic women were given vancomycin inappropriately at a rate of 30%. A similar trend has been shown at other institutions and indicates that there is still room for improvement in GBS prophylaxis protocol adherence.
A retrospective review of ultrasound guided advanced tendon procedures for various tendinopathies.

Daniel Stover, BS, Mederic Hall, MD & Andrew Peterson, MD

ABSTRACT

BACKGROUND: Historically, tendon pain was thought to be primarily an inflammatory problem and anti-inflammatory agents (such as NSAIDs and corticosteroid injections) were the mainstay of therapy. However, it is now better understood that much tendon pain is due to a chronic, degenerative process better termed as tendinosis. Along with this better understanding has come a wave of mostly unproven treatment modalities, many of which are meant to cause a pro-inflammatory change in the tendon or change the mechanical structure of the tendon. The treatment modalities reviewed in this study include tendon scraping, percutaneous needle tenotomy, percutaneous ultrasonic fasciotomy, and platelet rich plasma injections.

HYPOTHESIS: Ultrasound guided advanced tendon procedures will improve patients’ symptoms associated with tendon injury.

METHODS: This study reviewed the charts of 409 UI Sports Medicine patients treated with the various forms of ultrasound guided advanced tendon procedures from 6 different injury locations: patellar tendon (94), Achilles tendon (43), insertional Achilles tendon (41), common extensor tendon (112), common flexor tendon (25), and the plantar fascia (94). After reading the medical records, patients were grouped into three categories with respect to their follow-up post-surgery: better, worse, or no change. Additionally, the SF-12, satisfaction, and joint specific outcome scores were evaluated for patients that underwent percutaneous ultrasonic fasciotomy. The following joint specific outcome scores were used: Kujala (patellar tendon), Mayo elbow performance score (common extensor & common flexor), AOFAS (Achilles tendon, insertional Achilles tendon, plantar fascia).

RESULTS: The data was categorized by injury location and will be presented in the following format (%better,%same,%worse). Patellar tendon (73%,26%,1%), Achilles tendon (81%,16%,3%), insertional Achilles tendon (84%,16%,0%), common extensor tendon (72%,26%,2%), plantar fascia (71%,25%,5%), and common flexor tendon (64%,36%,0%). The percutaneous ultrasonic fasciotomy data was also categorized by injury location and will be presented in the following format with 95% confidence intervals (PCS SF-12, MCS SF-12, satisfaction [1=very satisfied and 5=very dissatisfied], joint score change from baseline). Patellar tendon (1.80±3.69, 0.27±2.42, 2.00±0.31, 9.82±7.01), Achilles tendon (3.96±3.68, 1.63±1.77, 1.40±0.28, -9.20±17.59) insertional Achilles tendon (6.23±2.89, -0.12±1.62, 1.72±0.27,10.25±10.39) common extensor tendon (5.38±2.36, 1.03±2.43, 1.94±0.27, 20.19±6.46) plantar fascia (4.40±2.63, 1.92±2.60, 2.10±0.29, 15.72±6.83), and common flexor tendon (5.69±5.52, -2.00±4.57, 2.39±0.66, 5.00±8.49). All injury locations had statistically significant positive satisfaction scores. All injury locations except the patellar tendon had statistically significant improvements in the PCS SF-12. There were not any injury locations that had statistically significant worsening of symptoms by any category.

CONCLUSIONS: The outcomes of ultrasound guided advanced tendon procedures vary by injury location; however, they all improve patients’ reported symptoms the majority of the time.
Effects of Long Distance Cycling on Median Nerve Sonographic Appearance During RAGBRAI
N. Stoyles, C. Richardson, M. Hall, A. Peterson

**Background:** RAGBRAI is a 7-day, endurance cycling event attracting a broad demographic of participants. Overuse injuries are commonly reported in such events, with previous studies describing anywhere from 10-14% of subjects experiencing wrist-related symptoms. Ultrasound has become an effective diagnostic tool for entrapment neuropathies such as carpal tunnel syndrome (CTS). Subclinical detection of injuries may allow for earlier intervention and prevention of future disability in cyclists. This prospective cohort study aims to assess whether subclinical changes in median nerve cross-sectional area can be detected with ultrasound and correlated with self-reported symptoms.

**Hypothesis:** There is a difference in cross sectional area of the median nerve at the carpal tunnel inlet between day 1 and day 6 of an endurance cycling event

**Methods:** Twenty subjects were recruited on the first day of RAGBRAI and received follow up on day 6 after 358 miles of riding and 9,878 feet of climbing. A modified Levine-Katz Questionnaire (LKQ) provided for demographics, screening, eligibility, and functional assessment both before and after the event. In addition, a sonographic (Phillips Lumify - Royal Philips, Amsterdam, Netherlands) transverse view of the median nerve was obtained at the carpal tunnel inlet in both wrists on both days. Images were loaded into Adobe Photoshop (Adobe Systems Incorporated, San Jose, California) and the median nerve traced along the inside of the nerve sheath with a pixel-based scaling technique applied to obtain cross-sectional area (cm$^2$). Each subject received a symptom severity score (11-55) based on LKQ. Paired t-test was used to assess means of the study population for both metrics (LKQ and cross-sectional area) at each time point.

**Results:** Three subjects were lost to follow up and an additional 3 to data corruption. Data was analyzed for 14 subjects (28 wrists). Mean age of subjects was 49.6 years. All subjects reported riding road bikes, 12 of which had drop handlebars. 12 subjects also reported glove use. No significant difference in mean cross sectional area was found between day 1 and 6 (P=.228) across all subjects despite a significant increase in LKQ scores (P=.02).

