ABSTRACTS

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College of Medicine

Medical Student Research Day
September 4, 2015

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Neuroantigen-specific CD8+ T cells in experimental autoimmune encephalitis (EAE)

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BACKGROUND: Multiple sclerosis (MS) in an immune-mediated demyelinating disease of the central nervous system that affects more than two million people worldwide. Though its etiology is still unclear, the mechanisms underlying MS can be studied using experimental autoimmune encephalitis (EAE) animal models, in which MS-like ascending paralysis is induced by immunizing mice with myelin proteins or their derived peptides (such as MOG, PLP, and MBP). EAE studies suggest that neuroantigen-specific CD4+ T cells mediate EAE/MS pathology. However, CD8+ T cells may also play a pathogenic role, as they outnumber CD4+ T cells in MS lesions. Interestingly, evidence from our laboratory demonstrates that neuroantigen-specific CD8+ T cells play a novel regulatory role in EAE/MS. The purpose of this research experience was to become familiar with the EAE model and the potential regulatory role CD8+ T cells play therein.

HYPOTHESIS: EAE induction results in neuroantigen-specific, IFNγ-producing CD8+ T cells that are capable of playing a regulatory role in clinical disease.

METHODS: Active EAE induction: Female C57BL/6 mice were immunized (s.c.) with 50-100μg of OVA_{323-339}, MOG_{35-55}, or PLP_{178-191} in CFA on day 0. Pertussis toxin (250ng) was additionally injected i.p. on days 0 and 2. Mice were monitored for clinical disease according to a 5 point scale of ascending paralysis. CFSE proliferation assays: Splenocytes were harvested from EAE mice, labeled with 0.25μM CFSE, and cultured in the presence of cognate or non-cognate antigen (MOG_{35-55} or PLP_{178-191}), ConA or media alone for five days. CFSE dilution as a measure of T cell proliferation was subsequently assessed via flow cytometry. Intracellular cytokine staining: Splenocytes were cultured in the presence of antigen for 5 days as described previously. On day 5, cells were stimulated with leukocyte activation cocktail (BD) for 4 hours, stained intracellularly using Foxp3 detection kit buffers (eBioscience) and assessed for IFNγ production via flow cytometry. CD8+ T cell isolation: Active EAE was induced with 100μg of OVA_{323-339} or PLP_{178-191} as described. Spleens and inguinal lymph nodes were harvested 16 days post-immunization, and cells were cultured in the presence of cognate antigen and hIL-2 for 3 days. αCD8 (Ly-2) magnetic beads (Miltenyi) were used to isolate CD8+ T cells. Population purity was assessed by flow cytometry.

RESULTS: IFNγ-producing CD8+ T cells can be detected in the spleens of EAE mice. When compared to non-cognate controls or media alone, stimulating splenocytes from EAE mice with cognate antigen induced a higher fraction of proliferation as well as an increase in IFNγ-producers within the CD8+ T cell population. Attempts to isolate the CD8+ T cells from culture proved difficult, although the qualities of these cells (which are neuroantigen-specific in nature) shows the potential for discerning effector regulatory function capability in future protection studies.

CONCLUSIONS: Neuroantigen-specific, IFNγ-producing CD8+ T cells may play a regulatory role in the context of MS, although the mechanisms underlying their regulatory potential must be further delineated, especially within the EAE model. Additionally, understanding the interplay between these CD8+ T cells and other immune cells in EAE/MS may be beneficial for the development of MS immunotherapies.
**Intramuscular hydrogen peroxide (H$_2$O$_2$) induces spontaneous pain via TRPA1 receptors**

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**Abstract**

**Background:** The transient receptor potential ankyrin 1 (TRPA1) receptors are important for the transmission and transduction of inflammatory and nerve injury pain, making them key targets for pain therapies; however, the role of TRPA1 receptors and the endogenous TRPA1 ligands in postoperative pain is not well understood. Hydrogen peroxide (H$_2$O$_2$) is known to activate the TRPA1 receptors in organs and is generated in wounds to recruit leukocytes and support the wound healing process. Our preliminary data from a Conditioned Place Aversion (CPA) experiment, which allows the affective component of pain to be measured, has found that intramuscular (I.M.) injection of H$_2$O$_2$ induces pain-related aversion. The purpose of this study was to further understand H$_2$O$_2$-induced spontaneous pain behavior in rats, by evaluating the effect of the TRPA1 antagonist 4-(4-Chlorophenyl)-3-methylbut-3-en-2-oxime (AP-18) on H$_2$O$_2$-induced CPA. Ca$^{2+}$ imaging was also performed to assess the effect of H$_2$O$_2$ on dorsal root ganglion (DRG) neurons after hindpaw incision.

**Hypothesis:** We hypothesized that I.M. injection of H$_2$O$_2$ would induce pain-related aversion, consistent with preliminary data, and that I.M. pre-treatment with the TRPA1 antagonist AP-18 prior to I.M. injection of H$_2$O$_2$ would block pain-related aversion. We further hypothesized that H$_2$O$_2$ would excite DRG neurons and that the percentage of responsive DRG neurons and amplitude of the response would change after hindpaw incision.

**Methods:** CPA was conducted in two separate groups of Sprague-Dawley (SD) rats: (1) I.M. injection of vehicle (0.3 ml) + H$_2$O$_2$ (0.3 ml, 60 mM) and (2) I.M. injection of AP-18 (0.3 ml, 50 mM) + H$_2$O$_2$ (0.3 ml, 60 mM). The CPA score was calculated by subtracting the time spent in the drug-paired chamber during testing from the time spent in the baseline chamber during baseline. Using Fura-2 Ca$^{2+}$ imaging, we also investigated the effects of H$_2$O$_2$ (100 µM and 1 mM) on calcium influx in Dil-labeled rat DRG neurons (L4-L5) innervating the hindpaw muscle after hindpaw incision vs. sham surgery.

**Results:** After conditioning, rats developed aversion to the chamber paired with I.M. injection of H$_2$O$_2$ (CPA score ±155 ± 37), but not to the chamber paired with I.M. injection of H$_2$O$_2$ and AP-18 (CPA score ±25 ± 19). Initial data from Ca$^{2+}$ imaging shows that some DRG neurons from incision and sham rats respond to H$_2$O$_2$.

**Conclusions:** Our results indicate that I.M. injection of H$_2$O$_2$ induces pain-related aversion, consistent with preliminary data, and that pre-treatment with the TRPA1 antagonist AP-18 prior to I.M injection of H$_2$O$_2$ blocks pain-related aversion, providing more evidence that H$_2$O$_2$-induced spontaneous pain behavior is mediated by TRPA1. These findings indicate that blocking TRPA1 may be an effective therapy for H$_2$O$_2$-induced spontaneous muscle pain.
Evaluation of APC’s and Tregs in Lesions from Patients with the Autoimmune Blistering Disease, Bullous Pemphigoid

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Abstract

Background: Bullous pemphigoid (BP) is an autoimmune disease targeting epidermal attachment proteins resulting in inflammation and blistering of the skin. Interestingly, BP manifests exclusively in the skin, despite the fact that the target antigen is expressed in many other tissues throughout the body. Immune responses to epidermal antigens are generated largely within the skin itself through antigen presenting cell (APC)-mediated activation of various T cell populations. Local activation and proliferation of regulatory T cells (Treg) is a critical component of peripheral tolerance via their ability to suppress autoreactive effector T cells. In BP, the origin of the autoimmune response is unknown; however, the advanced age of onset, localization of lesions to the skin, and the transient nature of the disease suggest a defect in tissue-specific immune regulation.

Hypothesis: It is our hypothesis that differences in dendritic and T-cell subset, frequency and/or function play a role in the loss of tolerance to skin antigens in autoimmune BP.

Methods: To address this, we evaluated the number of APCs and Treg cells (CD4+FOXP3+) in flaring BP vs. remission BP vs. age- and gender-matched control skin and blood. Skin biopsies were cryosectioned and stained with fluorescent antibodies specific for APC’s and T cells, then visualized using epifluorescent or confocal microscopy. To determine if these changes were tissue-specific, we examined these cell populations in the peripheral blood of BP patients and controls using flow cytometry.

Results: Staining for MHC class II and/or langerin, revealed a decrease in the overall number of APC and Langerhans cells in flaring BP skin, compared to control. However, when skin from BP patients in remission was examined, the frequency of APC’s and Langerhans was similar to the control skin. In addition, a significantly decreased number of Treg (CD4+FoxP3+) cells were observed in flaring BP skin when compared to control skin, but not in remission BP skin. Interestingly, the frequency of Treg cells was significantly elevated in the circulation of BP patients, compared to controls.

Conclusion: Overall, in the skin of flaring BP patients there was a decrease in Tregs and Langerhans cells; however, when looking at the peripheral blood, Tregs were elevated. Future studies are aimed at understanding the significance of these observations in relationship to lesion development and to understand the mechanisms responsible for the decreased Treg and LC numbers in the skin.
Telemedicine Provides Non-Inferior Research Informed Consent for Remote Enrollment in an Emergency Department-Based Clinical Trial

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BACKGROUND: Telemedicine through audio-visual conferencing can pair emergency physicians in a tertiary medical center with rural emergency departments (EDs), allowing for remote medical guidance and support to arrange inter-hospital transfer. Remote care telemedicine networks are beginning to provide an avenue for rural health research. Telemedicine-enabled patient enrollment could allow for easier recruitment of rural patients in community hospitals for clinical trials. However, patients’ comprehension of research goals and participant rights using the novel application of computer-enabled audio-visual communication to discuss informed consent is still unknown.

HYPOTHESIS: Patient comprehension of telemedicine-enabled research informed consent is not inferior to standard face-to-face research informed consent.

METHODS: A prospective, open-label randomized control trial was performed in a 65,000-visit Midwestern academic ED to test the effectiveness of a single dose of oral chlorhexidine gluconate 0.12% in preventing hospital-acquired pneumonia among adult patients with expected hospital admission. Prior to informed consent, potential participants were randomized in a 1:1 allocation ratio, concealed by sequential numbered opaque envelopes, to standard face-to-face consent vs. consent provided by audio-visual telemedicine. Both groups were approached by the same research assistant in another part of the ED for all interactions using a standard consent document and script. Telemedicine was provided using a hand-held computer and a commercially available telemedicine interface (REACH platform, Vidyo Inc., Hackensack, NJ). Comprehension of research consent was the primary outcome, and was measured using the modified Quality of Informed Consent (QuIC) instrument, a validated tool for measuring research informed consent comprehension (Joffe, 2001). Sample size was estimated to require 100 completed surveys using a non-inferiority design with a non-inferiority margin of 5 points (α = 0.05, power = 80%). Secondary outcomes included consent rate, survey completion rates, and subjective understanding of study informed consent. Three open-ended responses about participant’s opinions of telemedicine were recorded for the telemedicine group. A random sample of 10% of the total cohort was selected for independent scoring by two investigators to ensure adherence with QuIC scoring methods. Statistical comparisons were conducted with t-test, Mann-Whitney U test, and chi-squared test as appropriate and statistical significance was defined by threshold α < 0.05 using two-tailed tests.

RESULTS: One-hundred thirty one (131) patients were randomized, and 101 completed an evaluation of the consent process (n = 67 in the telemedicine group). Comprehension of research informed consent using telemedicine communication was not inferior to conventional face-to-face consent (QuIC scores 74.4 ± 8.1 vs. 74.4 ± 6.9 on a 100-point scale, p = 0.999). Subjective consent understanding were similar (93.8 vs. 89.3 on a 100-point scale, p=0.194). Consent rates (56% vs. 69%, p = 0.142) and survey completion rates (73% vs. 81%, p = 0.330) were similar between the two groups.

CONCLUSION: Audio-visual conferencing (telemedicine) is a non-inferior method of delivering research informed consent, with similar research comprehension and patient-reported understanding of research methods. Although consent rates were not statistically different, a larger study would be required to detect differences in consent rates using these two techniques. The results of this study will inform the design for future telemedicine-enabled clinical trials, especially in rural populations outside of academic medical centers.
Severity of Diabetic Microenvironment Differentially Impacts the Immunosuppressive Potency of Mesenchymal Stem Cells

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Abstract

BACKGROUND: Mesenchymal stem cells (MSCs) are a multi-potent stem cell population capable of differentiating into adipocytes, fibroblasts, chondrocytes, myoblasts and osteoblasts. In addition, MSCs exhibit dramatic immunomodulatory capabilities through the release of secretory factors that can mediate phenotypic changes in several immune cell types including: T lymphocytes, monocytes, dendritic cells and macrophages. The immunomodulatory axis of MSC function has been the central focus of hundreds of clinical trials investigating novel treatment paradigms for a diverse array of diseases including Type I Diabetes, diabetic wounds, spinal cord injury, graft vs. host disease (GvHD), and ulcerative colitis. Though the potential for MSCs as powerful players in cell-based therapeutics has been well demonstrated in both preclinical animal models and in some clinical trials, translation into the clinical realm has been delayed due to variability in MSC response to in vivo disease environments. Understanding the influence of specific disease microenvironments on MSC phenotype is vital to identifying strategies to enhance MSC immunomodulatory capabilities and survival for increased therapeutic potency in vivo.

HYPOTHESIS: The elevated serum concentration of palmitate, a sixteen carbon free fatty acid commonly elevated in patients with Type II Diabetes, will diminish the ability of mesenchymal stem cells to suppress the activation and proliferation of T cells.

METHODS: MSCs isolated from a human donor were cultured in vitro in either low, medium or high glucose (5.5mM, 15mM, or 25mM) conditions paired with no palmitate (BSA vehicle only), .100 mM, .200 mM, or .400mM palmitate. Cell viability after 24 and 48 hours was assessed via XTT assay. MSCs isolated from the same human donor were then co-cultured in vitro for 6 days at a ratio of 1 MSC to every 4 PBMCs (peripheral blood mononuclear cells) from an unrelated human donor at either low or high glucose (5.5mM or 25mM) concentrations paired with the previously described palmitate conditions. T cells within the PBMC population were activated via CD3/C28 Dynabeads and rhIL-2. Flow cytometry analysis of T cell proliferation was assessed using CFSE staining and quantified by percent of T cell population proliferation compared to total. The media from the culture wells was then analyzed by enzyme-linked immunosorbent assay (ELISA) to quantify production of interferon gamma (IFN-γ) as a marker of T cell activation.

RESULTS: At the 48 hour time-point, a significant decrease in MSC viability was observed in the higher palmitate conditions (~25% decrease at .200mM, ~50% decrease at .400mM palmitate in all glucose conditions). In the MSC and PBMC co-culture, suppression of T cell proliferation was observable in the low palmitate concentrations (0, .100mM), but not at the highest palmitate condition (.400mM, p<0.0001) in both the 5.5 and 25 mM glucose. The ability of MSCs to suppress IFN-γ production from T cells was diminished in both the .200mM and .400mM palmitate conditions regardless of glucose concentrations as measured by ELISA (.200mM, 5.5 & 25 mM glucose, p<0.0001; .400mM, 5.5 & 25 mM glucose, p<0.0001). Additionally, high palmitate concentrations decreased the ability of PBMCs to proliferate and produce IFN-γ, independent of the suppressive effects of MSCs.

CONCLUSIONS: Chronic exposure to high palmitate conditions adversely affects the viability and function of MSCs and decreases the proliferation and activation of PBMCs. Compromise of the immunomodulatory capacity of MSCs within high palmitate conditions highlights a pre-implantation therapeutic target in MSCs to ensure optimal therapeutic potency in vivo, when treating patients with Type II Diabetes.

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Assessment of KIT and Androgen Receptor Expression in Adenoid Cystic Carcinoma

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**Background:** Adenoid cystic carcinoma (ACC) is an uncommon tumor that arises in major or minor salivary glands or rarely in other organs (e.g., tracheobronchial tree, breast). It is a slow-growing, though clinically relentless tumor. Distinctive neurotropism leads to frequent, destructive local recurrences, and it has a propensity for hematogenous dissemination, often many years after the initial diagnosis. These recurrences/metastases are typically aggressive and often fatal. Because of its slow growth kinetics, ACC is resistant to conventional chemotherapy. Recently, MYB-NFIB translocation has been identified as the defining molecular genetic event in most ACCs, leading to constitutive expression of the MYB transcription factor. There is clinical interest in taking advantage of distinct signaling pathways activated in ACC, including those downstream of MYB, as targets for directed biologic therapy. Expression of the receptor tyrosine kinase KIT is characteristic of ACC, though this appears to be a better diagnostic marker than therapeutic target, based on clinical trials. Androgen receptor (AR) expression is typical of prostate cancer, and it is the basis of androgen-deprivation therapy. It is also characteristic of salivary duct carcinoma. Androgen ablation has been suggested as a potential treatment in advanced ACC, but there is conflicting data as to the frequency of AR expression in this tumor type, with reports ranging from 0-20%.

**Purpose:** Our goal was to construct a tissue microarray from a large cohort of adenoid cystic carcinomas, which will facilitate clinical and translational research with diagnostic and predictive biomarkers. Specifically in this study, we have examined ACCs for the diagnostic marker KIT and the predictive marker AR.

**Method:** We searched the University of Iowa Surgical Pathology database for all ACCs diagnosed from 1971-2015. Original glass slides were reviewed and the diagnoses confirmed. A best tissue block was identified for tissue microarray (TMA) construction. Tumors were arrayed in triplicate. We also arrayed 5 salivary duct carcinomas, a tumor type known to highly express AR. IHC was performed using a rabbit polyclonal antibody to KIT (catalogue number A4502, 1:300 dilution, Dako) and a mouse monoclonal antibody to AR (clone AR441, 1:300 dilution, Dako) after heat-induced epitope retrieval at high pH. KIT staining was scored semiquantitatively as follows: diffuse, multifocal, focal, rare cells positive, negative. AR was scored for extent (%) and intensity (0, 1+, 2+, 3+) and an H-score calculated (extent*intensity; 0-300). The following clinical data were obtained: tumor diagnosis, patient age, gender, anatomic location, primary/metastatic status. ACCs were graded according to convention, with tumors possessing >30% solid growth considered high-grade.

**Results:** We arrayed 83 ACCs from 61 unique patients (27M:34F; age range 22-93, mean and median age 56; 65 primary, 14 metastatic) and 5 salivary duct carcinomas (4M:1F; age range 41-79, mean and median age 66 and 67; 4 primary, 1 metastatic). Overall, KIT expression was detected in 97% of ACCs, generally diffuse or multifocal, and in 0% of salivary duct carcinomas. 8.4% (7/83) of ACCs were high-grade; all were KIT-positive (4 diffuse, 2 multifocal, 1 rare cells positive). Detailed KIT-expression data are provided in the Table. All 5 salivary duct carcinomas expressed AR, with mean and median H-scores of 227 and 240, respectively. Only 1 (1.2%) ACC expressed AR, with 1+ staining in 1% of nuclei.

**Conclusion:** ACCs nearly always express KIT, regardless of site of origin or grade, affirming the diagnostic value of KIT IHC, especially in crushed or small biopsies. Unlike salivary duct carcinomas, ACCs do not express AR to any significant extent, suggesting a lack of utility for androgen ablation in this class of tumors. Additional diagnostic and predictive markers of interest will include MYB, EGFR, and pS6rp, the latter a surrogate for mTOR pathway activation.
ROR1, an oncogenic target for therapy in basal-like breast cancer
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Background: Breast cancer, one of the leading causes of cancer-related mortality for women in the world, can be divided into four major subtypes based on genetic expression analysis. These subtypes are predictive of underlying genetic alterations, response to therapies, and long-term outcomes. Basal-like breast cancer (BLBC) is a highly aggressive subtype that lacks FDA-approved targeted therapy. The receptor tyrosine kinase-like orphan receptor 1 (ROR1) functions in embryogenesis and participate in the development of pharyngeal arch derivatives, neural crest cells, neural development, and limb/digit formation. The mechanistic function of ROR1 in embryogenesis is unclear, however in adult tissue, ROR1 is largely unexpressed. In contrast, ROR1 expression has been reported in a number of blood and solid malignancies, leading to its characterization as an oncofetal antigen and an ideal target for cancer therapies.

Hypothesis: ROR1 expression seen in BLBC drives the metastatic phenotype of BLBC and can be used as an efficacious target for therapy.

Methods: We performed bioinformatics analysis of Level 3 RNAseq and RPPA expression data for WNT/ROR1 signaling from breast cancer patients within the Cancer Genome Atlas (TCGA) NCI database. In addition, we performed immunofluorescence staining to corroborate our in silico expression data on deidentified human patient samples. Using breast cancer cell lines, we examined the signaling downstream of ROR1 through lipofectamine transfections of Flag-tagged ROR1 plasmid constructs and the use of small hairpin (sh) RNA targeting endogenous ROR1 mRNA. In NOD/SCID/IL2Rγ (NSG) mice xenografts, we evaluated the efficacy of several anti-ROR1 immunotoxins (ITX) against a highly metastatic BLBC cell line, MDA-MB-231.

Results: Across the breast cancer tumor samples in the TCGA, we found increased ROR1 expression in a subset of BLBC. We found that the highest ROR1 mRNA levels directly correlated to basal protein markers, and were inversely correlated with luminal subtype markers. In human patient samples from the Tissue Procurement Core, we found that ROR1 protein samples were present in triple-negative breast cancer samples, a clinical designation highly associated with BLBC. In BLBC cell lines, we found ROR1 protein levels were associated with the CD44HighCD24Low cancer stem cell phenotype. Treatment of the cell lines with antibody derivatives against ROR1 increased cell-surface CD24. Ectopic expression of ROR1 in MCF7 luminal cells, lead to an increased in pSTAT3, corresponding to bioinformatics observations we observed in the TCGA dataset. In NSG mice xenografted with MDA-MB-231 BLBC cells, we found sustained efficacy of ROR1 ITX on primary tumor growth, with minimal side effects.