**Discussion:** This data represents the second cohort of subjects studied using this design. The results of this cohort, similar to the previous (C. Richardson, 2016), indicate that sonography is likely not sufficient to meaningfully detect subclinical changes in the median nerve after a single endurance cycling event. A potential limitation of our study is that RAGBRAI is considered a “tour” rather than a race, reducing the intensity of the ride. Additionally, the distance may not be sufficient to induce noticeable change. Most of our subjects also used equipment designed to reduce injury (gloves and drop handlebars). However, the relative increase in LKQ scores suggests the race elicited discomfort out of many of our subjects that did not correlate with a general increase in cross-sectional area. A larger, longer longitudinal study in elite cyclists could potentially provide further insight into the natural sonographic history of cycling-induced neuropathy.
**Descriptive Epidemiology of Clubfoot in China: A Clinic-Based Study**

Eleanor Sullivan, BA, Jose Morcuende, MD, PhD, Li Zhao, MD, PhD, Paul Romitti, PhD

**Introduction:** Idiopathic congenital talipes equinovarus, or clubfoot, is a birth defect characterized by equinus of the ankle, varus of the hindfoot, and cavus and adductus of the forefoot. Worldwide, prevalence of clubfoot is estimated to be 1 in 1000 live births, varying across racial/ethnic groups. Prevalence of clubfoot also varies by sex, with an excess observed in males. Additionally, although bilateral and unilateral phenotypes present in similar proportions, unilateral phenotypes are predominantly right-sided. Despite decades of genetic and epidemiologic research, major etiologic factors for clubfoot remain elusive. Several genes have been reported to be associated with development of clubfoot, but replication of these findings is limited. Findings for environmental factors (broadly defined) have largely been equivocal. Maternal cigarette smoking during pregnancy has been the most consistently reported risk factor, although causal constituents in cigarette smoke have not been elucidated. In China, prevalence of clubfoot is estimated at approximately 1 in 2000 births, one-half the frequency observed in most countries. The reasons for this prevalence discrepancy are not well-studied, but may be due to differences in gene variants and/or epidemiologic characteristics between China and other countries. Improved understanding of the prevalence differences between China and other countries can aid our understanding of risk factors for clubfoot and allow for informed approaches to prevention. Herein, we describe our initial pilot work in one pediatric orthopedic clinic in Shanghai, China examining associations between selected child and parental characteristics and clubfoot.

**Methods:** Our case-control study included 117 cases and 86 controls. Cases were ages 0-8 years with physician confirmed diagnosis of clubfoot and no additional major structural birth defects. Controls were ages 0-10 years without clubfoot or another major defect. A consent letter and structured survey were administered to mothers of cases and controls via the clinic’s electronic messaging platform. The survey asked for information on child birth characteristics and family history, maternal pregnancy characteristics, medical history, and behaviors, as well as maternal and paternal sociodemographic characteristics. Recruitment occurred within an 8-week period from June 1, 2017 to July 28, 2017. Crude odds ratios and 95% confidence intervals were estimated using logistic regression analysis to investigate the associations between selected child and parental characteristics and clubfoot.

**Results:** We observed a male excess (2.3:1) of clubfoot. A positive, marginally statistically significant association for clubfoot was observed for delivery via C-section due to breech position compared to vaginal delivery. Positive, but non-significant associations for clubfoot were observed for twin compared to singleton births, for children with a first-degree relative with clubfoot compared to those without an affected first-degree relative, and for births in spring, summer, or fall compared to those in the winter. With regard to parental characteristics, we observed a significant positive association with clubfoot for mothers age <25 years at delivery and a positive but non-significant association for mothers age 35 years or older compared to those age of 25-34 years. We also observed significant inverse associations between clubfoot and mothers or fathers with a doctoral degree at delivery compared to those whose education ended after attending high school or a secondary technical school. Additionally, we observed a positive, marginally significant association between mothers exposed to second-hand smoke during the first trimester compared to those without such exposure; only one mother reported active cigarette smoking during pregnancy. Positive but non-significant associations were observed for mothers with a pre-pregnancy body mass index (BMI) of underweight or overweight compared to those with a pre-pregnancy BMI of normal weight. Compared to fathers aged 25-34 years at delivery, a positive, non-significant association was observed for those <25 years of age, and an inverse, non-significant association was observed for those 35 years or older.

**Discussion:** Our study of a hospital-based sample is one of the first to examine a large number of epidemiologic risk factors for clubfoot in China. Our results support previous research that reported positive associations for clubfoot with male sex, breech presentation, and young maternal age, as well as inverse associations with maternal and paternal education level at delivery. A possible explanation for the association with young maternal age is the relatively high proportion of first-time mothers in our study sample; thus, the underlying association may be one of parity. Low rates of maternal smoking precluded our study of the association between clubfoot and maternal active smoking; however, our study was the first to examine and find an association between clubfoot and maternal exposure to second-hand smoke during the first trimester of pregnancy. Due to our modest sample size, our findings are preliminary. Future analyses will examine additional child and parental characteristics reported in the maternal survey and their associations with clubfoot.
Procedural Outcomes in Hemophiliac Neonates

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Background: Hemophilia A (deficiency of factor VIII) and Hemophilia B (deficiency of factor IX) are X-linked inherited severe bleeding disorders. Specifically, factor VIII (FVIII) and factor IX (FIX) play key roles in the coagulation pathway, and if deficient will lead to unchecked bleeding, even during minor surgical procedures. Optimal preoperative management for the patients with hemophilia (PWH) is yet to be defined in many procedures. Circumcision is practiced worldwide, and the National Hospital Discharge Survey (NCHS) report in 2011 estimated that approximately 56% of male newborns are circumcised. Currently, there is no established protocol for effective preoperative therapy for PWH prior to circumcision. To date, case reports suggest treatment regimens consisting of a preoperative dose of factor replacement followed by daily factor replacement for 3-7 days after the procedure. These regimens may lead to hospitalization lasting up to 10 days. Here, we establish a peri-operative factor replacement protocol, which demonstrates improved patient outcomes with decreased hospitalization and medical costs.

Hypothesis: We hypothesize that one pre-procedural factor treatment will prevent bleeding complications in newborn PWH undergoing circumcision. This treatment will involve a one-time pre-treatment dose of FVIII or FIX replacement product given approximately 30 minutes to 1 hour prior to the procedure. This will in turn result in minimal bleeding post-circumcision. Additionally, we hypothesize that no post-circumcision factor replacement infusions will be necessary.

Methods/Results: We conducted a retrospective chart review. Variables analyzed include age at diagnosis, age at circumcision, reason for diagnosis, race, family history, surgical history, medical history, medication history, coagulation studies, blood counts, blood transfusions, pre- and post-op FVIII/FIX levels, number of factor infusions received, additional hemostatic measures needed, pre- and post-operative inhibitor levels, number and location of joint bleeds, age at first joint bleed, total hemorrhages since diagnosis. We will compare outcomes of PWH to those of normal newborn males undergoing circumcision based on length of procedure, major and minor surgical complications (pain, bleeding, inflammation, infection), age at surgical procedure, duration of hospital stay. We have collected data from seven PWH and our encouraged by our results to date.