Conclusions: As an oncofetal antigen, ROR1 is an ideal target for antineoplastic targeted therapy. Here we report for the first time that ROR1 is expressed in a subset of the highly aggressive BLBC and corresponds to a metastatic/relapse-promoting cancer stem cell phenotype. Introduction of ROR1 into luminal breast cancer cells increased STAT3 activation, which has been implicated in cancer progression and metastasis. Initial experiments into in vitro and in vivo targeting of ROR1 reduced the cancer stem cell populations and led to a reduction in primary tumor growth, respectively.
Early airway disease in cystic fibrosis pigs

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**Background:** Cystic fibrosis (CF) lung disease, the leading cause of morbidity and mortality in CF individuals, is characterized by chronic airway infection, inflammation, and mucus accumulation. Airway infections in CF are often difficult to treat due to the diversity and spatiotemporal dynamics of bacterial communities. Development of a porcine model of CF that spontaneously acquires lung disease enabled us to study the progression of lung disease over the first few weeks of life.

**Hypothesis:** We hypothesized that i) CF pigs are colonized at three weeks of age by one or multiple dominant bacterial species, and ii) the severity of colonization is directly related to airway inflammation, mucus accumulation, airway obstruction, and/or air trapping.

**Methods:** CF and non-CF pigs were studied at three weeks of age and assessed for lung disease using histopathology of airways, CT imaging of the lungs, and bacterial colonization using microbiology.

**Results:** Similar to previous newborn studies, 3 week old CF animals had a higher bacterial load present in their lungs (µ=3.0 x 10³ CFU/sample, range=0-2.2 x 10⁶ CFU/sample) than non-CF animals (µ=35.8 CFU/sample, range=0-2.5 x 10⁴ CFU/sample, p<0.05 non-CF v. CF) There were a comparable number of bacterial species present between genotypes. The diversity of lung bacterial communities were similar between CF and non-CF animals at birth and several weeks of age, but the species composition of the communities changed over time. If the lung bacterial species data were analyzed using a cutoff of 10⁴ CFU/sample, then we observed the emergence of a dominant bacterial species similar to humans with CF. Lung bacterial load was not related to airway inflammation, mucus accumulation, obstruction, or air trapping. However, air trapping was positively related to inflammation, mucus accumulation, and airway obstruction in three-week-old CF pigs.

**Conclusions:** CF pigs are more susceptible to lung infection than non-CF pigs at three weeks of age, and tend to be colonized by a dominant bacterial species. The lack of a relationship between bacterial load and the other indicators of CF lung disease suggest that bacterial-independent means, such as developmental structural changes, altered mucus properties, or an altered inflammatory response due to loss of CFTR function, contribute to CF lung disease.
Identifying Recurrent Enhancer Mutations in Cancer
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BACKGROUND: Cancer as a whole represents a large grouping of complex diseases which share a central phenotype—uncontrolled proliferation. Many of the drivers which have been described are mutations which fall within coding regions of the genome. Given the relatively recent developments of whole genome sequencing technologies, we are now poised to identify driver mutations in non-coding regions as well. For our study, we have chosen to focus on the non-coding regions known as enhancers. Enhancers are regulatory regions in our genome which influence gene expression via transcription factor mediated interactions with promoters. We have chosen them for study because of their proposed ability to influence the transcription of disease-associated genes. To identify potential driver mutations we make a basic assumption that recurrently mutated regions (statistically significant) are likely to have an influence on our phenotype of interest. The overall goal of our study is to identify recurrently mutated enhancer regions which may be cancer promoting.

HYPOTHESIS: There are a set of enhancers which, when mutated, contribute to the cancer phenotype.

METHODS: This was a meta-analysis using called somatic mutations (from WGS data) from two main sources: the Pediatric Cancer Genome Project, and the published dataset used by Alexandrov et al (2012). By combining these two sources, filtering on WGS data, we arrived at a final group of roughly 550 patients with 4.5 million mutations. We then defined several basic genomic bins (non-functional intergenic, intron, UTR, coding sequence, enhancer) using a combination of RefSeq and the Epigenetics Roadmap. Using this mapping we were able to filter out all mutations not falling within an enhancer region. Lastly, we applied a binomial probability model to determine if any of our enhancers were recurrently mutated to a statistically significant degree.

RESULTS: After applying our probability model and a multiple testing correction, we have identified 52 recurrently mutated (statistically significant) enhancer regions. Some of these regions are mutated in several forms of cancer, whereas others are only present in a single cancer type. Additionally, only a subset of these identified enhancer regions are considered to be active in the healthy forms of the tissue.

CONCLUSIONS: As we were able to identify 52 recurrently mutated enhancer regions from our dataset, there is a strong reason to believe that enhancers play a significant role in shaping the cancer phenotype. Our observation that some enhancer regions were mutated in multiple forms of cancer, whereas others were singular, implies that enhancer mutations could possibly be classified as either inconsequential, broadly cancer promoting, or cancer-type specific promoting. Lastly, from our enhancer activity analysis, we conclude that enhancers do not necessarily have to be active in the tissue of origin in order to be cancer promoting.
Factors Affecting Outcomes After ACL reconstruction
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Background: The anterior cruciate ligament (ACL) is the primary restraint to anterior translation of the tibia and provides both anterior-posterior and rotational stability in the knee. The medial and lateral menisci provide a concave surface, significantly increasing the congruence of the tibiofemoral joint and improving knee stability. If the menisci are not present, the convex femoral condyles articulate with the relatively flat tibial plateaus, decreasing the surface area of contact and increasing the pressure on the articular cartilage of the tibia and femur. Both medial and lateral meniscus injuries are frequently seen in conjunction with acute ACL injuries, with the medial meniscus more frequently surgically addressed at the time of injury. Anterior cruciate ligament reconstruction (ACLR) is an option for active individuals who have recurrent instability events after ACL injury. The goal of ACLR is to provide a stable knee joint while also serving to restore proper knee kinematics in order to prevent subsequent articular and cartilage injury. More than half of the patients undergoing ACLR will have concomitant pathology, including articular cartilage damage or medial or lateral meniscus tears. Understanding factors that predict outcomes following ACLR, including patient demographics, graft choice, surgical technique, and concomitant knee pathology, has become an area of interest over the last decade as ACLR techniques have continued to evolve.

Hypothesis: Patient factors and concomitant injury, including age, body mass index (BMI), smoking status, meniscal injury, and chondral injury will affect general health and knee-specific outcomes following ACLR at 6 months and 2 years postoperatively.

Methods: A total of 255 patients were prospectively enrolled in a cohort from January 2012 to December 2014 in order to track outcomes following ACLR at the University of Iowa. Following IRB approval, we performed a chart review of all ACLR patients. Patient demographic characteristics, concomitant pathology, comorbidities, and operative variables were obtained during the charge review. The primary outcome of interest for this study was general health and knee-specific outcomes measures including the SF-26, KOOS, and WOMAC at 6-month and 2-year follow-up. Additional outcomes of interest included reoperation following the index surgery. Univariate and multivariate analysis was performed to determine associations between patient factors and outcomes. Mean follow up time at 6 months was 165.34 ± 35.77 days and at 2 years was 743.55 ± 58.64 days.

Results: There were 255 patients identified who underwent ACLR and met the inclusion criteria for the study. Mean age was 26 years, BMI 26.2 kg/m², and 52% of patients were male. Of these patients, 31.8% had a medial meniscal tear and 43.5% had a lateral meniscal tear. ACL reoperation rate at 6 months was 4.1% and at 2 years was 6.7%. Univariate analysis looking at reoperation rate at 6-months and 2-years revealed that 18.2% of former smokers underwent reoperation compared to 3.5% of people who never smoked. Univariate comparison of patient reported outcome measures showed that age >25 years, former smoking status, use of allograft, and presence of articular cartilage injury (grade III or IV) were associated with worse outcomes at 6 months. At 2 years, these trends remained. Multivariate linear regression was used to assess predictors of patient reported outcome measures at 6-months and 2-years. It was discovered that these same predictive factors were associated with worse outcomes.

Discussion: This study examined the association between a number of different patient factors and outcomes following ACLR. The factors in this study that predicted inferior outcomes at 6 months and 2 years following ACLR provide valuable information to physicians and patients who consider the procedure. While previous studies have indicated that younger age is a risk factor for subsequent surgeries on the ipsilateral knee, we found that younger age was predictive of better outcome scores. Additionally, the results of this study suggest that the likelihood of re-injury is higher with allograft than bone-patellar tendon-bone (BTB) autograft. A limitation of this study is the availability of outcome data at 2 years due to the ongoing nature of the review. The low power at the 2-year time point makes it difficult to extrapolate this data, but provides future direction for the study.
Velvet, a New Mouse Model of Aortic Valve Disease

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Abstract

**Background:** Aortic valve disease (stenosis and regurgitation) is a potentially life-threatening heart condition especially to older population. Our lab recently reported that WAVE mouse with spontaneous point mutation in EGFR leading to decreased tyrosine kinase activity exhibits aortic valve regurgitation. In this study, we examine aortic valve structure and function in Velvet mouse, which has a dominant negative mutation in EGFR.

**Hypothesis:** Based on our recent study of WAVE mouse, we hypothesized that Velvet mouse, with a similar mutation in EGFR, may have aortic valve disease. We examined structural changes (including matrix) associated with functional abnormalities of aortic valve.

**Methods:** 7 Velvet and 7 C57/BL6 control mice were studied. Aortic regurgitation and aortic stenosis were determined using echocardiography. Histological staining was performed for calcification, collagen, and proteoglycan. Immunofluorescence staining/confocal microscopy was used to study several specific proteins. Photoshop and ImageJ were used for quantification. Student t test was used for statistical analysis, with p < 0.05 considered as statistically significant.

**Results:** Velvet mice have aortic valve dysfunction at a young age. The prevalence of mice developing aortic regurgitation or aortic stenosis was about 50%. Histology slides showed that Velvet mice valves were significantly larger than control valves. Velvet mice had significantly more valvular proteoglycan and collagen. Specifically, the leaflets had more collagen deposition and more proteoglycan staining than the controls. Further immunofluorescence staining was used to determine specific collagen and proteoglycan. Collagen 3 and intact versican (splice variant V0/V1) were not increased in Velvet valves. At the base of Velvet valves, versican tended to increase (p=0.056). Valve calcification was also assessed, but no calcification was found in Velvet or control mice.

**Conclusions/Discussion:** Velvet mice have aortic valve dysfunction starting at an early age, with aortic regurgitation (similar to WAVE) and aortic stenosis (different from WAVE), which is not calcific. Total collagen and proteoglycan were increased. However, collagen 3 and versican may not be associated with abnormal valve function in Velvet. More studies will be required to identify components of collagen and proteoglycan that are associated with diseased Velvet aortic valves.
Title: A Gene Expression Classifier for Gastroenteropancreatic Neuroendocrine Tumors
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Background: Gastroenteropancreatic neuroendocrine tumors (GEP NETs) are tumors with increasing incidence that originate from the islet cells of the pancreas or enterochromaffin cells of the small bowel. Pancreatic neuroendocrine tumors (PNETs) and small bowel neuroendocrine tumors (SBNETs) often metastasize to the liver or lymph nodes. Surgical resection and debulking even in the face of metastatic disease can improve survival, and PNETs also respond to targeted therapeutics such as the mTOR inhibitor everolimus and the multikinase inhibitor sunitinib. Although liver biopsy can make the diagnosis of a GEP NET, the primary tumor site may be unknown. There are currently few GEP NET-specific tools for determining the primary site from metastatic tissue. The Howe lab has developed a gene expression classifier (GEC) that uses four target genes and two controls to predict the primary site from metastatic tissue with 94.1% accuracy, but was limited by the number of tumors tested and the possibility that one of the primers for the control, GAPDH, could amplify genomic DNA. The goals of this study were to reconfigure the GEC based on a larger tumor set and a single control assay; determine its accuracy in predicting the primary site from metastatic tissue; and to compare its accuracy to the previously developed GEC.

Methods: Total RNA was extracted from 186 metastases using the TRIzol method and then reverse transcribed to cDNA. qPCR was performed in triplicate for four target genes (bombesin-like receptor-3 (BRS3), opioid receptor kappa-1 (OPRK1), oxytocin receptor (OXTR), and secretin receptor (SCTR)) and a control (Polymerase (RNA) II polypeptide A (POLR2A)). Mean expression for each gene was normalized to POLR2A and ddCt was calculated for all samples. A test set with lymph node metastases from 46 patients was used to derive four 2-gene models that comprise three steps in the GEC using R v3.2.1 (Vienna, Austria). The first step uses the genes BRS3 and OPRK1 to predict primary site, and when either BRS3 or OPRK1 do not amplify, OXTR replaces the missing gene for the second step. When BRS3 and OPRK1 both do not amplify, the third step utilizes OXTR and SCTR. Liver and nodal metastases not used in the test set were used to validate the set for the GEC. The accuracy of the current GEC using one control was then compared to the accuracy of the previous two-control GEC.

Results: 186 metastases from 145 patients were analyzed. 49 patients had PNET primaries and 96 had SBNET primaries. With the 46 test set samples removed, there were 140 metastases from 92 patients, and the one-control GEC correctly predicted 125/140 (89.3%). The previous two-control GEC correctly predicted 101/115 (87.8%), and this difference was not significant (t-test p-value=.89). For the new GEC, performance on SBNETs (95/105, 90.5% sensitivity) was better than performance on PNETs (30/35, 85.7% sensitivity), and lymph node predictions (41/43, 95.3%) were more accurate than liver predictions (84/97, 86.6%). The first step of the GEC correctly predicted 117/130 samples (90.0%), the second step correctly predicted two samples (100%), and the final step correctly predicted 6/8 samples (75.0%). To better understand how the new GEC would perform clinically, analysis was limited to 100 liver metastases from 97 patients. The new GEC correctly predicted 84/97 (86.6%) as compared to 52/56 samples (92.9%) with the previous GEC (p-value=.89). In the new GEC, SBNET liver metastases were correctly predicted in 64/73 (87.7% sensitivity) and PNET liver metastases were correctly predicted in 16/24 (66.7% sensitivity). Finally, the positive predictive values (PPV) in SBNETs was 95.0% (95/100 samples) versus 75.0% for PNETs (30/40 samples). For the old GEC, the PPV for SBNETs was 94/97 (94.9%) and for PNETs was 34/39 (91.9%). The PPV using the previous GEC was 94/97 (94.9%) for SBNETs and 34/39 (91.9%) for PNETs.

Conclusion: There was no significant difference in the sensitivity of the new one-control GEC compared to the old two-control GEC. The one-control GEC’s high accuracy and the increased simplicity makes it more accessible to clinical use for the prediction of the site of primary from biopsy samples of metastases. This could improve surgical exploration, as well as select patients who might benefit from targeted therapy.
Determining Correlation Between Thermal Images and Skin and Soft Tissue Infections

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**Background:** Skin and soft tissue infections are a major cause of morbidity and mortality. In 2012, over 575,000 patients were admitted to hospitals for treatment of cellulitis and other skin infections with a cost of over $3.8 billion. Although these infections are not difficult to diagnose clinically, it is difficult to monitor the natural history of these infections. Such infections are often subjectively tracked using simple approaches (e.g., marker outlines or feeling for warmth in the affected areas). These approaches are simple and inexpensive, but they are often inaccurate. For example, small changes in warmth over time are difficult to detect by touch alone. A more accurate and quantitative approach is needed. We propose the use of thermal imaging to track the progression of skin and soft tissue infections. Specifically, we will determine if images from a thermal camera correlate with daily improvement among patients hospitalized for skin and soft tissue infections.

**Methods:** After obtaining IRB approval, we built an automated paging algorithm to alert us when patients with cellulitis were admitted from the emergency department at UIHC. We also recruited patients referred to us by staff physicians on the Internal Medicine inpatient service. Eligibility criteria included English speaking adults between the ages of 21 and 100 with non-facial skin or soft tissue infections. Eligible patients were consented and thermal images of the affected area of skin were taken using an iPhone fitted with a FLIR One thermal camera. These images also included an unaffected portion of skin. A sterile coin of a fixed size was also included in all images for size reference. Daily images were recorded for the duration of each patient’s hospitalization.

The thermal images, recorded in the manufacturer's proprietary format, were unpacked to produce a 16-bit grayscale image with known min and max temperatures. A Canny edge detector was used to distinguish thermally disparate regions and a Hough transform ellipse detector was applied to identify the reference coin. Thermal contours were drawn at one degree intervals, and the sizes of each region enclosed by the contours was computed and normalized by the size of the reference coin.

**Results:** We approached 42 patients with an initial diagnosis of cellulitis and enrolled 38 people (28 males and 10 females) into the study. Data from 30 patients were analyzed. The average age for patients enrolled was 56.2 years (SD 21, range 21 to 94) and they were hospitalized for an average of 4.9 days (SD 3.2, range 1 to 13).

The thermal images showed dramatic improvement and the images showed a decrease in size despite continued redness and swelling. On average, the maximal temperature estimated by the thermal camera decreased by 2.4°C (SD 2.7, range -1.2 to 9.3) from admission to discharge. Analysis of images to determine surface areas and temperatures of affected skin and soft tissue are underway.

**Conclusion:** We found that thermal imaging at the bedside captures the clinical course (i.e. improvement) for patients admitted and treated for cellulitis. Future work will focus on providing robust measurements of surface area to compute a daily score that is easy to monitor by clinicians caring for patients with skin and soft-tissue infections. Furthermore, future work should examine if thermal imaging could accurately determine response to antibiotic therapy, specifically the lack of improvement, and the need for surgical intervention.
The Effect of Fine Motor Skill Activities on Surgical Simulator (Eyesi®) Performance

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Background:
Video game play has been shown to improve skill acquisition on laparoscopic simulators. However, few studies have analyzed the influence of demographic factors on ophthalmic surgical skills. EYESi® has shown significant construct validity for simulation of cataract surgery.

Purpose:
Determine if history of fine motor activity impacts EYESi® performance.

Materials and Methods:
Participants included medical students and ophthalmology interns. Demographic information, handedness, and time spent performing video games, musical instruments, and other fine motor activities were obtained. All participants performed 3 EYESi® tasks twice (navigation, forceps, and bimanual training) and scores were averaged.

Results:
Twenty-six participants (17 males, 9 females) completed 255 EYESi® tasks. Twenty-four participants were right handed. Nineteen participants reported regular video game history. Twenty-five participants played a musical instrument. Nine participants reported other fine motor activities including knitting and dissecting.

Those reporting regular video game play performed significantly better on navigation training compared to those that did not (score of 53 and 39 respectively, p = 0.03), and trended towards better performance on forceps training (33 and 21 respectively, p = 0.08). There was no correlation between video game quantity, musical instruments, or fine motor activities and simulation performance.

Conclusions:
Video game play, regardless of duration, improves ophthalmic surgical skills.
Clinicopathological predictors of chemoresponsiveness in epithelial ovarian cancer
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Objective: One-third of women with epithelial ovarian cancer are resistant to standard platinum-based chemotherapy, and insufficient data exist in predicting response to chemotherapy. We describe the clinical and pathological factors of patients with complete and incomplete response to treatment.

Method: In this retrospective study, data was reviewed from 75 medical charts of patients with primary epithelial ovarian cancers. All patients underwent chemotherapy and cytoreductive surgery for primary disease. Fifty-six patients had complete response (CR) to chemotherapy and 19 had incomplete response (IR). Fifty-eight and 17 patients had optimal and suboptimal cytoreductive surgery, respectively. Clinical and pathological factors were compared in patients with complete and incomplete response to treatment, and optimal and suboptimal surgery. Overall survival (OS), cancer-specific survival (CSS), and time to recurrence (TTR) were estimated using the Kaplan-Meier method for patient groups.

Results: Majority of patients in both CR and IR groups were diagnosed at advanced stage ovarian cancer. CR group had significantly lower preoperative CA125, and was more likely to have optimal chemotherapy. CR group also was more likely to have lymph nodes removed during cytoreductive surgery. A significantly lower percentage of CR patients died from the disease, and they had statistically longer time until recurrence and death. Patients who underwent suboptimal surgery had significantly shorter time until death but no difference existed in the time until recurrence between patients with optimal and suboptimal surgery. OS, CSS, and TTR were significantly increased in CR group and in patients that had optimal surgery.

Conclusion: It is important to achieve complete response during treatment and have optimal surgery because it will significantly increase OS, CSS, and TTR. Preoperative CA125 and lymph node removal during surgery may be predictive of complete treatment response.
Violence as a Mechanism of Ocular Injury in Women


Objective / Purpose: More than one third of women experience intimate partner violence (IPV) in their lifetime. IPV often escalates and over half of the women killed are killed by intimate partners. Despite the gravity of IPV, there is little training for physicians on how to identify and counsel these patients.

A recent study found IPV to be the third leading documented cause of orbital fractures in women (7.6%). In addition, 20% of orbital fractures had no documented mechanism of injury so the role of IPV is likely even more significant.

While the etiologies of orbital fractures in women have been well documented, the mechanisms of injury for the most common traumatic ocular injuries in women have not been fully evaluated. Knowledge of the incidence of IPV-associated ocular injuries and their common presentations will aid ophthalmologists in counseling these patients and getting them the assistance they need.

The purpose of this study is to determine the cause of traumatic ocular injuries in women and to evaluate the role of violence, specifically intimate partner violence, as the mechanism of injury.

Methods: A retrospective chart review was performed of all female patients who were evaluated at the University of Iowa Hospitals and Clinics between January 1995 and January 2015 for traumatic ocular injuries. Diagnoses in the study included: ruptured globe, corneal laceration, scleral laceration, hyphema, corneal abrasion, conjunctival laceration, retinal tear, and subconjunctival hemorrhage. The mechanism of injury was recorded for each patient and for those patients who were assaulted, the relationship of the perpetrator to the patient was noted. The clinical characteristics and course of the patients that sustained IPV were recorded as well.

Results and Conclusion: The selected ocular injuries occurred in 211 female patients during the study period. The leading cause of ocular trauma in women was accident with an inanimate object (31.8%, 67/211) followed by falls (25.6%, 54/211), motor vehicle collisions (10.0%, 21/211), assault (7.6%, 16/211), accidental traumas inflicted by another individual (7.1%, 15/211), and animal-related injury (3.8%, 8/211). Other causes were identified in 14.2% (30/211) of the cases.

Assault was documented as the fourth leading cause of ocular trauma and IPV accounted for 3.1% (5/16) of the assaults with a documented perpetrator. The perpetrator was documented in 81.2% (13/16) of ocular injuries from assault and relationships to the patient included: significant other (5), daughter's significant other (2), sister (2), cousin (3), and facility caretaker (1). In the remaining 18.8% (3/16) of violence-related cases, the identity of the attacker was not documented. All five IPV assaults resulted in multiple severe ocular injuries including scleral laceration and despite surgical repair, four of the five (80%) patients ultimately underwent enucleation for a blind, painful eye.

Overall, IPV was the documented mechanism of injury in 2.4% (5/211) of all ocular traumas and 3.3% (5/153) of ruptured globes. The true incidence of IPV may be even higher as the relationship of the perpetrator to the victim was undocumented in three cases of assault.

This study highlights the need for increased awareness of the relationship between severe ocular trauma and IPV in the ophthalmic patient population. Ophthalmologists should routinely discuss the mechanism of injury with ocular trauma patients and if violence was involved, document the relationship to the perpetrator. It is imperative to address this issue in order to ensure the safety and well-being of patients.
Cox-2 and 15-PGDH Play a Critical Role in Progression of Cerebral Aneurysms to Rupture with Evidence of Gender Differential Response to Aspirin in Humans and Mice

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Background: Human data suggests that aspirin attenuates inflammation in the wall of cerebral aneurysms and decrease the incidence of aneurysm rupture. We tested the hypothesis that aspirin modulated this process via the Cox-2 pathway. We also tested the role of gender and 15-PGDH on the effect of aspirin in reducing the risk of aneurysm rupture.

Methods: Cerebral aneurysms were induced with hypertension and a single stereotactic injection of elastase into the basal cistern of mice. Four groups (wild type [WT] receiving vehicle, WT receiving aspirin [25 mg/kg/day IP], WT receiving Cox-1 inhibitor [Sc-560 10mg/kg/day IP], and WT receiving Cox-2 inhibitor [NS-398 20 mg/kg/day]) were examined for incidence of aneurysm formation, rupture and asymptomatic survival. To test the role of Cox-1, three groups were used (Cox-1 KO, Cox-1 KO receiving aspirin [25 mg/kg/day IP], Cox-1 KO receiving Cox-2 inhibitor [NS-398 20 mg/kg/day]). Furthermore, two additional groups were tested (m-PGES-1 KO mice and m-PGES-1 KO mice receiving Cox-2 inhibitor). To test whether there is gender differential response to aspirin, four groups were tested (WT female receiving aspirin [25 mg/kg/day IP], WT male receiving aspirin [25 mg/kg/day IP] and 15-PGDH inhibitor [CAY10638], and WT female receiving aspirin [25 mg/kg/day IP] and 15-PGDH agonist [CDDO-Me]). In addition, secondary analysis of data from ISUIA was used to analyze gender differential effect for aspirin in humans. Aneurysm wall tissue and STA were resected during aneurysm clipping and stained for Cox-2 and 15-PGDH. Gene expression in cerebral arteries from these mice was quantified with qRT-PCR.

Results: Cerebral aneurysm formation was similar in WT receiving vehicle and WT receiving aspirin, Cox-1 and Cox-2 inhibitors. Aspirin and Cox-2 inhibitor decreased the incidence of aneurysm rupture significantly (p < 0.05). The incidence of aneurysm formation in Cox-1 KO, and Cox-1 KO receiving Cox-2 inhibitor were similar. However, only Cox-2 inhibitor had a strong tendency to decrease aneurysm rupture in Cox-1 KO. Males receiving aspirin had significantly decreased aneurysm rupture when compared to females (p < 0.05), but not aneurysm formation. 15-PGDH agonist [CDDO-Me] significantly reduced aneurysm rupture in female mice who also received aspirin (p < 0.05). Inhibiting 15-PGDH in males receiving aspirin did not alter both the incidence of aneurysm formation and rupture. Male human patients receiving aspirin had 75% decreased aneurysm rupture vs. 40% in females. STA was significantly rich with 15-PGDH but not Cox-2. This was significantly reversed in aneurysm wall tissue. Gene expression showed the following: 1) Aspirin and Cox-2 inhibitor decreased significantly the gene expression of MMP-9; 2) CD68, MCP-1, and MMP-9, were significantly elevated in female mice receiving aspirin, however, 15-PGDH was significantly elevated in males; 3) When activating 15-PGDH in females and inhibiting it in males receiving aspirin, CD68, MCP-1 and MMP-9 became statistically not significant; expression of 15-PGDH did not change, however, Culin3 and KEAP-1 were significantly elevated and NRF-2 was significantly lower in females.

Conclusion: Aspirin decreases aneurysm rupture in mice via Cox-2 pathway. Incidence of aneurysm rupture is significantly less in male receiving aspirin than female receiving aspirin both in human and mice. Activating 15-PGDH in females receiving aspirin significantly reduces the incidence of aneurysm rupture and eliminates the gender differential response to aspirin.
Implementation of trauma informed care in a pediatric population assessed for child abuse and neglect
Greta Dahlberg, Devin McKissic, Jenna Benoit, Resmiye Oral

Background. Adverse Childhood Experiences (ACEs) have been shown to predict an individual’s future engagement in health risk behaviors and subsequent long-term health outcomes. ACEs are common among the population, with large studies reporting that around 55% of people experience at least one during their lifetime. Trauma exposure can lead to disruption of neural development, which leads to impaired emotional, social, and cognitive behaviors. The eventual outcome is adoption of health risk behaviors and development of acute and chronic diseases. Trauma Informed Care (TIC) is a model that has been developed to take trauma exposure into account when providing care with the goal of interrupting the trajectory towards negative outcomes.

Hypothesis/Aims. The aim of this study was to assess trauma screening practices in a pediatric population being evaluated for child abuse and neglect. There were two hypotheses: (a) Patients being seen as outpatients in 2015 will receive the highest level of TIC and those being seen as inpatients will receive the lowest level of TIC and (b) Caregivers with higher rates of trauma exposure are more likely to have children with more trauma exposure.

Methods. This was a retrospective chart review of 165 patients evaluated for child abuse and neglect from January 1, 2014 to June 5, 2015 at a large hospital and outpatient Child Assessment Clinic (CAC). 60 of the 165 patients had completed some form of TIC documentation. The patients were divided into three groups based on location and date of service: Outpatient 2015 (Group 1), Outpatient 2014 (Group 2), and Inpatient (Group 3). Four new trauma screening forms were implemented at the CAC starting January 1, 2015. Recorded data (when available) included demographics, the presence of TIC documentation, ACE factors to which children and caregivers were exposed, family resiliency factors, and referral rates to services. Data were analyzed using SPSS statistical software.

Results. Screening forms for ACEs revealed that only 3% of children and 2% of adults have experienced 0 ACEs during their lifetime. It was shown that group membership (outpatient 2015, outpatient 2014, and inpatient) was negatively correlated with the presence of TIC documentation by a factor of -0.289 (p<0.001). Therefore, patients being seen in the clinic receive TIC at a significantly higher level than those being seen as inpatients. It was also shown that caregiver ACE exposure (calculated as a sum of questions answered ‘yes’ in the trauma screening form) is positively correlated with the child’s ACE exposure by a factor of 0.489 (p<0.001). Therefore, parents with more significant trauma history are more likely to have children who are also trauma-exposed. An ANOVA was performed for both hypotheses and both were significant at a p<0.001 level.

Conclusions/Discussion. Trauma informed care is an important tool for assessing a patient’s trauma history when providing medical care. Implementation of TIC should improve with the use of more thorough screening forms. Due to the fact that ACEs are highly prevalent in the general population, these screening forms should be implemented in other clinical settings such as inpatient units and primary care clinics. Trauma informed care should include trauma screening of both caregivers and children in order to properly assess the needs of the entire family and refer them to necessary services.
Small Von Willebrand Factor (VWF) Multimers from a Patient with Type 2B Von Willebrand Disease Inhibit Platelet Aggregation Mediated By VWF with a Normal Multimer Pattern

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Introduction: Von Willebrand Disease (VWD) is the most common inherited bleeding disorder and is caused by quantitative or qualitative abnormalities of von Willebrand factor (VWF), a plasma glycoprotein that mediates the adhesive interactions between platelets and the injured blood vessel wall. One type of VWD, type 2B, is typically an autosomal dominant disease in which the VWF displays enhanced or spontaneous binding to platelets, causing the VWF–platelet complex to be cleared from circulation and resulting in thrombocytopenia and reduced concentrations of high molecular weight multimers of plasma VWF. In addition, the type 2B VWD mutations in the VWF A1 domain may affect the conformation of the A2 domain, making it more susceptible for ADAMTS13 cleavage (Nishio et al, *PNAS*. 2014; 101: 10578-10583). Little is known about the effects of the cleavage products and whether they play a role in the hemostatic defects of type 2B VWD. Because type 2B A1 domains can bind spontaneously to platelets, we hypothesize that high concentrations of small multimers and VWF proteolytic fragments may bind platelets but be unable to support efficient platelet aggregation, thereby contributing to the bleeding tendency of patients with this type of VWD.

Method: Platelets from healthy donors were isolated and resuspended in various plasmas for the experiments. Citrated plasma from a patient with type 2B VWD (R1308C) was collected per IRB-approved protocol. Citrated pooled normal plasma (PNP) was used as a control. Platelet binding to VWF in PNP, type 2B plasma, or the mixture of both under shear was examined using a cone-and-plate viscometer. Platelet counts in each sample were measured before and after shearing, and the decrease in platelet count was proportional to the degree of platelet aggregation.

Results: Plasma VWF from the type 2B patient contained only small multimers (only 4 bands were visible on the multimer gel, corresponding to dimers to octamers), 75% of which were dimers and tetramers. VWF multimers in the citrated PNP contains at least 12 bands with dimers and tetramers making up only 34% of the total. At equivalent VWF concentration, PNP was much more effective than type 2B plasma in aggregating platelets under shear (76% aggregation vs 18%, respectively). Type 2B plasma diluted 1:1 with Tyrode’s buffer produced 36% aggregation, whereas PNP and type 2B plasma mixed at equal volumes yielded 33% aggregation, indicating that the effect of PNP was completely blocked by the type 2B plasma. The fact that undiluted type 2B plasma produced less aggregation than when it was diluted to 50% concentration likely indicates that the higher concentrations of small multimers and proteolytic fragments in undiluted plasma was more effective in blocking the contribution of VWF released from platelet granules.

Summary: Plasma from a patient with type 2B VWD, containing exclusively small VWF multimers and proteolytic fragments, inhibits the ability of normal multimers to aggregate platelets under shear. This inhibition is likely related to the ability of these multimers to bind platelets spontaneously, interfering with subsequent binding of large multimers. Our data suggest that, in addition to the deficiency of large multimers, interference by small multimers and proteolytic fragments contributes to the bleeding diathesis seen in type 2B VWD. These findings also suggest that simply infusing VWF during bleeding episodes may not be as effective as in VWD associated with pure quantitative deficiency of VWF.
Factors Affecting Turnover Time in a Hospital Surgical Suite  
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**Background:** Operating room (OR) costs are substantial. OR turnover time is the interim period between surgical cases. Turnover time can be measured reliably as the time a patient is wheeled out of an OR following a surgical case until the subsequent patient in the OR that day is wheeled into the same OR. Reducing turnover time can increase OR labor productivity and the efficiency of use of OR time. Because anesthesiologists, surgeons, physician assistants, advanced registered nurse practitioners, and OR nurses are waiting for the turnover, each extra few minutes can result in $100,000’s of excess cost annually. However, because there are many tasks performed in parallel, observational study is needed to determine principal causes of long turnover times.

**Purpose:** Identify bottlenecks to reductions in the mean OR turnover time at the University of Iowa’s main surgical suite.

**Methods 1:** During N = 19 OR-days, all turnovers in an OR were observed, totaling 65 cases; each OR was scheduled for at least 3 surgical cases for the day. These 19 OR-days represent independent events, unlike individual turnovers within the same OR-day, because each OR-day generally has the same surgical, nursing, and anesthesiology team throughout the day. For N = 12 of the 19 OR-days, the observer (D.E.) remained in the OR during the turnover to document times taken for the OR to be cleaned and for the surgical tools to be setup on the surgical table(s) for the next case.

**Results 1:** The mean ± standard error of turnover time was 33.2 ± 2.1 minutes (N = 19 OR-days). The mean time for surgical tool setup was 17.7 ± 1.9 minutes.

**Methods 2:** Results 1 showed that the mean turnover times exceeded the mean times for tool setup. Therefore, for the next N = 7 OR-days, anesthesia providers (nurse anesthetists or resident physicians) were followed out of the OR. They go to the post-anesthesia care unit (PACU) with their patient; pharmacy to acquire medications for their next patient; OR to setup their workstation; and then to the Day of Surgery (arrival) Area to pick-up their next patient. Milestone times were recorded.

**Results 2:** The mean ± standard error of time for the anesthesia provider to arrive at the Day of Surgery Area was 21.9 ± 2.1 minutes (N = 7 OR-days). Being greater than the mean time for surgical tool setup, one apparent bottleneck to turnover was the time it took anesthesia providers to arrive at the Day of Surgery Area. The mean time for the patient to be stabilized in the PACU was 7.8 ± 0.7 minutes (N = 6 OR-days). The mean time to acquire drugs was 2.2 ± 0.6 minutes (N = 6 OR-days). The mean time to draw up drugs and prepare the OR workstation was 7.1 ± 0.8 minutes (N = 6 OR-days).

**Methods 3:** Since the time of the anesthesia providers appeared rate limiting for the turnover, N = 40 cases were selected randomly for tracking from the moment of arrival of the patient in the PACU until the next patient in the OR that day was wheeled in. These cases’ times were independent because they were selected at random and from many ORs. For each of the cases, the extent of completion of the anesthesia, surgical, and nursing preoperative material before arrival of the primary anesthesia provider was documented.

**Results 3:** The mean ± standard error of the time for the anesthesia provider to stabilize the patient in the PACU and to reach the next patient was 19.9 ± 1.3 minutes. Patient stabilization in the PACU averaged 7.2 ± 0.3 minutes. Time to acquire drugs averaged 2.8 ± 0.2 minutes. Time to draw-up the medications and prepare the OR workstation averaged 6.8 ± 1.0 minutes. The latter two periods were underestimate of mean times to perform these tasks, because the periods included the 17.5% of cases with 0 minutes, occurring when the anesthesia provider either did not prepare the workstation before picking up the next patient or was working with another anesthesia provider who did it for them. Time spent in the Day of Surgery Admissions area with patients before transport to the OR averaged 13.9 ± 2.2 minutes. For 42.5% of the 40 cases, the anesthesia preoperative evaluation was not complete when the anesthesia provider arrived at the Day of Surgery Area. The limiting factor to a briefer turnover time was the anesthesia provider’s arrival in the Day of Surgery Area for 35.0% of cases (i.e., the nurses had the OR ready and the preoperative evaluation was already complete); completion of the anesthesia preoperative evaluation for 22.5% of cases; OR not ready for 27.5% of cases; patient not ready for 7.5% of cases; surgeon preoperative evaluation incomplete for 5.0% of cases; and nursing preoperative evaluation incomplete for 2.5% of cases.

**Conclusion:** The average turnover time for the studied OR-days was the same as the overall mean turnover time for the main surgical suite of the University of Iowa Hospital a decade ago (Dexter et al. Anesthesiology 2005;102:1242-8); in other words, the conditions are typical and generalizable. Anesthesia providers’ arrival to the day of surgery area and time to complete the preoperative evaluation were the limiting factors for OR turnover for 57.5% of turnovers. Two worthy strategies for targeted turnover time reduction are reducing the time for anesthesia providers to obtain and setup medications, and completion of the anesthesia preoperative evaluation before arrival of the anesthesia provider at the patients’ location.
A Practical Lower Limit in the Incidence of Postoperative Residual Paralysis with Electromyographic Neuromuscular Monitoring

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Background: Nondepolarizing neuromuscular blocking (NMB) agents are used in \( \approx 65\% \) of surgical anesthetics. However, multiple studies have shown that as many as 30\%-40\% of patients given these drugs show evidence of residual paralysis in the Post Anesthesia Care Unit (PACU). Blockade can be assessed by the use of quantitative electromyographic "Train of Four (TOF) monitoring. This involves the delivery four 2Hz electric stimuli to the ulnar nerve, while recording the response of the hypothenar muscles; fade (the fourth twitch being smaller than the 1st twitch) is evidence of residual paralysis. Monitoring can be used to guide drug administration and to verify adequate recovery. Since mid-2011, when the Department implemented universal TOF monitoring, the incidence of residual PACU paralysis has decreased from \( \approx 17\% \) to 5\% of patients. However, careful case reviews suggested that not all providers were adopting this monitoring; as many as 5\% of patients given NMB drugs were not monitored. Therefore, beginning in September 2014, all main operating room cases at the University of Iowa Hospitals and Clinics were examined to determine whether ANY form of monitoring was used. After a 3 month baseline period, and for the next 26 weeks, daily feedback to each provider involved in a "no-monitoring" case was contacted by the Chair. The result was a decrease in the average weekly incidence of "no-monitoring" from 2.4\% during the baseline period to 0.3\% after implementation of feedback. But the question was whether this practice change was associated with a further reduction in the incidence of residual paralysis.

Purpose: To determine if there would be significant improvements in TOF ratios following an intervention program to target anesthesia providers that were not using neuromuscular blockade monitoring.

Methods: This work was conducted as a prospective Quality Improvement project, registered with QUISPI. In July 2015, a survey was conducted in PACU to determine the current incidence of residual paralysis. A convenience sample of patients receiving general anesthesia were examined upon arrival to PACU. Quantitative electromyographic TOF was determined in duplicate using the same system as used in the OR (Datex-Omeda ElectroSensorTM EMG system). Of 202 patients surveyed, 153 of them received a non-depolarizing agent during their surgical procedure. All but 3 patients received rocuronium exclusively as a nondepolarizing agent. Patients that did not receive a nondepolarizing agent were used as controls to ensure correct measurements of the quantitative blockade system were being taken. These results were compared with data obtained from 287 similar patients in June-July 2014 (by DD).

Results: In June-July 2014, 13.2\% of PACU patients had a TOF ratio of \( \leq 0.9 \) (and 5.2\% \( \leq 0.8 \)). In the July 2015 survey, these incidences were 18.3\% and 3.3\%. These were NOT significantly different compared with 2014. Only 2\% of patients (1 patient) who DID NOT receive an NMB had a TOF ratio \( \leq 0.9 \), and 0\% \( \leq 0.8 \) indicating appropriate monitoring methodology.

Conclusion: In spite of a dramatic reduction in the incidence of "no-monitoring", the incidence of residual paralysis did not change significantly. We believe that this suggests we have reached a lower asymptote with further improvement limited by either the nature of the available monitoring method (which is difficult to use and interpret correctly in all patients) and/or due to the drugs available to reverse the effects of these relaxant drugs (neostigmine). This is supported by at least one rigorous randomized trial of monitoring that showed a similar incidence among monitored patients \( \approx 15\% \) in spite of careful monitoring. While the obvious goal is to reduce the incidence of residual paralysis to zero, this may not be possible without either better monitoring technology or better reversal drugs.
Child Welfare Professionals’ Determination of When Certain Unsafe Activities and Lack of Child Protection Constitutes Child Neglect
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ABSTRACT

BACKGROUND: Baseline norms for safe child rearing are established by federal and state laws, and enforced by governmental agencies and the legal system. Child Protective Services within the Department of Human Services investigates reported cases of possible child abuse and neglect. Although laws provide guidelines for the evaluation of these cases, the language and substance of child safety laws and guidelines varies greatly among states. The objective of our study was to identify factors that influence the determination of child neglect by child welfare experts, and develop some consensus regarding what constitutes child neglect with respect to child safety issues.

HYPOTHESIS: 1) Various factors including age of the child, legality of a behavior, and presence of injury will influence how a child abuse and neglect expert or a child welfare social worker evaluates a case of potential child neglect. 2) Child welfare professionals will be stricter in their determination of child neglect than the child safety laws and regulations presently described by states.