Conclusion: Treatment of hemophilia can be costly, and optimal procedural treatment protocols are desperately needed to provide patients with the highest quality care. From our study, we anticipate to establish a circumcision treatment protocol applicable to all newborns with hemophilia.
Effect of Dietary Oils on Peripheral Neuropathy Related Endpoints in Dietary Obese Sprague-Dawley Rats

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Objective: Determine the effect of dietary oils enriched in different mono- or poly-unsaturated fatty acids (olive (18:1, oleic acid), safflower (18:2 n-6, linoleic acid), evening primrose (18:3 n-6, gamma-linolenic acid), flaxseed (18:3 n-3, alpha-linolenic acid), or menhaden (20:5/22:6 n-3 eicosapentaenoic/docosahexaenoic acids) on peripheral neuropathy in diet-induced obese Sprague-Dawley rats.

Methods: Rats at 12 weeks of age were fed a high fat diet (45% kcal primarily lard) for 16 weeks. Afterwards the rats were fed diets with 50% of the kcal of fat derived from lard replaced by the different dietary oils. In addition, a control group fed a standard diet (4% kcal fat) and a high fat fed group (45% kcal primarily lard) was maintained. The treatment period was 32 weeks. The endpoints evaluated included motor and sensory nerve conduction velocity, thermal sensitivity and innervation of sensory nerves in the cornea and skin and vascular relaxation by epineurial arterioles.

Results: Our findings show that menhaden oil provided the greatest benefit for preventing peripheral nerve damage caused by dietary obesity. Similar results were obtained when we examined acetylcholine-mediated vascular relaxation of epineurial arterioles of the sciatic nerve. Vascular relaxation as were neural deficits was nearly fully protected when the high fat diet was enriched with menhaden oil. Enriching the diets with fatty acids derived from the other oils provided none to partial improvements.

Conclusions: These studies further support n-3 polyunsaturated fatty acids derived from fish oil could be an effective treatment for peripheral neuropathy.
Title: Overlapping Surgery in Primary Total Knee Arthroplasty: Are Outcomes Worse Than Single Operating Room Scheduling?

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Introduction: Overlapping surgery is common in high-volume total knee arthroplasty (TKA) practices and has come under recent scrutiny in the lay press. The aim of this study was to evaluate for differences in 30-day clinical and 1-year clinical and radiographic outcomes for primary TKA patients between single operating room (OR) and overlapping OR days. We hypothesize that there will be no differences in outcomes between patients receiving their primary TKA on single OR vs overlapping OR days.

Methods: We retrospectively reviewed individual patient records of a consecutive series of primary TKAs with 30-day clinical follow-up performed by a single academic orthopaedic surgeon between 2008-2016 (n = 462). A smaller cohort of patients with complete 1-year clinical and radiographic follow-up was also analyzed (n = 268). Patients were stratified by single OR vs overlapping OR days. Age, body mass index (BMI), Charlson Comorbidity Index (CCI) and American Society of Anesthesiologists (ASA) class were recorded to assess for confounding variables. Outcomes included anesthesiology time, 30-day readmissions, return to OR, complication rates (30-day and 1-year), patient reported outcomes (WOMAC pain, WOMAC functionality, and SF-36), 1-year radiographic coronal alignment and periprosthetic radiolucencies or loosening.

Results: In the 30-day cohort, 183 patients (40%) had an overlapping surgery while in the 1-year cohort 97 patients (36%) had an overlapping surgery. In the 30-day cohort, there were no differences in complication rates (10.4% vs 6.6%, p = 0.25), 30-day readmissions (4.3% vs 1.6%, p = 0.11), or return to OR (2.2% vs 1.6%, p = 1.00) between non-overlapping and overlapping surgery. In the 1-year cohort, there were no significant differences in anesthesiology time (165.5 vs 164.5 min, p = 0.85), complication rates (10.7% vs 4.1%, p = 0.06), 30-day readmissions (4.7% vs 1.0%, p = 0.16), or return to OR (2.9% vs 2.0%, p = 1.00), before and after adjusting for age, BMI, gender, ASA and CCI. WOMAC pain (86.5 vs 86.2, p = 0.92), WOMAC functionality (75.2 vs 74.4, p = 0.70), and SF-36 scores (41.1 vs 40.9, p=0.93) were similar at 1 year. There were no differences in number of knees in neutral coronal alignment (97% vs 95%, p = 0.50) or presence of radiolucencies on 1-year radiographs (6.5% vs 9.3%, p = 0.43). In both groups, 2 of the 5 knees outside neutral coronal alignment were due to post-traumatic deformity. There was one case of femoral aseptic loosening at 3 years in the single OR group. The remaining patients did not show any progression of radiolucency or loosening at last follow-up.

Discussion: This study demonstrates no differences in 30-day clinical or 1-year clinical and radiographic outcomes between patients undergoing primary TKA on single OR versus overlapping OR days, including patient-related outcome scores and radiographic outcomes. These results support the safe practice of overlapping surgical scheduling in primary TKA.
Effect of Intranasal Oxytocin on Maternal Behavior
Naomi Vather, BA, Guifeng Xu, MD, Patrick Breheny, MS, PhD, Lane Strathearn, MBBS, PhD

Background: The neuropeptide hormone, oxytocin is endogenously produced and is well-known for inducing uterine contractions during labor and allowing milk ejection during lactation. However, central oxytocin is also involved in mediating attachment by bringing about social and parental behaviors. Endogenously produced oxytocin responses have been positively associated with mother-to-infant gaze time and increased motherese vocalizations. Likewise, exogenous oxytocin results in increased social gaze, social reciprocity, and touch. Nevertheless, previous studies have not specifically evaluated the effect of exogenous oxytocin on maternal sensitivity in a mother-infant dyadic interaction.

Hypothesis: We hypothesized that the administration of oxytocin would increase maternal sensitivity to infant cues.

Materials and Methods: The age range of the mothers recruited was 20.6–42.4 years (mean = 29.3 years, n = 30) and the age range of the babies were 3.6–5.7 months (mean = 4.9 months, n = 30). Breastfeeding mothers were asked to feed their infants at least one hour prior to the visit. During separation from their child, mothers self-administered the nasal spray, which was randomized to be either oxytocin or placebo. After fifty minutes, the mothers rejoined their infant and participated in a videotaped “free play” interaction on the floor for 3 minutes. The recordings were coded using the 14-point sensitivity scale in the CARE-Index. A model fit linear regression was performed on SPSS to evaluate differences in maternal sensitivity when receiving either placebo or intranasal oxytocin and also to evaluate whether mothers shifted their behavior to be more sensitive, controlling or unresponsive after receiving either placebo or intranasal oxytocin. For the latter, the differences between controlling and unresponsive scores were calculated and tested.

Results: The number of mothers whose sensitivity scores increased after oxytocin (n=13) were comparable to those that decreased (n=14). Three of the moms had no change in their sensitivity scores after administration of oxytocin. Sensitivity scores after administration of oxytocin were not significantly different from those after administration of the placebo (p=0.92). There was no significant shift in behavior from unresponsive to controlling or vice versa (p=0.42).

Discussion: Our results suggest that maternal sensitivity, as measured by the CARE-Index, is not significantly altered after oxytocin administration, compared with placebo. However, the following limitations should also be considered: the study had a small sample size (n=30) and did not measure peripheral oxytocin levels in mothers at baseline or after receiving the intranasal placebo or oxytocin spray. This would have allowed the effect of oxytocin on maternal sensitivity and behavior to be more accurately assessed. Mothers who had increased sensitivity after oxytocin administration may have had lower basal levels of oxytocin compared to the mothers whose sensitivity score was the same or lower after oxytocin administration. There is evidence that individuals with lower pretreatment blood oxytocin concentrations may show greater improvement in their social responsiveness after receiving intranasal oxytocin administration.

Engineering a Polycaprolactone (PCL) Film to Deliver Stem Cell Derived Retinal Precursor Cells (RPCs) for Retinal Regeneration

Joseph Vecchi
Rotating with Dr. Budd Tucker and Collaborating with Dr. Kristan Worthington

**Background:** For retinal degenerative diseases where the photoreceptor cell layer is destroyed, like retinitis pigmentosa (RP), no effective therapies are currently available. Furthermore, once diseases such as RP have progressed enough this cell layer can be completely eliminated. The absence of cells renders traditional pharmaceuticals and gene therapy as non-viable treatment options. The only efficacious option would be to introduce new functional photoreceptor cells to the retina and for new synapses to form. Retinal cell replacement therapy has been a hot topic in the field of ophthalmology for years, but early studies showed that introducing cells as a bolus leads to low cellular attachment at the site of interest and poor cell organization overall. This observation has led the development that the replacement cells can be encapsulated in or seeded on a biomaterial to improve viability and cell attachment at the site. Currently, the biomaterials being explored for retinal engineering are poly(lactide-co-glycolide), which degrades too quickly and damages surrounding tissue, or are too stiff like poly(methyl methacrylate). A biomaterial like polycaprolactone (PCL), which degrades more slowly and is softer, holds potential for this application.

**Purpose:** The goal of this project was to tailor the elastic modulus, thickness, and pore features of a PCL film, which stem cell derived retinal progenitor cells (RPCs) will be seeded on. This film would then be delivered to the subretinal space in an attempt to reintroduce functional photoreceptor cells and restore vision. Various thicknesses and pore diameters were explored to optimize the amount of cells to be seeded and delivered by the film as well.

**Methods:** The film was composed of acrylated PCL monomer and polymerized via exposure to UV light in the presence of photoinitiator. Photomasks with variable pore size were used to create different circular through-pore geometries in the film. In order to modulate the mechanics of the film, the variables of photoinitiator concentration, UV light intensity, film thickness, pore diameter, and pore spacing were altered. Films were analyzed via scanning electron microscopy (SEM) to assess pore formation and thickness while dynamic mechanical analysis (DMA) was used to determine elastic modulus. The films were also assessed by transplantation into rat models. Surgeon feedback from the procedure was used to ascertain how the films handled and the results from follow up imaging informed biocompatibility. A Plackett-Burman experiment design was used to study which variables play a role in modulating the properties of interest.

**Result:** SEM revealed that the lowest possible UV light intensity, a larger pore diameter, and a lower photoinitiator concentration created through-pores most consistently. DMA revealed that decreasing the UV light intensity by a factor of 1.5 had a half effect of -115 MPa while decreasing the photoinitiator concentration from 0.5 wt% to 0.1 wt% had a half effect of -192 MPa. The surgeons were able to handle any film that sufficiently polymerized and noted more favorable biocompatibility with thinner and softer films.

**Conclusion:** The data suggest an optimized recipe would be one that minimizes the thickness and elastic modulus, while maximizing the surface area in the pores to protect the cells from flowing shear forces. This leads to the chosen recipe of 75wt% PCL, 0.1wt% photoinitiator, exposed to UV light for 10 seconds using a photomask with 75um pores separated by 25um spaces made to be 50um thick. Further optimization should be done studying the cell seeding to finalize this recipe prior as the experiments next step.
DNA Methylation Patterns of Brain and Peripheral Tissue in Drug-Resistant Epilepsy

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BACKGROUND: Epilepsy is a common neurological disorder affecting 0.5-1.0% of the U.S. population. 20-40% of these patients cannot obtain seizure control after 2 or more trials of anti-epileptic drugs (AEDs) and are subsequently diagnosed with refractory or drug-resistant epilepsy (DRE). The pathogenesis and etiology of DRE are not well understood and continues to be a challenge for the medical management of these patients. Although genetics have elucidated the role of drug metabolizing enzyme, transporter and ion channel mutations in DRE, it alone cannot account for the progressive pathogenesis of AED resistance. Epigenetic analysis of DNA methylation patterns in brain tissue of DRE may demonstrate continuously altered expression of epilepsy genes over the duration of DRE due to seizures and AED use. The impact of these epigenetic changes on DRE prognosis is relatively unexplored and could guide future clinical decision-making provided easily accessible peripheral tissue (blood, saliva, buccal) can adequately represent brain DNA methylation patterns.

HYPOTHESIS: The duration of epilepsy in DRE will correlate with significant hypo-methylated or hyper-methylated states in brain tissue samples at sites known to play a role in epilepsy. Further, peripheral tissues may serve as adequate surrogates for identifying these changes.

METHODS: Blood, buccal, saliva and brain tissues were collected from 13 patients with drug-resistant epilepsy that underwent epilepsy surgery for seizure focus resection. DNA from the samples were isolated and purified. DNA methylation analysis of the samples was performed with Infinium MethylationEPIC BeadChip Kit containing over 850,000 CpG methylation sites per sample at single-nucleotide resolution. Site methylation signals were measured with iScan (Illumina) giving β-values (proportion of site methylated) per sample as output. Pearson coefficients (r) were calculated between normalized β-values from brain tissue and each peripheral tissue (blood, saliva and buccal) at the CpG site level to determine methylation patterning similarities between tissues. The age of seizure onset/duration of epilepsy (seizure onset to seizure focus resection) and site level methylation were correlated for each tissue as well. Genes associated with CpG sites that had significant (p<0.05) correlations in the brain tissue and the comparison peripheral tissue for age of seizure onset and duration of epilepsy were analyzed for biological functional groupings using DAVID software.