METHODS: A survey was developed with questions related to demographic variables and six scenarios of potential child neglect. The study requests respondents to decide whether they consider the scenarios to be child neglect with varying age of the child involved, ranging from 4-14 years of age, and with alterations in the scenario regarding legality of the situation and the presence/absence of injury to the child. The scenarios included children left at home alone, unrestrained in a motor vehicle, having access to or possession of a firearm, and operating an all-terrain vehicle. The survey was entered into REDCap (Research Electronic Data Capture), a web-based application designed to support data capture for research studies. Ten social workers from the University of Iowa Hospitals and Clinics completed the survey both on-line and through an investigator interview to validate the survey. Changes were made to the survey based on validation feedback. The survey was distributed to all members of the American Academy of Pediatrics Section on Child Abuse and Neglect (SOCAN) and to 5000 members of the National Association of Social Workers (NASW) who reported their practice to be child/family welfare.

PRELIMINARY RESULTS: Of 523 SOCAN members, 152 have completed surveys to date including 45 males and 103 females with ages ranging from 31-86 years old (Mean age = 52, SD = 13). In the child left at home alone scenario, respondents were significantly more likely to declare the situation child neglect for those 8-14 years of age if the child had been injured. In addition, for children ages 10 - 14 years old, SOCAN members were significantly more likely to declare child neglect if leaving that aged child at home alone was against the law. In a scenario where a loaded firearm was allowed to be accessible to children, the legality of the situation significantly affected the evaluation of child neglect for every age category (4 – 14 years old). For a scenario in which a child had possession of a loaded firearm in the yard of his home, 100% of child abuse and neglect experts believed that the situation constituted child neglect for those aged 4 – 8 years old, and over 85% thought the scenario constituted child neglect for children 10 – 14 years old. No significant differences in child neglect determination were seen by sex, age, ethnicity, or presence of child abuse and neglect certification.

CONCLUSIONS: Our study shows that a number of factors including age of the child, presence of injury and the legality of a situation all affect how child abuse and neglect experts view a case of potential child neglect. This suggests that cases of potential child neglect may be evaluated differently from state to state, due to varying child safety laws across states, even though the risk to the child is the same. Moreover, large percentages of experts indicated certain scenarios warranted child neglect designation, even when no current state laws regulate those particular situations. These results call for child safety law reform to provide greater uniformity in the evaluation of potential child neglect cases and to better protect the safety of children.
Optimizing Design Features of a Novel Device for Joint Distraction in the Ankle

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Background: While total ankle arthroplasty is a successful procedure for treating osteoarthritis, it is typically less successful in young and active patients. Ankle distraction arthroplasty is an alternative joint preserving technique in which the bones of an arthritic ankle joint are held apart for several weeks, creating a gap between the articulating surfaces and allowing soft-tissue regeneration in the joint. The device currently used for this procedure, the Ilizarov fixator, has many shortcomings, therefore a new prototype device has been custom-designed for ankle joint distraction to solve some of these shortcomings. Computer modeling of this prototype provides a method for optimizing specific design features to ensure that it meets its target goals, without the time and expense required for physical modeling.

Hypothesis: Finite element (FE) analysis of the device will allow for determining device stiffness and ensuring that device deflection does not result in a tibial displacement greater than 5mm. Further optimization of the prototype design with respect to material stiffness, placement of the pins, thickness of support structures, and weight of the materials will improve the device stiffness and minimize device weight and bulk.

Methods: Finite element models were created by importing a combination of Vectorworks drawings and TrueGrid meshes into Abaqus. The final combined mesh models included a tibia with a simplified cross-section for ease of modeling. For each model, the tibia was statically loaded vertically with 1120 N, the 90th percentile weight of male patients under 45, in a configuration approximating the midstance phase. The base of the footplate was fixed to prevent translation in any direction. The moving parts were fixed using tie constraints, and the model was solved with results calculated in Abaqus. Results of tibial displacement, max stress in the pins, max stress in the remainder of the device not including the pins, volume of the device, and weight of the device were determined according to the variables of pin length, material properties, and part thickness. For the material properties and part thickness variations, 30mm was used as the standard pin length. A basic configuration of material properties and geometry was used for comparison in the pin length and part thickness tests and included ABS plastic for the footplate; steel for the pins, the rotating, and the locking mechanisms; and aluminum for the remainder.

Results: The results of pin length’s effect on tibial displacement, max pin stress, and max stress not in the pins are shown in Table 1. The tibial displacement was well below 5mm for any of the pin models, and the max stresses were well below the yield stresses of 400 MPa for the steel pins, and 110 MPa for the aluminum parts in the prototype device not including the pins. For the results of the lightest configuration, the device did not exceed 5mm displacement. The result of the lightest configuration based on varied material properties had a combination of carbon fiber, aluminum, titanium, and ABS. The weight of this configuration was 2.40 lb. vs. 3.22 lb for the basic configuration. The result of the lightest configuration based on varied the thickness of the device involved a configuration with reduced diameter side rods, reduced width and thickness support bars, and reduced width ring supports. The lightest reduced thickness configuration was 2.99 lb. vs. 3.22 lb for the basic configuration.

Conclusion: The prototype ankle distraction device has sufficient stiffness to prevent a tibial displacement of 5mm under a static load approximating midstance. Furthermore, with design modifications of thinner supports and different materials, the device is still able to provide the proper stiffness to prevent excess tibial displacement.
Alcohol Abuse: a role for ASICs?

Margaret Fuller in the lab of John Wemmie, MD, PhD

**Background/Rationale:** Alcohol use disorder is an addiction and a major public health problem, which contributes to over 200 diseases and 5.9% of deaths globally each year. However, the mechanisms underlying alcohol use disorder are unclear and treatments remain inadequate. Better treatment options may come from a better understanding of the molecular etiology of alcohol use disorder. Toward this end, other addiction models may provide important insight. Recently, addiction-related behaviors in mice to cocaine and morphine were found to depend on acid-sensing ion channels (ASICs). ASICs are pH-sensitive members of the degenerin/epithelial Na⁺ channel family that contribute to synaptic transmission and cocaine-associated synaptic plasticity in the nucleus accumbens. Thus, ASICs might also contribute to other addictions including alcohol-related addiction behaviors.

**Purpose of the study:** Test the hypothesis that ASICs may contribute to alcohol consumption in mice.

**Method:** Asic1a⁺/+ and Asic1a⁻/⁻ mice were given 24-hour unlimited access in the home cages to both water and ethanol. Consumption of each solution was measured daily over a period of one month. Ethanol concentrations were increased from 0% to 20%.

**Results:** Total ethanol consumption (volume x concentration) increased as concentration increased. However, Asic1a⁻/⁻ mice did not differ from Asic1a⁺/+ controls in either water consumption or ethanol consumption at any of the concentrations tested.

**Conclusion/Discussion:** ASIC1A disruption in mice did not affect alcohol consumption. However, consummatory behavior is only one aspect of alcohol addiction. Craving, the urge to seek out alcohol, is also a critical component of alcohol use disorder which often leads to relapse of uncontrollable alcohol consumption even years after achieving sobriety. Therefore future experiments will aim to determine if ASIC1A contributes to craving.
A lipogenic-switch shifts metabolism from glycolysis to mitochondrial respiration during aging

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Background: The recent growth in number and proportion of older adults in the United States is unprecedented, due to longer life spans and aging baby boomers [1]. As the population reaches a higher average age, age-related health issues are also anticipated to increase. Although the cellular and molecular mechanisms of aging are not completely understood, it is believed that changes in cellular metabolism may have a significant role during the aging process and age-related health issues. Cellular metabolism includes glycolysis, pentose phosphate pathway and mitochondrial respiration. Our unpublished results suggest metabolic reprogramming from glycolysis to mitochondrial respiration during aging of normal human fibroblasts. Mitochondrial respiration receives substrates from either glycolysis (pyruvate) or from lipolysis (fatty acids). The rate-limiting step of lipolysis is regulated by a multi-subunit enzyme complex consisting of G0/G1 switch gene 2 (G0S2), adipose triacylglycerol lipase (ATGL), and comparative gene identification-58 (CGI58). G0S2 is a negative regulator of ATGL, while CGI58 is a positive regulator of ATGL. An age-associated change in lipolysis could contribute to changes in mitochondrial respiration.

Hypothesis: A lipogenic-switch shifts metabolic reprogramming to mitochondrial respiration during aging via G0S2 mediated enhancement of lipolysis resulting in an increase in the amount of free fatty acids.

Methods: Normal human fibroblasts (NHFs) from individuals of different ages (3-d, 12-y, and 61-y) and NHFs from the same individual at different ages (29-y, 36-y, and 46-y) were obtained from Coriell, National Institute on Aging, and cultured following supplier’s protocol. Quantitative RT-PCR and immunoblotting assays were used to measure expression of G0S2, ATGL, CGI58, and fatty acid synthase (FASN). Boron-dipyrromethene (BODIPY) fluorescent dye, confocal-microscopy and flow cytometry methods were used to assay lipid droplets. Mitochondrial respiration was determined by measuring oxygen consumption rate (OCR) in control and palmitate or orlistat (FASN inhibitor) treated NHFs using Seahorse XF96 analyzer.

Results: Compared to young NHFs, older NHFs showed: (1) higher basal OCR; (2) decreases in G0S2 expression and increases in CGI58 and FASN expression; (3) increases in the size and number of lipid droplets; and (4) increases in OCR following palmitate treatment and decreases in OCR following orlistat treatment.

Conclusions: These results indicate that an increase in lipolysis may contribute to increases in mitochondrial respiration during aging of normal human fibroblasts. Molecular manipulations of G0S2, CGI58, and FASN are planned to test the causality of these genes in age-associated increases in mitochondrial respiration.

Reference


Difference in Primary Pulmonary Fibroblast Traction Force in Response to Initial Culture Conditions

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ABSTRACT

BACKGROUND: Idiopathic Pulmonary Fibrosis (IPF) is an interstitial lung disease of unknown etiology in which scar tissue makes gas exchange more difficult, usually resulting in death within five years of diagnosis. Much effort is being made to characterize pulmonary fibroblasts, which play a critical role in fibrosis. Such research is complicated, however, because it appears that the conditions in which fibroblasts are cultured greatly influence their phenotype. It is therefore unknown how well cultured cells resemble their in vivo counterparts.

HYPOTHESIS: Cells which are initially cultured in plastic wells will respond to their stiffer environment by exerting a greater traction force than those cells which are directly seeded onto the softer, hydrogel matrix.

METHODS: Lungs were harvested from healthy, C57BL6 mice and the tissue was dissociated using an enzyme cocktail and Dissociator from Miltenyi Biotech. We then conducted a negative selection for CD45-/CD31- cells using Miltenyi Biotech’s conjugated microbead technology. These surface markers are specific for hematopoietic and endothelial cells, respectively. We then conducted positive selection for cells expressing Thy-1 (CD90.2), which is a known fibroblast marker.

Polyacrylamide gels were created in glass-bottomed, 35-mm, 6-well plates. The gels were then incubated with dopamine (for anchoring), followed by fluorescent beads and finally collagen.

Upon isolating the Thy-1+ cells, we seeded half of them on the gels directly. The other half were placed in plastic 35 mm plates. Both groups were grown in DMEM with 10% FBS and 1% antibiotics. The cells adhered to the bottom within a day and changed to a branched morphology in 4-7 days.

Cellular Force Microscopy (CFM) was conducted on the hydrogel cohort 8 days after seeding (4 days after morphological changes were found). The cells were stained with DiI and then imaged using an inverted fluorescent Nikon Ti microscope and Nikon imaging software. The cells were then removed from the gel with trypsin and the gels were imaged again. We transferred the second group of cells from the plastic to hydrogels, waited two more days for them to re-adhere, and then analyzed them with the same method.

RESULTS: Cells which were first cultured on plastic and then passaged onto gels exerted significantly greater traction forces than cells seeded directly onto the gels (Mann-Whitney Test: Root-Mean-Squared Traction: P=0.0246; Peak Traction: P=0.0018).

CONCLUSION: There is a phenotype shift of fibroblasts in response to culture in plastic dishes that allows them to exert greater tension forces than those seeded directly onto polyacrylamide gels. This gives warning that data derived from serially-passaged fibroblasts may have limited translatability to in vivo conditions. Studying freshly-isolated primary cells may yield more useful data.
Molecular Analysis of Metastatic Lung Adenocarcinoma to the Pancreas: Case Presentation and Literature Review

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Background: Metastatic adenocarcinomas to the pancreas comprise approximately 2% of total pancreatic neoplasms. However, autopsy series have revealed an increased frequency of pancreatic metastases ranging up to 15% of all malignancies. This may be an indication that secondary pancreatic neoplasms are under-diagnosed. Therefore, it is important to consider in certain circumstances that an initial diagnosis of primary pancreatic ductal adenocarcinoma (PDA) may indeed be a secondary pancreatic neoplasm. Herein we present a patient with lung adenocarcinoma who presented with pancreatic metastasis where the lung metastasis mimicked the clinical scenario of primary PDA. The aim of the study is to review the literature on lung cancer metastasis to the pancreas and to define the pathologic studies necessary to confirm lung cancer in this complex clinical scenario.

Methods: A patient with pancreatic metastasis from lung cancer was reviewed following IRB approval. A PubMed search limited to English was performed using search terms, “pancreas,” “metastases,” “metastasectomy,” and “autopsy.” A comprehensive pathologic analysis was undertaken to confirm the diagnosis of lung cancer metastasis.

Results: A 52F presented with abdominal pain, vomiting, and obstructive jaundice. A CT scan showed a mass in the pancreas with dilatation of the biliary and pancreatic ducts. The patient then underwent ERCP with stenting to relieve the obstructive jaundice and subsequent endoscopic ultrasound with biopsy and pathology confirmed the lesion to be adenocarcinoma. The pancreatic mass had abutment with the superior mesenteric artery and therefore neoadjuvant chemotherapy was initiated. Repeat imaging following three cycles of chemotherapy showed disease progression and a new lytic lesion in the pelvis. A PET scan was obtained, which showed multiple sites of FDG avid uptake including the pancreatic head, right adrenal gland, multiple bone lesions, and a new right upper lobe lung nodule and suprahilar, mediastinal and supraclavicular lymph node metastasis. A core biopsy of the right supraclavicular lymph node demonstrated metastatic adenocarcinoma with focal mucinous features. The tumor expressed CK7 and TTF-1 and not CK20, CDX2, or GATA-3. Tumor was morphologically similar to that in the prior pancreas aspirate. A TTF-1 immunostain subsequently performed on a cell block prepared from that initial pancreas aspirate was similarly positive.

From the review of the literature, it was found that of the 647 autopsied patients with secondary pancreatic neoplasms, 163 (25%) had malignancies that originated in the lung. This was the highest frequency in the autopsy series, followed by breast malignancies (13.6%). PDAs have a CDKN2A mutation present in 95% of cases and a KRAS mutation in 83-95% of cases. Primary lung neoplasms have loss of CDKN2A expression in 45% of cases and KRAS mutations in 30-46% of cases. The molecular analysis revealed that this patient’s tumor was positive for the KRAS c.34G>T, p.G12C variant, which has some evidence of overrepresentation in adenocarcinoma of the lung. Additionally, the MET c.3029T:p.T1010I variant was identified which is frequently reported in lung but not in PDAs. An additional single nucleotide variant of unknown significance was found within the TP53 gene. 65-75% of pulmonary adenocarcinomas are Thyroid Transcription Factor-1 (TTF-1) positive, while 0-4% of PDAs are TTF-1 positive. This patient’s biopsy was TTF-1 positive, and combined with the comprehensive molecular analysis confirms adenocarcinoma of lung origin.

Conclusions: Secondary pancreatic neoplasms cause a diagnostic challenge to clinicians and may be under-diagnosed because of how they can simulate primary pancreatic cancer. However, a comprehensive pathologic analysis as demonstrated here can facilitate a diagnosis with high specificity and sensitivity in such a complex clinical scenario.
Impact of Early Expansion of the Affordable Care Act in Select States

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ABSTRACT

BACKGROUND: With the enactment of the Affordable Care Act (ACA) in 2010 there has been an increase in the amount of people that are eligible for Medicaid in the U.S. Some states and one district implemented a full or partial version of the ACA in 2010-11: California, Connecticut, Minnesota, New Jersey, Washington, and the District of Columbia. Full expansion of the ACA was implemented in 2014 in these states and other states that chose to expand the ACA.

HYPOTHESIS: In states that chose to expand the ACA early there will be an increase in the percentage of people insured and an increase in diagnosis and therefore prevalence of chronic diseases and their treatment when compared to states that did not implement an early expansion of the ACA.

METHODS: This was a retrospective cross-sectional study using a Center for Disease Control and Prevention (CDC) survey given through the Behavioral Risk Factor Surveillance System (BRFSS). The BRFSS conducts an annual, cross-sectional, adult survey via telephone in all fifty states, the District of Columbia, and three U.S. territories. This survey is composed of a core group of questions about health and behaviors and annually over 400,000 adults complete the survey. These publically accessible data sets were downloaded from the CDC’s site and put into a statistical program, SAS. In SAS the data was processed and a binary logistic regression model was used to analyze the survey data from the years 2009 and 2013 to determine the impact of income and early state ACA expansion (2010-11) on variables of interest. The outcomes were measured as an odds ratio (OR).

RESULTS: Higher income groups were more likely to: have health insurance OR=19.50 (95%CI 17.42, 19.50), have an established primary physician OR=2.60 (95%CI 2.46, 2.60), have outpatient rehab after a heart attack OR=3.62 (95%CI 2.52, 3.62), have their blood cholesterol checked OR=3.48 (95%CI 3.18, 3.48), have a class on how to manage diabetes if they have diabetes OR=2.14 (95%CI 1.86, 2.14), and to get their yearly flu shot OR=1.63 (95%CI 1.56, 1.63). Higher income groups were less likely to: have to defer going to a doctor because of cost OR=0.09 (95%CI 0.08, 0.09), have high blood pressure OR=0.38 (95%CI 0.36, 0.38), have high cholesterol OR=0.61 (95%CI 0.58, 0.61), have diabetes OR=0.24 (95%CI 0.22, 0.24), have diabetes affect their eyes OR=0.42 (95%CI 0.36, 0.42), and have their health limit their daily activities OR=0.16 (95%CI 0.15, 0.16). States that expanded the ACA early had citizens that were: more likely to have health insurance OR= 1.34 (95%CI 1.17, 1.34), more likely to take a class on how to manage diabetes if they have diabetes OR=1.16 (95%CI 1.05, 1.16), more likely to have outpatient rehab after a heart attack OR=1.54 (95%CI 1.23, 1.54), more likely to have daily activities limited by health OR=0.86 (95%CI 0.76, 0.86), less likely to have an established primary physician OR= 0.73 (95%CI 0.64, 0.73), less likely to defer a doctor visit due to cost OR=0.97 (95%CI 0.85, 0.96), less likely to have high blood pressure OR=0.73 (95%CI 0.61, 0.72), less likely to have blood cholesterol checked OR=0.88 (95%CI 0.77, 0.88), less likely to be told they have high blood cholesterol OR=0.95 (95%CI 0.84, 0.95), less likely to be told they have diabetes OR=0.88 (95%CI 0.76, 0.88).

CONCLUSION: Higher income groups were more likely to be insured and consistently received more care than lower income groups. These higher income groups also were less likely to be told they have chronic diseases, possibly in part due to the increased amount of preventative care they received. States that expanded the ACA early showed both an increase and decrease in both care and detection of disease when compared to states that did not expand the ACA early. Decreased prevalence of diseases may be the result of many things, including preventative medicine practices and/or fewer tests run to detect disease.
**Poster Title:** Identification of Sudden Cardiac Death Candidate Genes by Whole Exome Sequencing  
**Student:** Alexander M. Greiner  
**Mentor:** Barry London, M.D., Ph.D. Potter Lambert Chair in Internal Medicine; Director, Division of Cardiovascular Medicine; Director, Cardiovascular Research Center; Profess of Internal Medicine; University of Iowa  
**Additional Collaborators:** Haider Mehdi, Ph.D. (London lab, University of Iowa); Gina Morgan, M.S. (London lab, University of Iowa)

**Background:** Arrhythmias are a major cause of morbidity and mortality, resulting in more than 250,000 deaths annually in the United States. Life threatening arrhythmias most often accompany structural heart disease from myocardial ischemia, myocardial infarction, and nonischemic cardiomyopathy. Inherited arrhythmia syndromes such as long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada Syndrome (BrS), among others, are important causes of sudden death, especially in young otherwise healthy individuals. Mutations in ion channels responsible for the syndromes in some families have been identified by positional cloning and/or a candidate gene approach. Of note, the identification of the genes responsible for these inherited arrhythmia syndromes has led to rapid advances in the understanding of the mechanisms of the more common causes of sudden death. To date, the genes/mutations responsible for Brugada syndrome can be identified in only ~30% of patients.

**Aims:** We aimed to identify novel candidate genes/mutations in families with inherited arrhythmia syndromes using whole exome sequencing, and to determine the mechanisms by which the mutations cause arrhythmias.