RESULTS: The Pearson coefficient averaged over all CpG sites for brain tissue vs. blood, buccal and saliva were 0.10, 0.10 and 0.06, respectively. The brain-buccal comparison had the highest percentage of sites with significant (p<0.05) positive correlations (9.6%). Only the brain tissue had a significant overall correlation with duration of epilepsy and methylation (r=-.57). None of the tissues had significant overall correlation with age of seizure onset and methylation however. Shared in all four tissues types at the site level, 678 genes were found to be significantly negatively correlated with epilepsy duration. DAVID results showed these genes are involved with dopaminergic, cholinergic and glutaminergic synapse regulation and long-term potentiation.

CONCLUSIONS: The pathogenesis of DRE appears to be a progressive disease characterized by increased overall hypo-methylation (overexpression) of genes in brain tissue over time. Peripheral tissues overall do not predict this DNA methylation for epilepsy duration, but 678 genes in all tissues share this progressive hypo-methylation pattern. AED use and cumulative seizure activity may account for the epigenetic changes seen in genes associated with neural synapses and receptors and long-term potentiation responses. Buccal tissue may be the best surrogate for observing these methylation changes in the brain and may provide a framework for characterizing DRE pathogenesis non-invasively.
A Forward Genetic Screen to Investigate Vemurafenib Resistance in BRAF$^{V600E}$ Melanoma
Andrew Voigt, Hayley Vaughn, Charlotte Feddersen, and Adam Dupuy

**Background:** BRAF is a human gene that encodes the B-Raf protein, a serine/threonine protein kinase in the MAP kinase signaling pathway. A point mutation in this gene results in a single amino acid substitution at position 600 from valine to glutamic acid. This BRAF$^{V600E}$ mutation results in substantially increased kinase activity, and is found in roughly half of all cutaneous melanomas. The drug Vemurafenib (Zelboraf) is a B-Raf inhibitor administered to patients with BRAF$^{V600E}$ melanoma, and has an initially high response rate. However, resistance to Vemurafenib regularly develops within months of initial treatment. The mechanism that leads to acquired Vemurafenib resistance cannot be defined in the majority of melanoma patients.

**Purpose:** To investigate the genetic mechanisms that lead to Vemurafenib resistance by employing a cell-based forward genetic screen.

**Methods:** A375 human-derived BRAF$^{V600E}$ melanoma cells were cultured in 8 mL of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% Penicillin/Streptomycin. A375 cells were transfected with the transposase SB100. Transposase-transfected cells reached 80% confluence and were transfected with the transposon pT2/Onc3. Each plate was subsequently treated with 5 uM Vemurafenib (a cytostatic concentration) in 8 mL of DMEM every three and half days for a total of 17 days. This process was repeated for a total of 51 Vemurafenib-treated plates, 15 untreated controls, and 12 unmutagenized controls.

On day 17, DNA was extracted from 45 Vemurafenib-treated plates. DNA was extracted from the 15 untreated controls once plates reached confluence. DNA was sonicated to 300 base pair fragments. Fragmented DNA was then used as template in a process that generates amplicon libraries that represent the insertion sites for the mutagenic SB transposon in each plate of cells. The remaining plates were stained with trichrome blue to elucidate differences in the number of colonies formed between Vemurafenib-treated plates and unmutagenized controls.

**Results:** Unsurprisingly, A375 cells mutagenized with transposase/transposon and treated with Vemurafenib grew significantly more colonies than unmutagenized A375 cells treated with Vemurafenib (90.7 colonies per plate vs 0.917 colonies per plate, p<0.001).

A total of ~29,000 and ~213,000 unique insertional mutations were identified in control and Vemurafenib-treated cells, respectively. Analysis of the mutations in Vemurafenib-treated cells identified only five candidate genes which demonstrated significantly higher insertion events than random integration. These five recurrently mutated candidates associated with Vemurafenib resistance will be presented.

**Conclusions:** Only mutagenized A375 cells were able to meaningfully proliferate and form colonies in the presence of a normally cytostatic concentration of Vemurafenib. SB- induced mutagenesis provides a reproducible, high-throughput model of somatic mutations implicated in Vemurafenib resistance. Identified candidate gene mutations highlight novel mechanisms associated with Vemurafenib resistance. These five candidates will be modeled to confirm Vemurafenib resistance.
Risk Factor Differences in Patients by Age and Stroke Type Over Time With a Focus on the Young (≤45 years) In A Rural State, 2010-2017

By: Kristin Weeks  
Mentor: James Torner

Introduction
While stroke is the 5th leading cause of death in Iowa, incidence has been decreasing overall. However, the incidence has increased in younger populations 2010-2017. The risk factors responsible for the change in stroke in the young are not well understood.

Hypothesis
As the incidence trends of stroke differ by age, risk factors may vary by age for stroke types.

Methods
Using the Iowa Stroke Registry, data from 21,888 patients from 2010-2017 was collected from 32 hospitals. We investigated past medical, social, and family history by age (≤45, 46-59, ≥60 years) over time (2010-2017) by stroke type (overall, Subarachnoid, Intracerebral, Ischemic, Transient Ischemic). Categorical data statistical analysis used a Chi-Square test with an alpha=0.05.

Results
Past medical, social, and family history factors varied by age over time for stroke types and by stroke type over time. In descending order the most prevalent risk factors for stroke overall in ≤45 year olds are: history of hypertension (29%), anti-hypertension medication (27%), smoking (21%), dyslipidemia (17%), antiplatelet medication (15%), diabetes (13%). Compared to those ≥60 years, overall stroke patients that are ≤45 years old have increased histories of smoking (chi=0.008), drug abuse (0.05), obesity (0.001). Compared to those 46-59 years and ≥60 years, patients ≤45 years old have decreased histories of diabetes (chi=0.03), TIA (0.0179), MI/CAD (0.03), antiplatelet regimes (<0.001), anti-hypertension regime (0.003), dyslipidemia. For Ischemic stroke, the percentage of ≤45 year olds with each risk factor is higher than overall stroke. In subarachnoid hemorrhages, a larger proportion (8% vs. 4% overall) of ≤45 year olds have migraines (chi=0.001) and history of drug abuse (9% vs. 5% overall). Compared to stroke in general, in intracerebral hemorrhages, a larger proportion of ≤45 year olds have hypertension/take antihypertensive medication (chi=<0.001) and more than double the proportion of patients ≤45 years old in the TIA group have dyslipidemia.