**Methods:** Whole exome sequencing was completed on 20 affected individuals from 6 families (3 or 4 per family) with inherited arrhythmias/sudden death at the Iowa Institute of Human Genetics (IIHG). Whole exome sequencing yielded 15,000-75,000 variants/mutations per sample after alignment and using the Genome Analysis Toolkit (GATK) pipeline to identify valid variants. Variants were annotated using wAnnovar, which incorporates data from the 1000 Genomes project, the Human Gene Mutation Database (HGMD), and others. Variants were eliminated that were common (allele frequency >1%), synonymous SNPs, not in coding regions or splice sites, and not present in the heterozygous state. Variants were further identified by assessing the type of variants (nonsense/frameshift, missense, etc.), whether the affected amino acid is phylogenetically conserved, and whether the putative mutation would disrupt protein function as predicted by SIFT (Sorting Intolerant from Tolerant) and PolyPhen-2 (Polymorphism Phenotyping v2). Finally, variants were eliminated that were not expressed in the heart at the RNA and/or protein levels. Remaining putative mutations were confirmed by direct sequencing of DNA from all available family members.

**Results:** A SCN10A variant (S1060A) was identified in two affected individuals from a family with a history of BrS. The variant has not been reported in the NIH 1000 Genomes Project or the NHLBI Exome Sequencing Project, nor was it present in healthy members of the family. SIFT and PolyPhen indicate this variant is tolerated, but FATHMM, a similar tool, indicates this variant may be damaging. The 1060 residue is conserved with polar side chains (serine and tyrosine). SCN10A encodes a subunit of Nav1.8, which modulates the late sodium current during the cardiac action potential. Variants within SCN10A have recently been reported as being causative for BrS.

We also identified a variant in MSX1 (MSH Homeobox 1) in 4 affected individuals from a family with a history of left ventricular non-compaction cardiomyopathy (LVNC), aortic valve disease, and sudden cardiac death. The variant, E135D, is highly conserved and has not been reported by the 1000 Genomes Project or the NHLBI Exome Sequence Project. In addition, SIFT classified this variant as damaging. MSX1 plays a role in aortic valve development, and has been found to be expressed at high levels in calcified aortic valves. In addition, MSX1 has been determined to be expressed in the endocardium of the left ventricle, which is most affected in LVNC.

The initial analysis of the other four BrS families did not identify putative disease-causing variants.

**Conclusion/Discussion:** We have identified putative causative mutations in one family with BrS and in one family with LVNC and valve disease. Further analysis of these variants, through the use of heterologous expression systems (HEK cells, rat neonatal cardiac myocytes), induced pluripotent stem cells, and transgenic mice will yield valuable information regarding their pathogenicity.
Identification of novel genes with mutations leading to hypertrophic cardiomyopathy (HCM) in ENU mice

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ABSTRACT

BACKGROUND: Human molecular genetics has provided a substantial amount of etiological information regarding heritable cardiovascular disorders. However, considerable gaps remain with respect to the most common heritable cardiovascular disorder, hypertrophic cardiomyopathy (HCM, prevalence 1:500). This project focuses on HCM as it accounts for a large proportion of sudden cardiac death in the young and has an autosomal dominant Mendelian mode of inheritance, creating the potential for substantial clinical impact if discoveries are made. Currently, mutations in at least 19 genes have been associated with HCM. Most of these genes encode sarcomere proteins, while a few also encode for Z-disc and calcium homeostasis proteins. However, it is has been shown in clinical genetic screenings that substantial gaps exist within our knowledge. Only ~50% of HCM patients test positive for mutations in known HCM-associated genes, suggesting that novel genes associated with HCM remain to be discovered. Thus, we hypothesize that additional variants in genes related to the sarcomere, the Z disc, and calcium homeostasis can directly cause the disease. In addition, we suggest that shifting to a broader approach by considering metabolic variants, such as those resulting in increased glucose uptake or excessive glycogen storage, may increase the likelihood of finding clinically relevant correlations.

HYPOTHESIS: Variants in novel genes encoding proteins involved in the sarcomere, the Z disc, calcium homeostasis, and metabolic regulation can lead to HCM.

METHODS: A recessive (homozygous) ENU mutagenesis screen for congenital heart disease in C57/BL6 strain mice was conducted by collaborators at the University of Pittsburgh. Four ENU mouse lines exhibiting HCM in the absence of other structural heart disease were selected for further study. Each line underwent whole exome sequencing (WES) to identify all coding and splice site variants. Most recent offspring (~3 months of age) of the four ENU mice lines were screened phenotypically via transthoracic echocardiography. Measurements of left ventricular anterior wall (LVAW) thickness, left ventricular posterior wall (LVPW) thickness, left ventricular end diastolic dimension (LVEDD), left ventricular end systolic dimension (LVESD), heart rate (HR), and mass were taken from 2D and M-mode images to determine evidence of cardiac hypertrophy. Those with a mass >100 mg and/or LVAW >1 mm were then selected for breeding and subsequent Sanger sequencing for variants previously identified in each line by WES. Variants prioritized for study were heterozygous variants in genes involved with sarcomere and Z disc construction, calcium homeostasis, and metabolism. Sequencing data was analyzed using DNAStar software to determine the presence of expected heterozygous mutations when compared to wild-type July 2007 (NCBI37/mm9) assembly, to identify variants that co-segregate with the phenotype and therefore may be disease-causing.

RESULTS: No variants that have been genotyped thus far have been found to co-segregate with the HCM phenotype. Line 1: 31 variants genotyped out of 148 variants identified in the line by WES did not co-segregate. Line 2: 16 variants genotyped out of 28 variants identified in the line by WES did not co-segregate with HCM. Line 3: variants in Hjurp, Ugt1a1, Gm9733, and Socs5 were present but did not co-segregate with HCM. Line 4: variants in Celsr2 and Card11 were present but did not co-segregate with HCM.

CONCLUSION/DISCUSSION: Variants selected thus far for genotyping were shown not to co-segregate with HCM in each ENU line. Thus, an even broader approach must be taken in order to identify variants that track with this phenotype, genotyping genes not functionally associated with the aforementioned categories. Overall, by detecting HCM-causing mutations in genes not previously considered, we hope to facilitate development of novel methods of prevention, diagnosis, and treatment for HCM, giving patients an enhanced quality of life.
Pharmacological agents that generate H$_2$O$_2$ radiosensitize human lung and breast cancer cells to ionizing radiation

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Background:
Cancer cells, relative to normal cells, demonstrate evidence of chronic metabolic oxidative stress as evidenced by increased steady-state levels of O$_2^-$ and H$_2$O$_2$ that can be exploited to enhance radiosensitivity (PMID: 18937644; PMID: 17967311). Cancer stem cells (CSCs) also show increased metabolic oxidative stress and are thought to facilitate tumor relapse following chemotheraphy and radiation therapy due to the relative ineffectiveness of these therapies to target CSCs. Agents that increase oxidative stress could target both populations.

Hypothesis:
The current study tests the hypothesis that agents known to generate H$_2$O$_2$ or inhibit metabolism are capable of further increasing radiosensitivity in human lung and breast cancer cells.

Methods and Results:
Pretreatment of H292 human lung adenocarcinoma cells with 100 uM genuine H$_2$O$_2$ was found to significantly enhance clonogenic cell killing following 2 x 2 Gy exposures to ionizing radiation. Treatment of H292 cells with 100 uM D-penicillamine + 10 uM Cu (D-PEN) as well as pharmacological ascorbate (ASC, 5 pmol/cell), that are known to generate H$_2$O$_2$, were also found to sensitize H292 cells to clonogenic killing following radiation exposure. Furthermore treatment of SUM159 breast cancer cells with D-PEN also sensitized the cells to radiation-induced cell killing. FACS analysis of aldehyde dehydrogenase positive H292 cells treated with H$_2$O$_2$, DPEN, or ASC showed no selective depletion of CSCs. When metabolic inhibitors of mitochondrial metabolism (500 nM 12-triphenylphosphonium, 12TPP) or thioredoxin reductase (500 nM Auranofin; AUR) were used, radiosensitization was only seen in H292 cells with AUR and there was no correlation of sensitivity with markers for CSCs.

Conclusions/Discussion:
These results support the hypothesis that agents that generate H$_2$O$_2$ can act to radiosensitize breast and lung cancer cell lines in vitro. These experiments will be continued both in vitro to determine precise mechanisms for enhanced cell killing as well as in vivo to determine the potential clinical utility of these approaches in cancer therapy. (supported by 2T35HL007485-36 and R01 CA182804)
**Helicobacter pylori modulates neutrophil morphology and lifespan**  
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**Background:** *Helicobacter pylori* is a Gram-negative spiral-shaped bacterial pathogen that inhabits the gastric mucosa of approximately 50% of the world’s population, causing a chronic gastritis that increases the risk of developing peptic ulcers and gastric cancer. Bacterial and host-derived factors lead to a chronic neutrophil-rich inflammatory response, but this response fails to clear the infection. Neutrophils (PMNs) are able to phagocytose *H. pylori* but have a reduced capacity to kill ingested bacteria. Details of this interaction remain poorly understood. The purpose of this study was to address knowledge gaps regarding the phenotype and fate of infected PMNs and their bacterial cargo.

**Hypothesis:** Previous experiments have shown that *H. pylori* prolongs the lifespan of infected PMNs and causes phenotypic changes, including nuclear hypersegmentation, indicative of PMN subtype differentiation. This study sought to 1) identify the role of major *H. pylori* virulence factors in this process, 2) determine whether secreted bacterial factors could bring about these changes, and 3) determine whether PMN transcription and translation were required for *H. pylori* modulation of PMN phenotype and lifespan.

**Methods:** *H. pylori* wild-type and mutant strains were grown on sheep blood agar plates overnight at 37°C under microaerophilic conditions. PMNs, isolated from the peripheral blood of healthy donors, were left uninfected or infected with wild-type or mutant *H. pylori* at an MOI of 5:1. In some experiments, PMNs were incubated in the presence of absence of an inhibitor of transcription or translation, or exposed to varying concentrations of supernatant collected from an *H. pylori* culture. Annexin V-FITC/PI staining flow cytometry was used to assay PMN apoptosis. Cytospins were used to assess apoptosis by analysis of PMN nuclear morphology.

**Results:** Approximately half of uninfected PMNs, which undergo constitutive apoptosis, showed signs of an apoptotic morphology by 24 hours, whereas the lifespan of PMNs infected with wild-type *H. pylori* was significantly prolonged. None of the *H. pylori* mutants studied significantly altered the extent of PMN apoptosis relative to wild-type. In contrast, *H. pylori* supernatant was shown to be capable of inhibiting PMN apoptosis in a concentration-dependent manner. Finally, transcription and translation inhibitors ablated the ability of *H. pylori* to extend PMN lifespan, yet did not prevent induction of nuclear hypersegmentation by 4 hours post-infection.

**Conclusions:** The major *H. pylori* virulence factors tested do not appear to be required for delaying PMN apoptosis or inducing nuclear hypersegmentation. The studies with *H. pylori* supernatant suggest that secreted factor(s) from *H. pylori* can inhibit PMN apoptosis. In addition, we show that PMN transcription and translation are required for the prevention of PMN apoptosis in *H. pylori* infected neutrophils but not for nuclear hypersegmentation. In summary, *H. pylori* has complex effects on PMN phenotype and fate and our data begin to provide insight into the complex underlying mechanisms.
Gene sets implicated in the etiology of eating disorders

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BACKGROUND: Eating disorders, including anorexia nervosa, bulimia nervosa, and binge-eating disorder, are characterized by severe disturbances in food intake and self-perception of physical appearance. Although current treatment strategies, focusing on normalizing meal patterns and addressing cognitive distortions, successfully help many patients, eating disorders still have the highest mortality rates of all mental illnesses. The risk of developing an eating disorder is highly heritable and many research efforts have focused on elucidating genetic variations that might contribute to the risk of developing the illness. Although several genes have been previously implicated in the etiology of eating disorders, results of molecular genetic studies have often lacked statistical power, resulting in failure to replicate many of the initial findings. The purpose of the current study is to analyze the exomes of 98 cases of patients with anorexia, bulimia, and binge-eating disorder in order to identify damaging mutations that cluster into shared biological pathways.

PURPOSE: To identify gene sets and pathways that potentially contribute to the etiology of eating disorders.

METHODS: Ninety-eight unrelated participants were recruited from the UIHC eating disorder inpatient unit, the Psychiatric Partial Hospital, and from community volunteers via email. 57 patients had anorexia nervosa, 30 had bulimia nervosa, and 11 had binge eating disorder. Whole exome sequencing was performed by the Broad Institute on DNA collected from saliva samples. Samples were quality-controlled using FastQC, then aligned to the hg19 human reference genome using the Burrows-Wheeler Aligner (BWA). Platypus, a haplotype-based variant caller, was used to call single nucleotide variants and indels. We then used the software tool ANNOVAR to functionally annotate the genetic variants. To estimate the impact of the genetic variants, we used the Combined Annotation-Dependent Depletion (CADD) score. Variants with less than 5% minor allele frequency and phred-scale CADD scores of greater than 15 were identified and considered putative loss of function (LoF) variants. After genes containing LoF variants were identified, we then determined how many individuals in our cohort carried LoF variants in each gene. This cohort-level burden per gene was compared to 10,000 individuals simulated from the Exome Variant Server (EVS). A proportion test was performed using the statistical software R to determine if the observed cohort burden differed significantly from the data simulated from EVS variant characteristics. The genes in our cohort identified as having significantly excessive burden were then sorted into gene sets, as defined by Gene Ontology (GO) annotations. Gene set enrichment analysis (GSEA) was then performed to determine if any pathways, processes, or functions displayed excess burden.

RESULTS: GSEA returned 320 gene sets that displayed excess burden in our cohort. Some gene sets had over 20 genes with excess burden, whereas other sets had only one gene with excess burden. Several gene sets that had the highest genetic burden were dendrite development, synapse organization, negative regulation of cytokine production, and carbohydrate catabolic processes.

DISCUSSION: Analysis of the genetic data identified several biological pathways potentially relevant to the development of eating disorders that warrant further exploration. One gene set of interest was “process of negative cytokine regulation.” Numerous studies have found that the overexpression of cytokines can suppress appetite (anorexia) and lead to the loss of lean body mass. Further analyses implicated mutations in the NOD (nucleotide-binding oligomerization domain) gene, whose protein product plays an important role in innate immunity as sensors of microbial components in the gut. Mutations in this gene occur in a subpopulation of patients with Crohn’s disease, another disorder associated with inflammation and appetite suppression. Another gene set that has received high prioritization includes “processes of dendrite development and morphogenesis.” Aberrant dendritic development can have detrimental effects on processes such as pruning, myelination, and branching – all of which significantly impact development and behavior. Identifying LoF dendritic development genes in individuals with eating disorders could allow for earlier intervention and better treatment outcomes. One limitation of the current study is the use of simulated data from the EVS as control data to compare our findings. As the sequencing data from the EVS did not go through the same process and analysis pipelines as our data, the results should be interpreted with caution. Additionally, the statistical analyses used in this study do not allow for the identification of unique variants that have not been reported previously and may contribute to sporadic cases of eating disorders in individuals or families. In future studies, we plan to use an individualized approach and compare genetic results to phenotypic observations in order to determine if unique genetic variants could help explain individual symptoms. Additional future plans include Sanger sequencing to confirm novel variants. We hope to be able to use knowledge on the genetic contributions to eating disorders to refine the development of effective treatments, reduce stigma, and inform the public.
Abstract

Anticoagulation medications are increasingly used in the pediatric population, and due to the limited clinical studies, dosing regimens are derived from the adult protocol. The most commonly used anticoagulant is Warfarin, which requires close monitoring via INR due to its narrow therapeutic range. Fluctuation in INR values brings safety and efficacy risks to the pediatric population, including bleeding and clotting complications. These risks lead anticoagulants to be named as a National Patient Safety Goal by the Joint Commission. The purpose of this study is to describe the epidemiology of Warfarin usage at UI Children’s Hospital, understand the current practice patterns, describe the safety and efficacy of Warfarin therapy, and identify any barriers to high quality Warfarin therapy. To identify patients included in this retrospective cohort we used Warfarin med orders for pediatric patients admitted between January 1, 2013 through May 31, 2015 and no patients were excluded. EMR review, data collection and descriptive analysis was performed on variables including patient demographics, Warfarin and interacting medications dosing, pertinent comorbidities, lab values, INR monitoring, interruptions in therapy, bridging regimens, complications, and patient and family education. 26 patients and 38 hospital admissions were included in this study, 85% of which were Cardiology patients. Of the 38 admissions, the average time spent in therapeutic range was 31.58% and 5 of the 26 patients experienced a bleeding and/or clotting complication while taking Warfarin. Following careful analysis of these complications, we identified four main quality improvement areas: Communication between specialty clinics, pharmacies, and families; documentation of anticoagulation therapy; frequency of INR monitoring to prevent unnecessary lab draws and expenses; and bridging and dosing education to prevent unnecessary dose changes and better maintain therapeutic range. On August 12th, this study was presented to the UIHC Children’s Safety and Quality Committee with the goal of impacting the quality of therapy provided to future Warfarin pediatric patients.
Youth Tackle Football Players Experience Lower Injury Rates Than Flag Football Players
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Background/Rationale
Sports-related injuries are the leading cause of injury in adolescents. There are an estimated 2.8 million youth football players between the ages of 7 and 14 in the U.S. Previous studies on youth football injuries have been limited by small sample sizes or conflicts of interest and produced a wide range of data. Injury rates in youth football have been reported from 8.5/1000 to 43/1000 exposures (games or practices) in prior studies. Additionally, it is unclear whether flag football provides a safer alternative to tackle football with relation to injury rates.

Purpose of the study
This prospective cohort study aims to measure the rates and types of injuries in youth football. It compares injury rates between tackle and flag football leagues and examines how age, gender, and position affect the incidence and types of injuries. It is hypothesized that there is not a difference in injury rates between youth tackle football leagues and youth flag football leagues.

Methods
This two-year prospective cohort study has enrolled two tackle football leagues and one flag football league with an electronic attendance and injury form. League personnel enter attendance and injury data from teams, which provides the leagues with a database to track players and injuries. Data is de-identified prior being evaluated by research team members. Data collected from the forms included: gender, age, grade, position, date of injury, location and description of injury, type of league, setting (practice, scrimmage), type of play/drill, type of injury (contact, non-contact), return to play, and final diagnosis of injury. Injury rates (total injuries per 1000 exposures) were evaluated using descriptive statistics and injury, severe injury, and concussion rates were compared using regression analysis.

Results
We collected data on 46,416 exposures and 128 injuries, resulting in an overall injury rate of 2.76 injuries per 1000 exposures. When separated by league type, tackle football leagues had an injury rate of 2.60 injuries per 1000 exposures while the flag football league had an injury rate of 5.77 injuries per 1000 exposures, which was significantly higher than the tackle football league (p=0.0065). In addition, there was a non-significant trend towards lower concussion rates in tackle football leagues.

Conclusion/Discussion
This is the largest ever cohort study of youth football players. Injury rates were lower in youth tackle football league and there is a non-significant trend towards lower rates of concussion in tackle leagues. A second year of data collection is underway, which is expected to allow us to better describe the types of injuries suffered by youth football players as well as expand our conclusions about concussion risk.
Systematic review of the role of surgical margins on sarcoma recurrence and survival
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Background/Rationale: Sarcomas are tumors of mesenchymal origin that commonly arise from soft tissue and bone in the extremities and pelvis. Surgical removal of sarcomas via amputation or limb-salvage surgery, often accompanied with adjuvant therapy, is the mainstay of treatment, and this requires a team of specialized surgeons, oncologists, radiation oncologists, radiologists, and pathologists. An important measure of successful sarcoma treatment is the surgical tumor margin, yet defining and reporting the tumor margin has remained a source of controversy. Currently, at least six different margin classification systems exist, and there is not a universally accepted method of margin reporting. Following sarcoma excision surgery, the tumor margin is classified in various ways, typically as some iteration of adequate (negative) or inadequate (positive). The simplest systems use a dichotomous terminology in which the presence or non-presence of tumor cells at or near the inked margin of tissue sample indicates positive or negative margins. Meanwhile, other systems are more complex, such as the Enneking system that classifies margins as intralesional, marginal, wide, or radical. Not only does this confound analysis of oncologic results, but also limits comparison between investigative reports. There is a large amount of literature addressing sarcoma excision and local recurrence, although the margin classifications used in the studies are variable. Lack of congruity between classification systems remains an area of concern.

Purpose: In this study, we hypothesized that there is a margin classification system that is most predictive of recurrence and survival following sarcoma excision. The aim of our study was to perform a systematic review of literature to investigate the association between sarcoma excision margins and oncologic outcomes (recurrence and survival).

Methods: We developed a comprehensive literature search strategy of the following databases: Ovid MEDLINE, CINAHL, Cochrane Library, Web of Science, Embase, and ClinicalTrials.gov. The search strategy and search were performed under guidance of a clinical education librarian specialist (AB). All studies generated from the initial search were reviewed based on title and abstract. Studies eligible for inclusion consisted of those that reported on at least ten patients with a primary sarcoma of the extremities or pelvis who received limb-salvage or amputation surgery with a report of the final surgical margin. Only studies that provided recurrence and/or survival outcomes with a minimum follow-up of two years were included for review. If these criteria were not met, the studies were excluded. Additional exclusion criteria included radiation-induced sarcomas, recurrent sarcomas, and sarcomas with multiple surgical procedures.

Results: Our initial database search generated 13,518 articles to be reviewed. After duplicates were removed, 10,422 articles remained. These articles were then screened based on title and abstract. After title and abstract, approximately 1,200 articles currently remain for full text review.