Conclusions
Younger strokes experience risk factors at different proportions than older strokes. Overall, young strokes have a higher attribution to behavioral/lifestyle factors and are also associated with stroke type.
Are Rural Sarcoma Patients at Risk for Worse Outcomes?
Ryan Wendt, Yubo Gao PhD, Benjamin Miller MD, MS

Background
Orthopaedic oncology is a subspecialty of orthopaedic surgery specifically designed to manage musculoskeletal neoplasia. Treatment of sarcoma, the primary malignancy originating in the extremities, is complex and requires a multidisciplinary approach with coordination of oncologic, radiation, and surgical subspecialists to provide the best outcome. Given the rarity of sarcoma, accounting for 1% of all cancer diagnoses, there are many issues of access and volume that may be unique and important. Specifically, there is a yet undefined relationship between access to regional referral centers and whether the eventual oncologic outcomes are influenced by either distance, travel time, or residing in a rural community.

Purpose
The purpose of this study was to investigate if patients that reside in rural counties, or at greater distances removed from comprehensive cancer centers, experience adverse outcomes (e.g. higher stage at presentation, larger tumors at presentation, or diminished overall survival).

Method
We used the Surveillance, Epidemiology and End Results (SEER) Program Database as our primary data source. This commonly used tool for rare cancer analysis collects data from seventeen geographically variable United States populations. These registries represent approximately 26% of the U.S. population. The patient population assessed included patients of all ages with high grade osteosarcoma residing in New Mexico, Utah, or Iowa. These states were chosen because they were in the top 25 most rural states in the United States, representing the rural areas included in SEER. All patient data came from 1990-2014. SEER provides county-level data, and the centroid distances from the county of a patient’s residence to the nearest comprehensive cancer center, as defined by the National Cancer Institute, was calculated. The cases were analyzed based on both their time required to travel and distance to travel to the nearest comprehensive cancer center within their state of residence and to the nearest center despite state of residence. The distances and times to travel were calculated using county centroid coordinates and Google maps. SEER does not provide patient treating centers, so the nearest in-state center and nearest center despite state of residence were used as a surrogate. The cases were also grouped based on designation of counties using a Rural-Urban continuum code found within SEER*stat. Patients were compared by urban vs rural and very rural vs not very rural status. Very rural patients resided in rural counties that were not adjacent to urban counties.

Results
There was a total of 476 patients that met the study criteria. Of those 476, 131 patients were missing data for the size of tumor and were excluded from the size of tumor analysis. There was no statistical significance found between the distance or time to treating center and the size of tumor at presentation. There was an increase incidence of metastases found for patients residing in a county with a greater than 2 hour drive to the nearest in-state treating center (p=0.0208). Using a Kaplan Meier analysis, it was shown that patients residing in rural counties showed a decreased five-year survival (p=0.007). Also, patients residing in very rural counties showed decreased five-year survival (p=0.003) when compared to not very rural patients. Cox regression was analysis showed that a patient’s rural status was still a risk factor for mortality after controlling for both size of the tumor and the presence of metastasis, hazard ratio 1.579 (95% CI: 1.027-2.427).

Discussion
Diseases like osteosarcoma have been shown to have very poor prognostics when presenting at high stage. Despite advances in care, survival rate has remained unchanged since the 1990s. Overall, the distance and the time to travel to the nearest comprehensive cancer center showed little effect on the size of the tumor or the presence of metastasis at presentation for osteosarcoma patients. However, a patient’s rural status was shown to be a risk factor for mortality. This work is important to show that patients living in rural counties are at risk of dying sooner than a patient living in an urban setting despite the size of their tumor or whether or not they have metastatic disease. It may be important to assess what makes a patient’s mortality risk increase due to rural status versus just their distance to comprehensive centers, and this could be addressed in future studies.
Ambulatory Activity Surveillance in Postoperative Orthopedic Patients

Matthew Wilson, Nicholas Evans, Christopher Anthony, Jacob Simmering, Philip Polgreen

**Background:** Sedentary activity and prolonged bed rest are common among hospitalized patients and are associated with increasing insulin resistance, bone loss, blood clots, pulmonary embolisms, and skin breakdown. Even a single day of prolonged sedentary time has been shown to reduce insulin action, and physically active adults who substantially reduce their number of steps for three days can experience significant increases in average postprandial glucose levels. In addition, low levels of activity during hospitalization are associated with functional decline, readmission, and mortality for older adults. Surgical patients are frequently sedentary following their procedures. The benefits of ambulation for patients are increasingly recognized yet there is a strong disconnect between recommendations and practices. A range of relatively inexpensive and easy to use devices are now available that are capable of measuring granular activity (e.g. steps per minute). Furthermore, it is now possible to easily gather this information so that it could ultimately be fed back to healthcare workers and patients to inform decision-making and ultimately interventions to decrease sedentary activity among hospitalized patients.

**Purpose:** The objective of this project was to pilot an ambulatory activity surveillance system following orthopedic surgical procedure.

**Methods:** After consenting, patients were given a wrist-based Fitbit (Flex 2 model), a small wearable, tri-axial accelerometer similar to a pedometer. Devices were then collected by a member of the research team on the day of discharge. To avoid relying on participants’ personal devices we used custom software to collect each subject’s data via the Fitbit API, which was then stored in a database for future analysis. Subsequently, we computed the average number of steps, ambulatory episodes and the average longest ambulatory episode in the first 12 and 24 hours following the initiation of data collection.

**Results:** 39 subjects were enrolled following definitive orthopedic surgical intervention following traumatic injury, upper extremity orthopedic procedures, and spinal orthopedic procedures. Only subjects with a minimum of one weight-bearing lower extremity were included. Of the 39 subjects enrolled, 17 (63%) had at least 12 hours of continuous data collection following surgery, and 12 (44.4%) of patients had at least 24 hours of continuous data collection. During the first 12 hours, the mean number of steps recorded was 349.8 (SD = 279.3), the mean number of active minutes was 28.5 (SD = 22.1), and the mean length of the longest ambulatory episode was 4.0 min. (SD = 2.9 min.). During the first 24 hours, the mean number of steps recorded was 608.7 (SD = 389.9), the mean number of active minutes was 51.3 (SD = 32.4), and the mean length of the longest ambulatory episode was 4.5 min. (SD = 3.2). The majority of ambulatory activity occurred between the hours of 8:00am – 8:00pm. The most active period on average occurred from 11:00am – 12:00pm with a mean of 104.7 steps/hour; approximately double the overall mean hourly step count of 51.0 steps/hour.