Conclusions/Discussions: Our initial search and screen based on title and abstract review was successful. We have identified a number of articles that look promising for data analysis. With this information, we hope to identify the margin classification system that is most effective in predicting recurrence and survival outcomes for sarcoma patients.
Interoceptive sensitivity is associated with affect, personality, and memory in older adults

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ABSTRACT

BACKGROUND: Interoception can be defined as the perception of signals from within the body, whether visceral or proprioceptive. Interoceptive sensitivity, then, is how sensitive an individual is to those signals. Interoceptive sensitivity has been found to be associated with emotional and cognitive variables among younger adults. Past studies have shown that greater interoceptive sensitivity is related to higher levels of trait anxiety, and more recent studies have found positive relationships between interoceptive sensitivity and memory for emotional stimuli. However, little is known about whether these relationships persist in older adults.

HYPOTHESIS: In the present study, we take an exploratory approach to examine how interoceptive sensitivity relates to emotion and cognition in neurologically normal adults.

METHODS: Participants (N = 53) were recruited from an existing registry of older adults living independently in the Iowa City/Johnson County area. Participants were aged 59-91 years old, with a mean age of 76.6 years old. A heartbeat-counting task was used to measure interoceptive sensitivity, and self-report questionnaires measuring affect, personality, and mindfulness skills were administered. Neuropsychological data from prior research were also available for each participant. Data were analyzed using SPSS statistical software using Pearson partial correlations.

RESULTS: Data analysis revealed several relationships: 1) higher interoceptive sensitivity was associated with lower levels of positive affect (r = -.28, p = .03); 2) higher interoceptive sensitivity was associated with lower levels of trait extraversion (r = -.38, p = .004); and 3) higher interoceptive sensitivity was associated with stronger anterograde memory ability (r = .34, p = .01).

CONCLUSIONS: These results suggest that, among older adults, interoceptive sensitivity is facilitative for aspects of cognition but perhaps disruptive for certain aspects of emotional experience. This study also lays the framework for future studies examining how interoceptive awareness may influence higher-order cognitive abilities (e.g., decision-making) in normal elderly.
Triphenylvinlypyridine derivatives for the treatment of metastatic melanoma

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Background and Rationale: Melanoma incidence has increased dramatically over the past decade. Although melanoma can be cured with surgical resection if detected early, progression to the metastatic stage is often lethal, with a five-year survival of less than 20%. Recent discoveries of genetic mutations (e.g. BRAF) have lead to new treatment options for patients; however, rapid development of resistance limits their effectiveness. This presents a critical need for novel therapies that can effectively treat melanoma and circumvent the substantial challenges drug resistance poses. Previous studies in our laboratory demonstrate the potential for lipophilic-cations to target melanoma mitochondria, disrupt oxidative metabolism, and induce cell death. Promising lipophilic cation triphenylvinlypyridine (TPVP) derivatives have been developed to target melanoma cells relative to non-malignant cells. These molecules offer great potential for side chain manipulation, allowing us to explore how properties such as hydrophobicity alter drug efficacy. Our data suggest that TPVP derivatives preferentially accumulate in, alter the redox state of, and decrease survival of melanoma cells.

Hypothesis: Triphenylvinlypyridine (TPVP) derivatives modified with linear-aliphatic side chains can be developed to exploit differences in mitochondrial oxidative metabolism and selectively kill melanoma cells relative to non-malignant cells.

Methods: We assessed a small library of TPVP derivatives modified with alkyl side chains of different length, overall charge, and position around the TPVP molecular head group. We examined the effects of these TPVP modifications on mitochondria oxidative metabolism and melanoma-cell cytotoxicity. In vitro clonogenic survival assays and ATP bioluminescence assays were performed with TPVP compounds to test drug toxicity. JC-1 assays were done to assess the effects of TPVP compounds on mitochondrial membrane potential. DHE assays were done to determine how TPVP affects the redox state of melanoma vs. non-malignant cells. Responses to treatments were evaluated in A375 cells, a BRAF mutant primary melanoma cell line. Assays were performed at 21% O₂ and 4% O₂, in order to mimic the tumor microenvironment.

Results: Our results suggest that TPVP derivatives decrease clonogenic survival and ATP concentration in melanoma cells. TPVP derivatives were also found to disrupt mitochondrial membrane potential as assessed by JC-1 accumulation. Furthermore, TPVP derivaties significantly alter the redox state of melanoma cells compared to nonmalignant cells as measured by DHE oxidation. Exploration of structure-activity relationships between side-chain properties and compound efficacy determined that a singly charged molecule with a ten-carbon length alkyl chain has the greatest influence on clonogenic survival, ATP concentration, JC-1 accumulation, and DHE oxidation.

Conclusion and Discussion: This study suggests that lipophilic cation TPVP derivatives with reactive alkyl side chains promote melanoma cell death. Although the exact mechanism is not fully understood, initial data suggest that one component is disruption of mitochondrial oxidative metabolism and alteration of the normal redox state of the cell. Investigation of structure-activity relationships in TPVP side-chains has also lead to further understanding of specific properties that may enhance in vivo biodistribution. This research is innovative because the approach exploits inherent differences in melanoma metabolism relative to normal cells. This research is significant because success could result in novel treatments and improved therapeutic outcome for a rapidly increasing number of patients with a highly aggressive, often lethal form of skin cancer.
Early Life Exposure to General Anesthetics Reduces Sleep in Adult *Drosophila melanogaster*

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Abstract

**Background:** Every year 6 million children undergo anesthesia, including 1.5 million children under the age of 12 months. Human epidemiological studies and rodent models suggest that exposure to general anesthetics (GAs) during early childhood may permanently damage the developing brain resulting in long-term behavioral impairments. Growing concern over the potentially harmful side effects of GAs warrants a basic understanding of how GAs affect brain development and ultimately influence behaviors.

The fruit fly *Drosophila melanogaster* is a valuable experimental model for examining fundamental problems in neurobiology. The sleep-like state in adult flies, defined by 5 minutes of inactivity, is a conserved and well-studied behavior. Fly sleep can also be quickly and easily assessed in a non-subjective and quantitative manner. Studying the effects of early life exposure to GAs on adult sleep behavior in fruit flies can provide basic biological insight into how GAs can affect the development and function of specific neural structures within the brain. Furthermore, identifying the molecular and cellular targets of GA-induced changes in the brain will provide a foundation for investigating the possible mechanisms behind behavioral alterations seen in previously anesthetized pediatric patients.

**Hypothesis:** Exposing fruit flies to GAs during the pupal stage and immediately after eclosion will impair adult sleep behavior.

**Methods:** Two particular time windows for *Drosophila* brain development were studied: the end of the pupal stage and immediately following eclosion. Wild-type female pupae and female virgin flies within 5 hrs post-eclosion were collected for anesthetization. The flies were exposed to different doses of sevoflurane, a commonly used inhalational anesthetic, for 60 minutes in air-tight glass chambers (35 mm x 10 mm). Following GA exposure, the flies were allowed to mature for three to four days in an environmental chamber at 25°C with 65% humidity. 24-hour sleep behavior was then assessed using a video-assisted positional tracking system. Sleep and wake parameters, including sleep latency, sleep-bout length, and number of sleep episodes, were calculated using a custom Microsoft Excel-based program.

**Results:** Flies treated with 15µL of sevoflurane within 5 hours post-eclosion slept 10% less than control flies over a 24-hour period. Significant reduction of sleep amounts in these flies occurred only during the day in which anesthetized flies slept 20% less than controls. Nighttime sleep was not affected. Interestingly, pupal exposure resulted in a slightly different sleep phenotype. No significant changes to 24-hour sleep amounts were seen in late stage pupae exposed to 20µL sevoflurane. However, anesthetized flies slept 18% less than control flies during the day and nighttime sleep increased 11%.

**Conclusion:** Early life GA exposure in fruit flies disrupts normal sleep behavior in adulthood. Flies treated with sevoflurane shortly after eclosion only exhibit reduced sleep during the daytime while flies treated during the pupal stage sleep less during the day and sleep more during the night. This suggests that the nature and severity of GA-induced sleep impairment varies depending on the neurodevelopmental stage of the fly. These data lend support to previous studies suggesting that early-life GA exposure can interfere with normal brain development in a dose-dependent manner and result in persisting behavioral abnormalities in adulthood.
Succinate Dehydrogenase Deficiency in Pheochromocytoma and Paraganglioma

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Brigham and Women’s Hospital and Harvard Medical School2

Background: Succinate dehydrogenase (SDH) is a heterotetramer (composed of SDHA-D subunits) that resides in the inner mitochondrial membrane, participating in the Krebs cycle and the electron transport chain. SDH-subunit mutations have been implicated in the autosomal dominant hereditary paraganglioma-pheochromocytoma syndrome and have also been described in a subset of gastrointestinal stromal tumors and rare renal cell carcinomas. SDH-deficiency has also been associated with increased biologic aggressivity in pheochromocytomas and paragangliomas. Pheochromocytomas are neuroendocrine non-epithelial neoplasms that arise in the adrenal, often secreting symptomatic amounts of catecholamines. Paragangliomas are their extra-adrenal counterparts, arising at sites of physiologic paraganglia and in solid organs; they are less likely to be functional. Unlike many other tumors, the biologic potential of pheochromocytomas and paragangliomas is difficult to predict based on histology alone.

Purpose: There is clinical interest in identifying SDH-deficient pheochromocytomas and paragangliomas, as an indicator of a potential hereditary cancer syndrome and as a prognostic marker. Any SDH-subunit mutation destabilizes the SDH complex, resulting specifically in markedly reduced to absent SDHB protein expression, detectable by routine immunohistochemistry (IHC). We sought to validate SDHB IHC as a means of detecting SDH-deficient tumors in a large cohort of pheochromocytomas and paragangliomas. We hypothesized that deficiency would be common and that it would be more frequent in metastases than primary tumors.

Method: We searched the University of Iowa Surgical Pathology database for all pheochromocytomas and paragangliomas diagnosed from 1991-2015. Original glass slides were reviewed and the diagnoses confirmed. A best tissue block was identified for tissue microarray (TMA) construction. Tumors were arrayed in triplicate. IHC was performed using a mouse monoclonal antibody to SDHB (clone 21A11AE7, 1:100 dilution, Abcam) after heat-induced epitope retrieval at pH 6.0. SDHB expression was scored as normal (intense, granular cytoplasmic staining) or abnormal (markedly reduced or completely absent staining). The following clinical data were obtained: tumor diagnosis, patient age, gender, anatomic location, primary/metastatic status. Two-sided Fisher’s exact and Mann-Whitney tests were used to analyze categorical and interval data.

Results: We arrayed 111 pheochromocytomas from 101 unique patients (46M:55F; age range 11-88, mean and median age 46; 103 primary, 8 metastatic) and 149 paragangliomas from 131 unique patients (53M:78F; age range 14-85, mean and median age 48 and 47; 111 primary, 6 metastatic, 32 unspecified). Abnormal SDHB IHC was more common in paragangliomas than pheochromocytomas (p<0.0001), and although it appeared more common in metastases than primaries of both tumor types, neither comparison achieved statistical significance. Patients with metastatic tumors were more likely to be male (0.024); patients with metastatic paragangliomas were younger than patients with primary tumors (0.0053). Detailed data are presented in the Table.

<table>
<thead>
<tr>
<th>Tumor Type (Subcategory)</th>
<th>n</th>
<th>M:F</th>
<th>Median Age</th>
<th>% SDHB IHC Abnormal</th>
<th>% SDHB IHC Completely Lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pheochromocytoma (Total)</td>
<td>111</td>
<td>46:55</td>
<td>46</td>
<td>13%</td>
<td>6%</td>
</tr>
<tr>
<td>Pheochromocytoma (Primary)</td>
<td>103</td>
<td>40:53</td>
<td>45</td>
<td>12%</td>
<td>6%</td>
</tr>
<tr>
<td>Pheochromocytoma (Metastatic)</td>
<td>8</td>
<td>6:2</td>
<td>50.5</td>
<td>25%</td>
<td>13%</td>
</tr>
<tr>
<td>Paraganglioma (Total)</td>
<td>149 (131 pts)</td>
<td>53:78</td>
<td>47</td>
<td>50%</td>
<td>17%</td>
</tr>
<tr>
<td>Paraganglioma (Primary)</td>
<td>111</td>
<td>40:71</td>
<td>50</td>
<td>47%</td>
<td>14%</td>
</tr>
<tr>
<td>Paraganglioma (Metastatic)</td>
<td>6</td>
<td>4:2</td>
<td>32.5</td>
<td>83%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Conclusion: SDH-deficiency is common in pheochromocytoma and especially in paraganglioma. We advocate screening all pheochromocytomas and paragangliomas for SDH-deficiency using SDHB IHC, as the identification of SDH-deficiency should direct genetic counseling and may have prognostic significance.
Validation of Linearly Ramped Protocol for the Determination of Lactate Threshold
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University of Iowa Sports Medicine

Background/Rationale: Lactate threshold is a tool that has been utilized for performance and training purposes by many endurance athletes. The lactate threshold, or maximal lactate steady state, is the exercise intensity at which lactate, a byproduct of anaerobic glycolysis in cells, will unavoidably increase in the blood stream as a result of anaerobic glycolysis in cells. This is due to the rate of glycolysis outpacing the rate of oxidative phosphorylation. Lactate threshold can be determined by measuring blood lactate through a linear (graded load) protocol, a stepwise (incremental) protocol, or a constant output protocol. For the direct measurement of blood lactate, the stepwise protocol is the most common approach found in existing literature. Few studies have been performed utilizing a linear ramp. In addition, heart rate and rate of perceived exertion inflection points are commonly used as non-invasive methods of determining lactate threshold.

Purpose of the study: This study aims to validate a linear ramp protocol lactate threshold test that will be used in an upcoming study of recovery aids. We compared the lactate threshold determined by a linearly ramped cycling protocol to a stepwise protocol used in previous recovery aid studies. As a secondary aim, we assessed whether heart rate and Relative Perceived Exertion (RPE) could be used as non-invasive methods to determine lactate threshold for both the linearly ramped and the stepwise protocols.

Method: Six well-trained cyclists performed the linear and stepwise protocols in a randomized order. The trials consisted of either the linear ramp of 1W/kg every 10 minutes until exhaustion or the stepwise ramp of 50 W increases every 2 minutes until failure. All testing was performed using a cycling ergometer (Wahoo KICKR). Blood lactate, heart rate, relative perceived exertion were recorded at fixed intervals. Lactate threshold was determined through the inspection method.

Results: The mean lactate threshold determined by linear ramp protocol (236 ± 10 W) and stepwise protocol (254 ± 20 W) were similar. In addition, for both the linearly ramped and stepwise protocols, the mean lactate threshold values determined by inspection of the non-invasive measures (heart rate and RPE) versus workload relationship were significantly lower than those from the blood lactate and workload relationship.

Conclusion/Discussion: The findings of this validation study indicate that a linearly ramped protocol may elicit a similar lactate threshold to the stepwise protocol. In addition, the non-invasive tools of heart rate and relative perceived exertion used to determine lactate threshold underestimated the work rate at which lactate threshold was reached. This suggests that these non-invasive measures do not accurately determine lactate threshold and athletes should consider alternative measurements of lactate threshold when being utilized for training purposes.
Why can’t patients with albinism see? Electrophysiology and structure of the central retina in albinism

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BACKGROUND: Albinism is a congenital condition characterized by complete or partial absence of melanin pigmentation in the skin, hair and eyes. Most patients with albinism are legally blind (less than 20/200 vision). In addition to reduced visual acuity, nystagmus, strabismus, photophobia, decreased stereopsis, and amblyopia are usually present. Treatment is supportive since there is no way to improve the structure or function of the retina when they have formed incorrectly in utero. A pathognomonic feature of albinism is foveal hypoplasia, a mal-development of the macula in the central retina that can be detected on optical coherence tomography (OCT). The correlation between macular function, as measured by electrophysiology, and structure, as measured by OCT, is unknown in albinism. Understanding this relationship will offer clues to therapeutic strategies to improve vision in these patients.

AIMS: Multifocal ERG (mfERG) and full field ERG (ffERG) will be used to measure and compare retinal electrical activity in patients with albinism and normal controls and to explore function-morphology-genotype correlation. The same hypothesis will be tested in a mouse model of albinism using the full field ERG, OCT and histology.

HYPOTHESES: The mfERG will be abnormal in patients with albinism and will inversely correlate with degree of mal-development of the fovea on OCT. The mfERG will show lower amplitudes in patients with albinism compared to normal age matched controls. The ffERG will have higher amplitudes in albinism patients than in normal controls.

METHODS: IRB approval was obtained for a prospective study of mfERG and ffERG in patients with albinism and normal controls, and for a retrospective chart review of clinical data. Participation was offered to all patients with albinism presenting to the Department of Ophthalmology and Visual Sciences from June 2014 to July 2015. The control group consisted of healthy individuals in the same age group recruited through a hospital flyer. Retinal function was evaluated by measuring electrical activity in the macula and fovea in response to light stimuli (mfERG and ffERG). Diagnostic OCT results in patients’ records were correlated with ERG and visual acuity. Patients were compared to controls using the nonparametric permutation test.

RESULTS: 12 patients with a molecular genetic and clinical diagnosis of albinism were enrolled (7 males, 5 females, mean age=15.1 yrs). 11 normal controls were enrolled (3 males, 8 females, mean age=28.5 yrs).

- mfERG: 7 out of 12 patients had Ring 1 (central) amplitudes within 1 SD of the normal controls (85+/-24 nV/deg²) in at least one eye, even in the absence of a visible fovea on OCT. All 12 patients had higher amplitudes in the central than peripheral rings. There was no correlation between visual acuity and Ring 1 amplitudes on mfERG. Compared to controls, patients had lower mfERG amplitudes and shorter latencies in the central ring (p=0.004, p=0.0074). Patients and controls were assigned to three age groups (6-15 yrs, 16-25 yrs, and 26-50 yrs). Within each group, patients still had lower mfERG central ring amplitudes than the controls.

- ffERG: In patients, greater central macular thickness (less well developed fovea) correlated with lower Ring 1 and 2 amplitudes on mfERG (p<0.05) and with lower a-wave amplitudes on ffERG in 3.0 light adapted, 0.01 dark adapted and standard combined response conditions (p<0.05), but not the 30 Hz flicker condition. There was no correlation between ffERG amplitudes and visual acuity. Patients averaged higher a-wave amplitudes than controls in all but the 30 Hz flicker condition (p<0.01). Patients had shorter latencies in the scotopic 0.1 condition for a-wave and b-wave (p<0.02). Patients also had higher b-wave amplitudes in the light adapted 3.0 condition (p=0.03).

- In mice with albinism there was no difference in ffERG amplitudes (n=10) or retinal thickness on OCT (n=5) compared to the wild type control. The cone density across the retina was higher in the wild type control than albino (p=0.0004).

CONCLUSIONS: Our study shows for the first time that albinism patients with foveal hypoplasia on OCT still may have normal electrical activity in the central retina. On average, however, the central mfERG amplitudes are lower than normal controls while the ffERG amplitudes are higher. This suggests that more light reaches the retina in albinism patients, increasing ffERG amplitudes, however the central cones cannot respond normally to the detailed stimulus of the mfERG. We found a relationship between mfERG amplitude and OCT, but not visual acuity, which is consistent with previous studies showing no correlation between OCT and visual acuity. However our results are diametrically opposed to two reports of children under anesthesia who had no recordable mfERG spike in the central retina. The etiology of decreased vision in albinism is complex and requires more study, but absence of electrical activity in the central retina is not a proximal cause of vision loss.
“Extra” tubes at UI Health Care: a work in progress. Robert M. Humble, Matthew D. Krasowski. Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242

BACKGROUND
Clinical laboratories frequently receive “extra” tubes of blood, urine, or body fluids. At the time of collection, these specimens have no testing associated with them. This may be done by nursing or phlebotomy to allow for specimens in the event that additional (‘add-on’) testing is later ordered or due to practices such as “drawing the rainbow” (collecting multiple tubes before specific laboratory orders are placed by provider). The primary downside of extra tubes is additional blood loss for the patient. In addition, over a period of time, misuse of extra tubes can result in substantial amounts of biohazardous waste. There is little to no literature investigating extra tube collection and use.

HYPOTHESIS/AIMS
The objective of this study was to analyze patterns of extra tube collection over an extended time period. We also analyzed utilization of extra tubes for add-on testing after implementation of new laboratory information system (LIS) software (Epic Beaker).

METHODS
In this retrospective study at an academic medical center, electronic health records were searched to obtain all extra tube orders that were placed in the time period of April 1, 2004 to June 15, 2015. Add-on orders placed after implementation of Epic Beaker were obtained from August 2, 2014 to June 15, 2015.

RESULTS
During the time period of retrospective study, 745,666 extra tubes were collected on 193,596 patients. The emergency department accounted for 37.5% of extra tubes (n=279,729). Outpatient clinics and services accounted for 32.9% of extra tubes (n=245,246). Inpatient units accounted for 29.4% of extra tubes (n=219,493). Operating rooms accounted for 0.2% of extra tubes (n=1,200). For the period during which we analyzed utilization of extra tubes for add-on testing, 48.6% of add-on orders associated with an extra tube were for chemistry tests and 43.6% were for hematology and coagulation tests. We observed extra tube totals decrease upon implementation of paperless ordering and implementation of a new LIS. In addition, interventions in outpatient cardiology and digestive diseases clinics in 2012 yielded significant decreases in extra tube collection. In the period since implementation of Epic Beaker, 10.7% of extra tubes were used for add-on testing. This translates to 43.8 extra tubes per day, with 4.7 tubes per day used for add-on testing.

DISCUSSION/CONCLUSION
Our study is the first to quantify extra tube collection over a period of several years. In our study, extra tubes are rarely used for any laboratory testing. Our data suggests that individual patient care areas are responsive to intervention by the clinical laboratory to reduce blood and body fluid loss, and to reduce waste of supplies.
GMPPB-Associated Dystroglycanopathy: Emerging Common Variants with Phenotype Correlation
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Background
Mutations in GDP-mannose pyrophosphorylase B (GMPPB), a catalyst for the formation of the sugar donor GDP-mannose, were recently identified as a cause of muscular dystrophy resulting from abnormal glycosylation of α-dystroglycan. To date, only 32 individuals with GMPPB-associated dystroglycanopathy have been reported in literature. We aim to provide further insight into the breadth of clinical manifestations and propose genotype-phenotype relationships based on clinical findings in individuals with emerging common sequence variants in GMPPB.

Methods
Retrospective cohort analysis through the Iowa Wellstone Dystroglycanopathy Natural History Study and outside collaborators.

Results
In this series we identified nine unrelated individuals with GMPPB-associated dystroglycanopathy. The most mildly affected individual has normal strength at twenty-five years of age after initially presenting with hyperCKemia on routine bloodwork, while three severely affected children presented during infancy with motor delay, intellectual disability, and epilepsy. Their current ages range from three to fifty years. All have serum creatine kinase levels exceeding five times the upper range of normal. Muscle biopsies of all individuals are dystrophic with abnormal immunostaining for glycosylated α-dystroglycan. Five novel and six recurring variants in GMPPB were identified.

Conclusion/Discussion
There is a wide range of clinical presentations associated with GMPPB mutations as has been observed in other disorders of O-mannosylation of α-dystroglycan. This cohort, together with previously published cases, allows preliminary genotype-phenotype correlations to be made for the emerging GMPPB common variants c.79G>C (p.D27H) and c.860G>A (p.R287Q). We observe that c.79G>C (p.D27H) is associated with a mild limb-girdle muscular dystrophy phenotype, while c.860G>A (p.R287Q) is associated with a relatively severe congenital muscular dystrophy typically involving brain development. Sixty-six percent of GMPPB families to date have one of these common variants.
Pharmacological Ascorbate Enhances Chemotherapeutics in Gastric Adenocarcinoma


Background and Hypothesis: Gastric adenocarcinoma (GAC) is the fourth most common cause of cancer in the world and is the second leading cause of mortality from cancer. High mortality is secondary to the late-stage diagnosis upon presentation, treatment regimens lacking efficacy, and high metastatic potential. New treatment regimens are needed to target this disease that are both less toxic and more efficacious. Pharmacological ascorbate (high doses of intravenous vitamin C aimed at serum concentrations >10 mM) is selectively cytotoxic to cancer cells by acting as a pro-oxidant by generating hydrogen peroxide ($\text{H}_2\text{O}_2$). This leads to cell death by inducing DNA damage, autophagy, and altering multiple other biochemical pathways essential to cancer cell survival. We hypothesize that pharmacological ascorbate will enhance the cytotoxicity of standard chemotherapeutic drugs currently used to treat GAC.

Methods: Human GAC cell lines MKN-45 and AGS were evaluated for clonogenic survival after treatment with pharmacological ascorbate (0.5-20 mM), cisplatin (0.05-2.5 uM), irinotecan (0.1-5 uM), alone and in combination. The normal human stomach/intestinal cell line, Hs738st/Int, was evaluated for toxicity by pharmacological ascorbate using MTT assay and clonogenic survival.

Results: Pharmacological ascorbate treatment had no effect on the normal cell line Hs738st/Int while selectively decreasing clonogenic survival of AGS and MKN-45 in a dose dependent manner. AGS clonogenic survival was decreased significantly following cisplatin and irinotecan treatment in comparison with cell control at 0.5 uM ($p \leq 0.0001$) and 2.5 uM ($p \leq 0.0001$), respectively. MKN-45 clonogenic survival was significantly decreased after cisplatin and irinotecan treatment at 0.1 uM ($p \leq 0.01$) and 1 uM ($p \leq 0.01$), respectively. Cisplatin and irinotecan combination treatment decreased clonogenic survival 40% ($p \leq 0.0001$) in comparison to cisplatin alone and 57% ($p \leq 0.0001$) when compared to irinotecan alone. When cisplatin and irinotecan were combined with pharmacological ascorbate in the AGS cell line, clonogenic survival was further decreased compared to cisplatin and irinotecan combination therapy (27%, $p \leq 0.0001$). In MKN-45, pharmacological ascorbate in combination with cisplatin was shown to decrease clonogenic survival significantly when compared to single agent treatments with cisplatin or pharmacological ascorbate (32%, $p \leq 0.001$; 31%, $p \leq 0.001$, respectively).

Conclusions: In this study, pharmacologic ascorbate in combination with standard chemotherapeutic treatments increased killing of GAC cells in vitro. These results demonstrate the exciting potential of pharmacological ascorbate use in the treatment of GAC. Further studies include transitioning the combined treatment modality into a mouse model, determining the role of H$_2$O$_2$ and other reactive oxygen species in the chemo-toxic response and combining pharmacological ascorbate with other chemotherapeutics.
The Association of Weight Loss and Cardiometabolic Outcomes in Overweight and Obese Children: A Systematic Review and Meta-regression


Background:

Excess body weight in children is associated with several immediate and long term medical comorbidities. We aimed to identify the degree of reduction in excess body weight associated with cardiometabolic changes (lipid panel, liver function tests, systolic (SBP), diastolic blood pressure (DBP), HgA1C and fasting blood glucose) in overweight and obese children.

Methods:

We included randomized controlled trials (RCTs) and cohort studies that evaluated interventions to treat pediatric obesity (medication, surgery, life style and community based interventions). Studies with less than 6 month follow up duration were excluded. We conducted a comprehensive search of several databases. Two independent reviewers screened and extracted data from eligible studies. We assessed the risk of bias of the included studies using Cochrane risk of bias tool and Newcastle Ottawa Scale. We used a random effects regression model to assess the association between BMI/weight and cardiometabolic changes.

Results:

We included 42 studies (37 RCTs and 5 cohorts) enrolling 3807 patients. Studies had overall moderate to low risk of bias. A one-unit decrease in SBP was significantly associated with a decrease of 0.16 units (p=0.04) and 0.61 units (p=0.05) in BMI and weight, respectively. A one-unit increase in HDL was significantly associated with 0.74 units decrease in weight (p=0.02). A one-unit decrease in triglycerides was significantly associated with 0.1 unit decrease in weight (p=0.03). The remaining associations were not statistically significant.

Conclusions:

Weight reduction in children is associated with significant changes in several cardiometabolic outcomes, particularly SBP, HDL, and triglycerides.
Title: The role of HDAC4 in eating disorders

Mentor’s name: Dr. Michael Lutter

Student’s name: Ian Kidder

Background/rationale: Anorexia nervosa (AN) and bulimia nervosa (BN) are serious eating disorders that affect up to 2-4% of women, yet their etiology remains unknown. Although environment plays a major role in eating disorder development, genetics are thought to be a predisposing factor. Our laboratory previously reported that the A786T mutation in the transcriptional repressor histone deacetylase 4 (HDAC4) is associated with a high risk of developing AN or BN in a large family with multiple members affected by eating disorders. We found that the A786T mutation in HDAC4 is a gain-of-function mutation that increases transcriptional repression of the transcription factor estrogen-related receptor alpha (ESRRA) by HDAC4, which may lead to neuronal dysfunction and altered eating behaviors.

Purpose of the study: To further test this hypothesis, we developed a knock-in mouse model of at the corresponding location in the mouse HDAC4 protein (A778T).

Methods: Knock-in and wild type mice were assigned to either single or group housing and weighed daily. Anxiety-like behaviors were assessed using the elevated plus maze and light/dark boxes. Time spent avoiding open spaces in these tests were used to score level of anxiety. To assess learning and memory, mice were subjected to the Barnes Maze and operant conditioning. Mice in the Barnes Maze were scored based on time spent searching for the exit point in the maze, with reduced time considered increased learning ability and memory. For operant conditioning experiments, mice were scored based on their ability to associate a behavior (lever press) with a stimulus (high fat, high sucrose food pellet) using a fixed ratio regimen.

Results: We found housing- and gender-dependent differences in body weight homeostasis between HDAC4 and wild-type mice. HDAC4 female mice exhibited significantly greater weight gain compared to wild-type littermates under group-housed conditions, but not in single housing. Furthermore, group housed female HDAC4 knock-in mice displayed increased irritability and anxiety-like behaviors, enhanced learning in the Barnes Maze, and decreased operant responding for a high fat diet reward compared to wild-type littermates. No differences in body weight or behavior were observed in male wild-type and HDAC4 knock-in mice.

Conclusion: These results suggest the HDAC4 A778T mutation may promote behavioral changes relevant to the development of eating disorders in female, but not male mice. This mouse model may be useful in future studies of the neurobiology of eating disorders and to identify new treatments.
Type 1 diabetic induced pluripotent stem cell-derived insulin producing cells rapidly correct hyperglycemia in diabetic mice

Emma Killoran, Gohar Manzar, and Nicholas Zavazava

**Background:** A potential treatment for type 1 diabetes (T1D) is replacement of the patient’s destroyed islets with functioning insulin producing cells (IPCs) to restore the ability to regulate and maintain physiological blood glucose levels. Cadaveric islets or whole pancreata can serve as the replacement; however, organ shortage makes this an impractical large-scale solution. Functional IPCs have been made from embryonic stem (ES) cells and, more recently, from induced pluripotent stem (iPS) cells that are able to correct hyperglycemia in diabetic mice. However, the derivation of IPCs from T1D patient-derived IPCs has not been successful.

**Purpose of Study:** We aimed to create functional, glucose-responsive IPCs from human iPS cells derived from T1D patient fibroblasts using a five-stage differentiation protocol that were glucose responsive and able to correct hyperglycemia in diabetic mice.

**Methods:** T1D iPS cells were differentiated into IPCs using a 27-day 5-stage protocol developed in our lab. IPCs were transplanted into diabetic mice on day 27. Mice were treated with streptozotocin to destroy their native β-cells and induce diabetes. Following the streptozotocin treatment, 1.2 million IPCs were transplanted subcutaneously in the shoulder flank of each mouse. Blood glucose levels were measured weekly. IPC-treated mice were subjected to a glucose tolerance test 6-weeks post-transplantation. 9-weeks post-transplantation, organoids were harvested and stained for insulin, glucagon, and somatostatin.

**Results:** In this study, we successfully differentiated T1D patient-derived iPS cells into functional IPCs that, when transplanted, are able to rapidly correct hyperglycemia in diabetic mice. Normoglycemia was achieved in a span of 28 days following IPC treatment. IPC-treated mice were also able to achieve normoglycemia following a supraphysiological glucose challenge showing that these IPCs are able to physiologically respond to glucose *in vivo*. Post-transplantation, these cells form vascularized organoids that have morphological features characteristic of glandular tissue and contain regions that express insulin, demonstrating that iPS cell-derived IPCs can be used as an alternative source of IPCs for the treatment of T1D.

**Conclusion:** T1D iPS-derived IPCs are able to rapidly correct hyperglycemia in diabetic mice in a span of 28 days. These cells are able to restore normoglycemia following supraphysiological glucose challenge. Following transplantation, the IPCs form vascularized organoids that express insulin showing that iPS-derived IPCs have the potential to be used as an alternative source of IPCs.
Interaction of HCN4 and Cav1.3 in SA Node Function
Qaadir (Sheps) King-McAlpin, Mei-lings Joiner, Matteo E. Mangoni, and Amy Lee

ABSTRACT

BACKGROUND: Cav1.3 and HCN4 are ion channels found in the sinoatrial node (SAN), the primary pacemaker of the heart. Both channels are depolarizing to the SAN and play an important role in pacemaking. They are involved in sympathetic β-adrenergic stimulation of heart rate (HR). Epinephrine binds to the β-adrenergic receptor, a G protein coupled receptor, leading to increased HR via mechanisms involving Cav1.3 and HCN4. Mutations in these channels lead to sinus bradycardia and sick sinus syndrome. A better understanding of how these channels function will provide for a better understanding of these diseases that affect HR. The β-adrenergic receptor interacts with both channels. Also, Cav1.3 and HCN4 have similar subcellular localization in SAN cells. Unpublished studies from a collaborator show that beta-adrenergic stimulation results in increased HR in mice with a single KO mutation of either Cav1.3 or HCN4. However, β-adrenergic stimulation of HR is inhibited in double knockout mice. These results suggest that one channel can compensate for the loss of the other. However, a remaining question is the degree of contribution of each channel to beta-adrenergic stimulation in normal hearts. To further understand the function of these two channels in regulating HR, I will show whether they interact in a signaling complex.

HYPOTHESIS: HCN4 and Cav1.3 interact with each other physically or via a protein complex

METHODS: We transformed plasmids containing vectors for the Cav1.3 channel pore forming subunit (a1D), Cav1.3 auxiliary subunits (b3 and a2d1), and wild-type HCN4 channel into E. coli bacteria to grow up sufficient quantities of DNA for transfection. DNA was extracted from bacteria and purified via midi prep. We then separately transfected Cav1.3 a1D and HCN4 DNA into human embryonic kidney (HEK) 293T cells to check for overexpression of each channel independently. After confirmation of overexpression we performed a co-immunoprecipitation (co-IP). Cav1.3 and HCN4 DNA were transfected together in HEK cells. After the cells expressed the channels we solubilized the channels in buffer containing Triton detergent. Following cell lysis we pulled down Cav1.3 using immunoprecipitation (IP). Western blot analysis of HCN4 was used to show if HCN4 interacts with Cav1.3. To control for nonspecific binding of the Cav1.3 antibody to HCN4, we transfected cells with only HCN4 DNA, pulled down with Cav1.3 antibodies, and probed for HCN4.

RESULTS: The co-IP experiments showed that there is an interaction between Cav1.3 and HCN4. Control IPs indicated an absence of non-specific binding between the Cav1.3 antibody and HCN4.

CONCLUSION: My results suggest that Cav1.3 and HCN4 do interact. Future directions for this project will be to show whether the proteins physically interact at a molecular level. It would also be interesting to see how the channels affect each other’s function by looking at the effects of either mutated Cav1.3 or mutated HCN4 in vitro and in vivo.

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Mentor: Dr. Nandakumar Narayanan  
Collaborators: Dr. Jeremy Greenlee, Dr. Kuan-Hua Chen, Eric Emmons, Katrina Okerstrom, Haiming Chen, Dr. YoungCho Kim

Background

Parkinson’s disease (PD) is a neurodegenerative disorder which involves the loss of dopamine in the subthalamic nucleus (STN), a midbrain structure. Common motor symptoms of the disease include rigidity, tremors, and balance issues. PD can also disrupt cognitive functions such as working memory and timing. The neural mechanisms for timing are unclear, and PD patients undergoing deep-brain stimulation are a unique opportunity to study timing in humans at a cellular level.

Purpose

The mechanism of human timing is unclear. This problem is of particular importance because several human diseases, including Parkinson’s disease, involve deficits in timing. The purpose of this experiment was to investigate the mechanism of timing in PD patients, specifically in the STN, during a simple interval timing task in hopes that an electrophysiological biomarker could be identified, aiding physicians in the diagnosis of the disease.

Methods

In this experiment, we employed a simple interval timing task, consisting of 3- and 12-second intervals, while simultaneously recording from the scalp and/or STN using EEG. 12 PD patients and 12 matched controls had EEG data; 8 PD patients were undergoing the first of two operations for unilateral or bilateral STN DBS, and 1 patient had EEG with and without DBS. Time-frequency analysis of the data, performed in MATLAB, included the following: timing behavior, event-related potentials, event-related band power, field-field coherence, spike-field coherence, and offline-sorted neuronal activity.

Results

First, PD patients had impaired timing relative to controls. PD patients also had attenuated ~4 Hz rhythms in frontal cortex. In the STN, PD patients had 4 Hz activity triggered by the cue. Single neurons in STN ‘ramped’, or increased their activity from the instructional cue over the interval until interval end. Field-field coherence of the center frontal scalp electrode versus the most active STN electrode yielded predominantly low-frequency (< 10 Hz) activity. Spike-field coherence of the same electrodes yielded a similar result, with a burst of activity appearing right after stimulus onset in the delta and theta ranges. Finally, 4 Hz activity in frontal cortex was modulated by DBS in one patients

Conclusion

These data demonstrate that 4 Hz oscillations are a mechanism of timing and provide insight into the mechanism of impaired timing in PD patients, and could contribute to neurophysiological biomarkers for this disease.
Role of hypothalamic angiotensin receptors in the hemodynamic and anorexigenic responses to leptin

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ABSTRACT

BACKGROUND: Hyperleptinemia increases the risk of adult hypertension and overreactive sympathetic tone. Recent studies have linked leptin signaling with angiotension receptor expression within the hypothalamus. A transgenic mouse model was developed to better understand and localize this interaction.

HYPOTHESIS: Microinjection of angiotensin II type 1A receptor (AT1A)-flox/flox mice with adeno-associated-virus (AAV) expressing Cre recombinase within the arcuate nucleus of the hypothalamus will suppress AT1A receptor expression and decrease the blood pressure and food intake responses to leptin supplementation.

METHODS: 4 month old male AT1A flox/flox Cre- mice were implanted with carotid radiotelemeters to monitor blood pressure, heart rate, and locomotor activity. After a 7 day recovery period, the protocol of baseline recordings of blood pressure, heart rate, and locomotor activity was recorded and food intake collected. Following baseline measurements, intraperitoneal leptin injections were given twice daily for 3 days while measuring blood pressure and food intake. The same group of mice were microinjected with AAV Cre bilaterally into the ARC. After 7 day recovery period, the same protocol described before was repeated. The brain was harvested for histological analysis.

RESULTS: Compared to baseline values, leptin administration significantly decreased the food intake of AT1A-flox/flox mice, but do not elicit a hypertensive response. Following AAV-Cre microinjection, AT1A receptor mRNA expression was decreased to 23% of baseline values, but the hemodynamic and anorexigenic responses to leptin were unaffected.

CONCLUSION: ARC-specific knock-down of AT1A receptor expression did not significantly alter the hemodynamic or anorexigenic responses to leptin. Our previously investigations revealed enhanced leptin sensitivity in mice with a history of neonatal growth restriction and future investigations will determine if the effect of neonatal GR is mediated by enhancement of an otherwise quiescent leptin-angiotensin interaction.
Can surgeons accurately predict the risk of perioperative complications?
James G. Kohler, Natalie Glass, PhD, Benjamin Miller, MD, MS

Background/rationale: Many clinical factors (age, BMI, co-morbidities, etc.) are known to increase an individual patient’s risk of perioperative complications and hospital readmission. There are risk calculators available that predict a risk of postoperative complications based entirely on objective measurements [1-3], designed as a counseling tool so patients can better assess their potential risks and benefits prior to an intervention. These models, such as the Risk Assessment and Prediction Tool (RAPT) [2-4], have significant strength in identifying very high and low risk patients, but they lose predictive efficacy for patients with intermediate scores. We questioned if a surgeon’s subjective intuition, or their "gut feeling," could provide an additional source of accurate risk assessment in the preoperative setting. Although it is difficult to quantify these subjective measurements, this does not imply that such measurements are not of worth. In fact, a global measurement that combines objective (age, co-morbidities, smoking status etc.) and subjective (personal experience, a “gut feeling”) values, though difficult to explain, will likely improve risk assessments. In a society transitioning more and more towards personalized medicine, it seems appropriate to explore this valuable customized, subjective evaluation to better quantify a patient’s true surgical risk.

HYPOTHESIS: The aim of this study was to assess the accuracy of a surgeon’s subjective clinical intuition in determining a perioperative risk assessment. We hypothesized that preoperatively, surgeons can accurately predict a patients’ perioperative complication risk.

METHODS: We administered surveys to surgeons prior to each operation as means for subjectively evaluating their patient’s risk for post-operative complications (Figure 1). Surgeons scored each patient’s likelihood, on a scale from <1-100, for experiencing a minor medical complication, major medical complication, minor surgical complications, major surgical complication, and unplanned 30-day all-cause readmission. Explanations and examples of the complication categories were provided for congruency across surveyors (Figure 2). Three surgeons were involved in the study, along with 10 associated residents. A total of 89 of 155 patients in the study were evaluated twice, once by the attending surgeon and also by the associated residents, with the others being evaluated only once. Data were collected over a three month period, from February- April 2015. Following a procedure, we searched each patient’s discharge summary and clinic notes within electronic medical records to determine any adverse events and readmissions experienced within the 30 days following surgery. We used simple descriptive analysis to determine association between predictor variables and complication outcomes, utilizing Chi square for categorical and simple t-test for continuous. We then included several clinical variables into a multivariate logistic regression for analysis.