**Discussion:** Despite the known benefits of postsurgical ambulatory activity, our results show that patients are minimally active following orthopedic procedures. In addition, we found that postoperative patients did not ambulate frequently or for considerable durations outside of formal physical therapy rounding times. We hope to use these results to develop further interventions designed to increase postoperative ambulation of orthopedic surgical patients. Furthermore, we believe this surveillance framework can be beneficially adapted to a wide variety of surgical and non-surgical inpatient populations.
A Comparison of Histologic and Radiographic Methods in Novel Sheep Model for Hearing Preservation Cochlear Implant Surgery

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BACKGROUND: Over 360 million people worldwide suffer from moderate to profound hearing loss. The hybrid cochlear implant (CI) is a type of hearing preservation surgery that combines electrical and acoustic stimulation (EAS) to improve speech perception ability and hearing in patients who have residual low-frequency hearing. Hybrid CI uses a shorter electrode implanted into the high-frequency basal cochlea while preserving the low-frequency apical region for acoustic stimulation. Currently, up to 50% of patients who undergo hearing preservation cochlear implant surgery experience delayed hearing loss post-surgery. Studies have hypothesized that it may be due to chronic intracochlear inflammation and fibrosis induced by trauma from insertion of the electrode array.

Recently we developed a custom prototype device which aims to reduce insertion trauma and account for post-surgical hearing loss by controlling electrode insertion rate and depth both during and after CI surgery. A pilot study was undertaken to evaluate the prototype device feasibility in a novel sheep model for CI surgery. Sheep cochleae were chosen for its similarities to human cochlea in length, volume, and number of anatomical turns. To assess the aims of the prototype device, it is necessary to develop a method for evaluating intracochlear electrode position, intracochlear structural integrity, and fibrotic response. Here we compare four different histologic and radiographic methods: frozen sectioning, paraffin sectioning, epoxy-resin, and 3D X-ray microscopy.

HYPOTHESIS: We hypothesized that the 3D X-ray microscopy and epoxy-resin methods are as effective as traditional frozen and paraffin sectioning methods for assessing intracochlear electrode position, intracochlear structural integrity, and fibrotic response.

METHODS: The sheep (n=8) were implanted bilaterally using prototype device insertion in one ear and manual insertion in the contralateral ear. The sheep were monitored and sacrificed after 30 days. Following the study, the cochleae were explanted and fixed with 4% formaldehyde solution. For frozen and paraffin methods, the electrode was removed and the cochleae were decalcified in 120mM EDTA. Following embedding, they were cut into 20µm and 7µm sections, respectively. The sections were stained with H&E and imaged under light microscopy. For the epoxy-resin method, the cochleae were fixed in 4% formaldehyde and the electrode was glued to the round window insertion site. The cochleae were dehydrated using serial ethanol solutions and cleared with acetone. The samples were dried and embedded in a degassed epoxy mixture which secured electrode placement within the cochlea. Using a materialographic grinder and bandsaw, the epoxy block was mounted, ground, and sectioned at 100µm. The sections were subsequently stained with eosin Y and toluidine blue. For 3D X-ray microscopy, a Zeiss Xradia Versa 520 3D X-ray Microscope was used to image the excised temporal bone with the electrode in-situ. The electrode was then carefully removed and temporal bone reimaged. The two scans were overlaid into a composite 3D image which shows electrode position and soft tissue resolution without radiologic electrode artifact.

RESULTS: For frozen and paraffin sections, we identified intracochlear trauma, presence of a fibrotic capsule, and inspected the architecture of the Organ of Corti. For the epoxy-resin embedded sections, we assessed the intracochlear electrode position in-situ along with surrounding tissue response. For 3D X-ray microscopy, we evaluated electrode position, basilar membrane trauma, and electrode dynamics such as fold over and kinking at a resolution of 12µm.

CONCLUSIONS: Frozen and paraffin methods allow visualization of the fibrotic response and intracochlear architecture while preserving the option to do further immunostaining but require electrode removal and a lengthy decalcification and sectioning process. The epoxy-resin method has the added benefit of showing intracochlear electrode positioning but destroys the sample and does not allow for further immunostaining. 3D X-ray microscopy provides excellent visualization of in-situ electrode position and intracochlear structural integrity and does not require decalcification. Compared to traditional frozen or paraffin sectioning methods, a combination of 3D X-ray microscopy and epoxy-resin methods may be a more appropriate, time-efficient alternative.
CRISPRi Knockdown of BAG3 Interactors in iPS-CM to Elucidate Mechanisms of BAG3-Related Dilated Cardiomyopathy

Authors/Affiliations: Perry Wu, Gladstone Institutes; Luke Judge, MD/PhD, Gladstone Institutes; Juan Perez-Bermejo, Gladstone Institutes; Mohammadali Mandegar, DPhil, Gladstone Institutes/Tenaya; Po-Lin So, Gladstone Institutes; Bruce Conklin, MD, Gladstone Institutes

Background: Recent biochemical and genetic studies clearly implicate BAG3 in certain genetic forms of dilated cardiomyopathy (DCM). BAG3 is located in the Z-disk of cardiac and skeletal muscles, where we hypothesize its function to be in maintaining sarcomeric integrity through strict regulation of critical steps of protein quality control including protein folding, proteasomal degradation, and autophagy. We previously demonstrated that both heterozygous and homozygous loss of BAG3 in induced pluripotent stem cell-derived cardiomyocytes (iPS-CM) led to irregular protein quality control with phenotype of decreased force production, sarcomeric disarray, and increased sensitivity to toxic stressors such as bortezomib. In contrast, heterozygous mice have not shown such phenotypes. Studies show that BAG3 is a stress-inducible co-chaperone that serves as a scaffold by binding and coordinating two classes of molecular chaperones: the HSP70s and tissue-specific small HSPs. Since BAG3 itself is merely a scaffold that holds together functional components, we want to determine which of these components are critical for causing BAG-related DCM. We recently used AP-MS studies in iPS-CM to map out a cardiac-specific interactome for BAG3. By developing and utilizing a CRISPR interference (CRISPRi)1 expression system with potent knockdown activity in fully differentiated iPS-CM, we can identify specific BAG3 interactors that recapitulate part of or all of the BAG3 phenotype to further elucidate the mechanisms through which BAG3 mutations relate to DCM.

Methods: iPS-CM were generated from the differentiation of induced pluripotent stem (iPS) cells with modifications of the WNT modulation method2. We generated a dual-component plasmid with constitutive expression of gRNA and dCas9/KRAB co-expressed with mCherry reporter. Plasmid was directly introduced in day >30 iPS-CM through nucleofection using Amaxa Nucleofector 4D system. RTPCR was used to quantify RNA levels and validate knockdown of target genes. Immunofluorescence (IF) was used for labeling of sarcomeric proteins and targeting of other proteins of interest to observe changes in expression. Phenotypes resulting from knockdown of BAG3 and putative BAG3 interactors were evaluated by co-transfection of CRISPRi plasmid specific for each target with CAG driven ACTN-GFP reporter that delineates the Z-disk followed by a blinded, manual sarcomere scoring system developed in the lab.

Results: Direct nucleofection of the dual-component vector system containing both gRNA and dCas9/KRAB in fully differentiated iPS-CM resulted in mCherry-reported expression of dCas9/KRAB, with specific knockdown of targets upon addition of gRNA. Previously validated BAG3-targeting gRNA was first incorporated into dual-component plasmid, demonstrating significant knockdown with RTPCR (Fig. 1) and IF (Fig. 2). Targeting of 10 genes by testing five guides per gene identified at least one targeting guide with potent knockdown activity at the RNA level. Validating knockdown with immunofluorescence demonstrated significant loss of target protein expression in cells containing plasmid with CRISPRi expression system with guide specific for the corresponding target gene. Knockdown of BAG3 with CRISPRi plasmid resulted in recapitulation of BAG3 knockout findings including loss of HSPB8 on IF staining and significantly increased sarcomeric disarray with bortezomib drug treatment when compared to control (Fig. 3).

Conclusion: The transient co-delivery of circular CRISPRi dual component system with an ACTN-GFP reporter in terminally differentiated iPS-cardiomyocytes is a high-throughput means of knocking down gene expression of BAG3 interacting partners to screen for the critical components that cause BAG3-related DCM.

1. CRISPRi utilizes catalytically dead Cas9 (dCas9) lacking endonuclease activity to sterically inhibit transcription in a RNA-guided manner

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**Figure 1:** In pooled populations where not all cells receive CRISPRi plasmid, 60% knockdown efficiency can be achieved when validated with RTPCR 1 week post-delivery.

**Figure 2:** Immunofluorescence 2 weeks post-delivery shows that uptake of CRISPRi plasmid with previously validated BAG3 gRNA sequence results in complete loss of protein in iPS-CM.
Figure 3: Co-transfection of CRISPRi plasmid for BAG3 knockdown and ACTN-GFP reporter plasmid results in a rare event in which, on a confluent plate of cells, only a small number of cells receive both plasmids. This effectively allows for single-cell microscopy to evaluate for sarcomeric disarray and can be applied to all other BAG3 interactors to determine which are critical for the BAG3 KO phenotype.
CRISPRi Knockdown of BAG3 Interactors in iPS-CM to Elucidate Mechanisms of BAG3-Related Dilated Cardiomyopathy

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Beyond the Bench: Translational Visual Biomarkers and Treatments in Multiple Sclerosis

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Background and Purpose
Multiple Sclerosis (MS) is an autoimmune-mediated, neurodegenerative and demyelinating disorder with various clinical phenotypes consisting of muscular, sensory, cognitive, and visual symptoms. In many MS patients, the first clinical presentation is optic neuritis (ON), an episode of optic nerve inflammation. Visual symptoms of multiple sclerosis include decreased vision and visual conductance due to demyelination and neuronal loss marked by accumulated axon degeneration and retinal ganglion cell apoptosis. Research on the pathophysiology of MS is enhanced by the animal model experimental autoimmune encephalomyelitis (EAE), which allows mechanisms underlying MS-related neurodegeneration and preclinical treatments, along with translational biomarkers of MS to be thoroughly investigated. The presented pilot study serves as a preclinical investigation of mesenchymal stem cells (MSC) to treat MS in a mouse model of EAE. The exact phenotype rescue and mechanism of MSCs is not understood even with the recent completion of a Phase I clinical trial of the treatment in MS patients.

Methods
To induce EAE, mice (n = 22) were injected with solution containing MOG emulsified with complete Freund’s adjuvant (CFA) containing mycobacterium tuberculosis. Control mice (n = 10) were sham immunized with an equal volume of PBS and CFA. All mice were injected with pertussis toxin on day 0 (day of immunization) and again on day 2. One group of EAE mice (n = 11) were treated with one million MSCs per mouse day 7 post immunization. Clinical progression of EAE was scored daily for 35 days post immunization using the standard clinical scale. Pattern-evoked electroretinography (PERG) was used to objectively measure the function of RGCs by recording the amplitude and latency of the PERG waveform at baseline, and 4, 11, 18, 25, and 32 days following induction of the model. Visual function was assessed weekly by the established optokinetic tracking response (OKR) and the pupil light reflex (PLR) using a novel testing paradigm that was also piloted in humans. OCT imaging centered on the optic nerve head and automated segmentation of the different structural layers of the eye were performed to measure retinal layer thicknesses to assess structural changes across the different experimental groups. These functional parameters are directly translatable to human study. Histological analysis was also performed post-sacrifice of the mice (day 36 post immunization) to assess immune-cell infiltration, demyelination, and RGC loss.

Results
A sub-clinical model of EAE was established with subtle clinical presentations. No significant differences were observed in the PLR, OKR, and nerve fiber layer or ganglion cell layer thickness over the course of the experiment. EAE mice exhibited a delay in PERG latency compared to controls as early as day 4 post immunization, before any known clinical onset, that persisted through the experiment. Interestingly, MSC-treated EAE mice fully recovered from the delay in latency within a week of treatment.

Conclusions
Even in a sub-clinical model of EAE, a delay in PERG latency serves as a valuable, translatable biomarker that can be detected prior to clinical onset or any other functional parameters. PERG latency likely indicates onset of inflammation, and shortly after, demyelination of RGC axons. The delay in PERG latency was fully rescued in MSC-treated EAE mice, demonstrating the immunomodulatory and remyelinating abilities of MSCs. Further investigations must ascertain the mechanism behind the therapeutic abilities of MSCs in a more severe model of EAE, and at-home, repeated measures of PERG and the PLR may prove useful in human, longitudinal studies to monitor the disease course and treatment of MS and other conditions (the latter objective is already in the works).