RESULTS: We found that surgeons had the ability to accurately predict the occurrence of minor medical complications, but sample size precluded any testing on the remaining four variables. By using surgeon-predicted complication rate cut-offs of <10% (low risk), 10-40% (intermediate risk), and >40% (high risk), one surgeon patients (BJM) were shown to significantly differ in observed complication rates (Intermediate versus Low: OR= 7.31, CI= 1.05-51.10, p=0.0449. High versus Low: OR= 58.50, CI = 6.88-497.29, p=0.0002. High versus Intermediate: OR= 8.00, CI= 1.00-63.96, p=0.0499). Applying these established cutoffs to a different group of patients and surveyors, these cutoffs were subsequently found to maintain strength and predictive power when identifying low risk patients (High versus Low: OR=5.12±3.89, 95%CI=1.15-22.72, p=0.0318. High versus Medium: OR=1.39±1.04, 95%CI=0.32-6.01, p=0.6561. Medium versus Low: OR=3.67±1.32, 95%CI=1.81-7.44, p=0.0003). When grouping patients from all surveyors into these cutoff ranges, patients had an actual complication risk of 10.9%, 47.4%, and 58.8% for the low, intermediate, and high risk groups respectively.

CONCLUSION: Subjective surgeon intuition was found to be an accurate tool when predicting the occurrence of minor medical complications. Utilizing the preoperative minor medical complication prediction ranges detailed previously, it is possible to effectively segregate low, medium and high risk groups, allowing for better management of preoperative counseling and effectively informing patients and families what to expect following surgery.
Residual Astigmatism After Toric IOL Placement

Student: Brent Kramer, M3
Mentor: Dr. John Berdahl, MD
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Background: Over 70% of patients that undergo cataract surgery have greater than 0.5 diapoters (D) of corneal astigmatism. Toric intraocular lenses (IOLs) give patients with astigmatism a viable option to pursue emmetropia and spectacle independence after surgery. Residual astigmatism is an unsatisfying result for patients who receive toric IOLs. Residual astigmatism after toric IOL placement occurs when the IOL isn't in the ideal position or doesn't have the correct power and therefore doesn't neutralize the corneal astigmatism. Such problems can arise for many reasons: inaccurate measurements, improper IOL placement, surprising surgically induced astigmatism (SIA), disregarded posterior corneal astigmatism, or undesired IOL rotation. To ease the difficulty of calculating the ideal position of toric IOLs for patients with residual astigmatism, a freely available online Toric Results Analyzer (astigmatismfix.com) was developed. Users are able to enter the current measurements of a post-toric IOL patient with residual astigmatism into this online calculator and it returns the ideal location of the toric IOL and the expected residual refraction.

Purpose: Analysis of the Toric Results Analyzer entries was performed to better understand toric IOL rotation and residual astigmatism.

Methods: Retrospective review of 7461 entries with residual astigmatism after toric IOL placement.

Results: In 73% of entries, the ideal axis shifted >5°, and in 64% of the entries, the IOL rotated from its intended location by >5°. Of the IOLs that rotated >5°, the average magnitude of duration was 26.4±21.1°. 42% rotated clockwise, and 57% rotated counterclockwise. After rotation, there was a 19% decrease in horizontally located IOLs, a 9% decrease in vertically located IOLs, and an 84% increase in diagonally located IOLs. Of the 7461 entries, it was determined that rotating the IOL to its ideal axis would decrease astigmatism in 96% of cases, correcting 1.02±0.92D on average (41±30%).

Conclusion: Surgically Rotating toric IOLs in patients with residual astigmatism can significantly decrease the amount of astigmatism. Even when the IOL is in the intended location, significant residual astigmatism can be present, and improved by proper rotation. For this reason, a toric IOL calculator such as the Toric Results Analyzer should be used when managing residual astigmatism in any patient.
Using Sideline Concussion Tests in the Emergency Department
Adam Kruse, MS; Andrew Nugent, MD; Andrew Peterson, MD, MSPH

ABSTRACT

Background: Traumatic brain injuries (TBIs) are a significant cause of death and disability in the U.S. The Center for Disease Control estimates that 1.7 million civilians sustain a TBI each year. While many of these TBIs are treated in Emergency Departments, there is no evidence-based method of detecting or grading TBI in patients who have normal structural neuroimaging. Recently, the University of Iowa has partnered with EPIC Research and Diagnostics (Scottsdale, AZ) to evaluate a device designed to detect abnormalities in cellular respiration and synaptic transmission in the setting of TBI. The project is industry initiated and industry funded.

Purpose: This project was an investigator initiated accompanying study to evaluate the validity of two common sideline concussion tests. The Concussion Symptom Severity Score (CSSS) and Balance Error Scoring System (BESS) tests are well-validated sideline tests for concussion, but have not been validated in the setting of non-sport-related concussion, in settings other than the sideline or athletic training room or in moderate or severe TBI.

Methods: This study used data collected during the industry study to analyze the BESS and CSSS. The industry study’s goal is to enroll 200 subjects: 100 subjects through the University of Iowa’s Emergency Department for potential TBI, and 100 control subjects who have not sustained a TBI. As a part of the industry study, each subject, if able, completed the CSSS and BESS. These CSSS and BESS scores were then analyzed separately from the industry study. All subjects are between the ages of 18-65. TBI subjects sustained their TBI within 72 hours of participating in the study. Physicians rated each of the TBIs as minor, moderate, or severe. Exclusion criteria include: current neurological or psychological disease, history of substance or alcohol abuse, cancer, any electronic device that cannot be removed (pacemaker), missing fingers, pregnancy, hand tremors, long fingernails, or a head injury in the past two years for TBI subjects or any history of TBI for control subjects.

Results: As of 14 August 2015, 21 subjects with a TBI have been enrolled and 31 controls have been enrolled. All of the 21 TBIs, 20 were rated as minor and 1 was moderate. The CSSS was only collected for 20 subjects and only 8 subjects were able to complete the BESS. The CSSS was collected for 31 control subjects and the BESS was conducted for 30 control subjects. The CSSS average for TBI subjects was 23.85 and the CSSS average for control subjects was 0.6452. This resulted in significantly different results with a Confidence Interval of 16.6232 to 29.7865 with a p-value of 4.8968x10⁻⁹. The BESS average for TBI subjects, who were able to complete the BESS, was 6.125 and the BESS average for control subjects was 8.333. These results were not significantly different.

Conclusions: The BESS is not sensitive to diagnosing a TBI in the ED, but the CSSS is sensitive. The majority of TBI patients were not able to perform the BESS, and the TBI subjects who were able to perform the BESS did not score significantly different from the control population. The CSSS scores between the control and TBI groups were significantly different, but the severity of the TBI could not be determined by the CSSS.
Anatomic Outcomes of Posterior Spinal Osteotomies with Anterior Lumbar Interbody Implants for Treatment of Adult Kyphosis and Scoliosis

Stuart Weinstein M.D., Nyle Larson B.Sc.

**Background:** Smith-Peterson osteotomies (SPO) and pedicle subtraction osteotomies (PSO) have been shown to correct lumbar kyphosis and in the last decade asymmetric PSO (APSO) has allowed for coronal correction of scoliosis. These invasive procedures provide good anatomic correction but present patients with a difficult recovery. Strategies that improve osteotomy effectiveness while decreasing invasiveness will result in better outcomes. New technologies in minimally invasive inter-vertebral body implants could be used to provide additive sagittal and coronal correction to osteotomies. So far, no studies have evaluated the results of adding minimally invasive interbody implants to osteotomies.

**Aims:** Compare the effects of APSO and SPO in adults with spinal deformity with a focus on the effect of adding interbody implants prior to osteotomy.

**Methods:** This was a retrospective case-control study of 52 patients treated with APSO compared to 38 patients treated with SPO. Image and data analysis software was used to measure and analyze Cobb angle, C7 plumbline and Distal Fractional angle in the coronal plane and Cobb angle, C7 plumbline, L1-S1, T10-L2, T5-T12 angles, Pelvic Tilt and Pelvic Incidence in the sagittal plane. Data was collected on the vertebral level of osteotomy, previous spinal surgery, timing of lumbar interbody implant surgery and vertebral rotation. Results are expressed as mean (95% Confidence Interval).

**Results:** APSO and SPO provided significant yet similar correction of coronal Cobb angle. APSO accomplished 13.5° (5.8°-21.2°) more correction in sagittal Cobb angle than SPO. APSO also provided 3x more correction per sagittal level than SPO. APSO and SPO patients that had implants placed before osteotomy experienced greater coronal Cobb angle correction than patients with osteotomies alone (APSO: 35.9° vs. 13.6, SPO: 33.7° vs. 22.7), but showed no significant difference in sagittal Cobb angle correction. The level of the APSO had no effect on sagittal Cobb angle correction, but SPO achieved more correction when applied in the thoracic spine (Lum: 9.58°, Thor/Lum: 12.8°, Thor: 17.8°). APSO and SPO patients with no previous spinal surgery experienced greater coronal Cobb angle correction than patients with previous spinal surgery (APSO: 32.5° vs. 19.9°, SPO: 32.1° vs. 15.4°), but had no significant difference in sagittal Cobb angle correction.

**Conclusions:** Adding minimally invasive interbody implants before osteotomy increases the coronal Cobb angle correction in both APSO and SPO but has no significant effect on sagittal Cobb angle correction. Depending on the parameter, APSO offers comparable or greater correction than SPO in the treatment of spinal deformity. Osteotomy patients with previous spinal surgery should not expect as much coronal correction as patients who are undergoing their first spine surgery. Placement of APSO makes no difference on the amount of sagittal Cobb angle correction, while more rostral SPO placement results in more correction.
Irradiance Measurement Abnormalities in Seasonal Affective Disorder

Scott A. Laurenzo, Ashley M. Schumacher, James B. Potash, Stewart Thompson, Randy Kardon, and Jess G. Fiedorowicz

BACKGROUND: Seasonal Affective Disorder (SAD) is a subtype of major depressive disorder, defined by the DSM-V as recurrent major depressive disorder “with seasonal pattern,” (1). Some studies found higher prevalence of SAD at higher latitudes, suggesting that the subtype is a biological vulnerability to respond to the environmental stress of changing light with disordered mood (2, 3). A shortened light/dark cycle increased depressive symptoms in mice due to the influence of intrinsic photosensitive retinal ganglion cells (ipRGCs), which contain the photopigment, melanopsin (4). Melanopsin-mediated pathways respond to blue light, entrains circadian rhythms, and causes a sustained pupil contraction. A red light stimulus will activate ipRGCs via rods and cones with a shorter contraction.

HYPOTHESIS: The pupillary light reflex to blue light will show decreased sustained contraction in participants with major depression, particularly SAD, compared to controls.

METHODS: We recruited participants with unipolar major depression who previously participated in a genetics study of major depression. Diagnosis was confirmed using the Depression and Seasonality Interview-Ontario version. Participants were assessed for seasonality, physical activity, sleep quality, sun exposure, anxiety and depression severity. A Neuroptic binocular pupillometer was used to analyze the participant’s pupillary light reflex. Assessments were made within one month of the winter and summer solstices.

RESULTS: Participants with unipolar disorder (N=19) were a mean (SD) of 47.8 (11.3) years old on study intake and 79% were female. Diagnosis of unipolar major depression was confirmed for all but two participants, one of whom was assessed as having biopolar disorder and the other subthreshold depression. These participants were compared to a group of healthy control participants were compared to a group of healthy control participants (N=11) with a mean age of 46.9 years (82%) female. The results show differences in the seasonal pattern for blue light sustained contraction between the participants with major depression and healthy controls.

CONCLUSIONS: The results indicate the pupillary light reflex may provide a measurable endophenotype that can be used to identify physiological differences in those with mood disorders as they relate to activity of melanopsin-mediated pathways, circadian rhythms and seasonality. Future studies can examine this association in order to help create better diagnostic measures, research tools, and treatments of the disease.

References
Co-localization of serotonin (5-HT) and thyrotropin-releasing hormone (TRH) within neurons of the ventral medulla

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Background: Serotonergic neurons within the ventral medulla are organized into four groups and some are chemosensitive to changes in blood CO₂ concentration. When there is a disruption in the network of 5-HT neurons Sudden Infant Death Syndrome (SIDS) and Sudden Unexpected Death in Epilepsy (SUDEP) may occur, in which there are problems in coordination of respiratory output with changes in sleep state and CO₂/pH levels. A subset of 5-HT neurons is known to co-localize and release TRH, which is a potent respiratory stimulant. It is possible that only certain groups of 5-HT/TRH neurons are chemosensitive to specific stimuli and send axonal projections to excite downstream respiratory neurons. The above disorders may involve dysfunction in a certain group or specific downstream projections. However, it is not known the presence or extent that TRH is co-localized within the 5-HT groups of the medulla or the respiratory groups that receive TRH axonal projections.

Aim and Hypothesis: Here we sought to identify the percentage of 5-HT neurons that co-localize TRH in each 5-HT cell group in the medulla, as well as the areas to which they send axonal projections. We hypothesized that 5-HT neurons co-localizing TRH (5-HT/TRH neurons) would comprise <5% of 5-HT neurons and that these neurons send projections to all the respiratory neurons of the lower brainstem: the retrotrapezoid nucleus (RTN), nucleus tractus solitarius (NTS), nucleus ambiguous, and pre-Bötzinger complex.

Methods: In transgenic reporter mice, the enhancer region for Pet1 was used to drive expression of cre recombinase (ePet-cre) in mice carrying an allele for tdTomato flanked by loxP sites. The promoter for TRH was used to drive expression of green fluorescent protein (GFP). The transgenic fluorescent reporter mice were used to analyze the brainstem distribution of 5-HT neurons (ePet-cre/tdTomato/TRH-GFP; n=4 mice) and to determine projections of 5-HT/TRH neurons to respiratory neuron groups (Phox2b-tdTomato/TRH-GFP; n=1 mouse). Mice underwent cardiac perfusion with 4% paraformaldehyde (PFA), and brains were isolated and stored in 4% PFA overnight. The brains were then immersed in 30% sucrose solution until being sectioned at 20 microns. Sections were analyzed on a Leica SP5 confocal microscope, with brainstem distribution determined by counting neurons in one 20 micron section at each location referenced in a mouse brain atlas (Paxinos & Watson, 2007) from Bregma -7.76 to -6.00 mm. Chemosensitivity of 5-HT/TRH neurons was also investigated with culture and medullary slices from 5-HT/TRH neurons. Under perforated patch clamp electrophysiology the change in firing rate was characterized with exposure to acidosis (pH 7.4 to 7.2), which would occur with an increase in blood PCO₂.

Results: 5-HT/TRH neurons comprised 4.36±0.94% of 5-HT neurons within the ventral medulla from Bregma -7.76 to -6.00 mm in the parapyramidal region, raphe pallidus, and raphe magnus. No 5-HT/TRH neurons were found in the raphe obscurus. The parapyramidal region contained the highest concentration of TRH neurons (4.87±1.59%, n=4 mice, p=0.05). In the rostral medulla, rostral to Bregma -6.85 mm, 5-HT/TRH neurons were equally distributed throughout the 5-HT cell groups: the parapyramidal region, raphe pallidus, and raphe magnus (p=0.1). In contrast, in the posterior medulla caudal to Bregma -6.85 mm, 5-HT/TRH neurons were found almost exclusively in the parapyramidal region (TRH was present in 7.53±3.09% of 5-HT neurons, n=4 mice, p=0.015). Projections of 5-HT/TRH neurons were found in the NTS, RTN, nucleus ambiguous, and near where the pre-Bötzinger complex is expected to be.

Conclusions: 5-HT neurons co-localizing TRH comprise <5% of 5-HT neurons within the ventral medulla and respond by increasing their firing rate to physiologic acidosis. These cells send axon projections to downstream respiratory nuclei in the medulla: the RTN, NTS, nucleus ambiguous, and region of the pre-Bötzinger complex. Therefore, we show that TRH-containing neurons are ideally suited to sense blood acidosis when breathing is reduced, and TRH neurons have the axonal circuitry to excite neurons needed to increase breathing. TRH axon projections were found in all respiratory nuclei, but the greater concentration in the parapyramidal region shows many 5-HT/TRH neurons may be ideally suited to maintain respiration through excitation of RTN neurons. Future directions will address the density of projections to the various respiratory nuclei, which will determine if certain regions receive more input from 5-HT/TRH neurons. Other future experiments will use optogenetics to selectively activate 5-HT/TRH neurons and measure the change in ventilation with plethysmography, as well as determining the effect 5-HT/TRH neurons have on respiratory neurons using patch clamp recordings.
Structure/Function correlation of Optical Coherence Tomography in the Central and Peripheral Visual Fields
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Introduction: Glaucoma is one of the leading causes of blindness worldwide. Perimetry, the quantification of the visual field, is used to both diagnose and monitor the progression of glaucoma. Currently, nearly all static automated perimetry testing investigates the central 24 or 30 degrees of one’s visual field, and uses size III (0.43 degree diameter) stimuli. Although this method is the clinical standard, a 20-30% loss of optic nerve fibers can remain undetected when focusing on this area of the visual field. The peripheral visual field, representing over 3 times the territory currently being evaluated, remains largely unexplored. Past constraints to testing the far periphery have been high variability in responses and the length of time needed to administer the test seen with size III stimuli. Previous work by the Wall laboratory at the University of Iowa showed that using a larger, size V (1.72 °) stimuli is easier for subjects to attend to and decreases retest variability in the periphery. Also used clinically is Optical Coherence Tomography (OCT) to evaluate structural changes within the eye. Together, OCT and perimetry data allow analysis of structure/function correlations. Areas of defect most frequently damaged by glaucoma as shown by OCT have been shown to cluster in three regions: 1) the temporal portion of the superior optic disc; 2) the temporal portion of the inferior optic disc; 3) the border of the nasal and superior quadrants. Thinning in the location of the supero-nasal optic disc are thought to fall outside the central visual field, which may mean that testing the peripheral visual field may offer a more comprehensive visual assessment for those with glaucoma.

Purpose
The peripheral visual field is largely unexplored in static automated perimetry. Consequently, the correlation between OCT structural data and function in the peripheral visual field hasn’t been possible. The aim of this study is to determine whether correlation exists between the Peripheral visual field and OCT to gain a more comprehensive assessment for those with glaucoma.

Methods: The Open Perimetry Interface, a recently evolved programming language run through the R-based computer software, was used to implement perimetry testing on the Octopus Perimeter. Initial efforts were focused on optimizing the OPI computer code in order to achieve a user-friendly perimetry test. Changes to the code involved implementation of a practice test for new testers, output file format, a window to pause the test if necessary, as well as many other subtleties. All coding changes were done through collaboration with Andrew Turpin’s team in Melbourne, Australia. Proper perimetry instruction was also learned and practiced prior to the start of testing. Next, 45 oculard-healthy control subjects were recruited to assemble a normative database for the Octopus Perimeter. Control subjects completed 2 visits where they were given 5 perimetry tests: 30-2 size III, 30-2 size V, 30-1 size V, peripheral size V, and peripheral size VI. 9 glaucoma subjects were also recruited. Subjects had to have mild vision loss (mean deviation < -4) on a SITA standard test for study entry, and were then given 4 perimetry tests: 30-2 size III, 30-2 size V, peripheral size V, and 30-2 size III SITA standard. Subjects were also tested on the Cirrus OCT instrument that was used to measure retinal nerve fiber layer (RNFL) thickness and ganglion cell layer thickness. The normative database was then created and used to analyze abnormal test locations of glaucoma subjects falling in the 5th, 2nd, 1st, and 0.5 percentile levels. Ophthalmologists at the University of Iowa qualitatively analyzed the OCT and visual fields for glaucoma subjects to determine how well OCT data correlated with central and peripheral visual fields. Qualitative grades of poor, fair, good, and excellent were assigned to each type of test based on how well they correlated with OCT RNFL thickness and ganglion cell thickness. Values were then assigned to each correlation grade to compare the success of test types (poor=1, fair=2, good=3, excellent=4).

Results: OCT data correlated best with the peripheral V test for 3 glaucoma subjects. Central visual field tests correlated best for 4 glaucoma subjects. On 2 occasions, central and peripheral tests correlated equally well with OCT data. Assigning values to each grade of correlation and summing the numbers for the 9 comparisons yielded the following results: SITA 30-2 III = 23, Peripheral V = 25, 30-2 III = 26, 30-2 V = 27

Conclusion: For the 9 glaucoma subjects analyzed, 30-2 V testing correlated the best with OCT data, reaffirming previous work in Dr. Wall’s laboratory showing that using size V stimuli is reliable in perimetry. Additionally, the peripheral V test correlated best with OCT data on 3 occasions, suggesting that the outer visual field periphery may be useful for clinical testing.
Conjugation of Pathogen Associated Molecular Pattern Molecules to Radiation Attenuated Plasmodium Sporozoites Enhances CD8+ T Cell Responses

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**Mentor:** John Harty, PhD  
**Additional Collaborators:** Samarchith Kurup, DVM, PhD

**Background/Rationale:** Malaria is a highly prevalent tropical disease caused by the protozoan parasite *Plasmodium spp.* In 2013 malaria caused an estimated 198 million clinical cases and over 500,000 deaths, mostly of children. Anti-malarial drugs are not a long-term solution because of associated safety risks and constantly developing drug resistance. This makes an effective anti-malarial vaccine a superior method to contain malaria. However, developing a malaria vaccine that provides long lasting, sterilizing immunity has proved difficult. Even for short lived protective immunity, vaccines require frequent high doses of live-attenuated parasites.

Pathogen associated molecular patterns (PAMPs) are molecules the immune system recognizes as a sign of a foreign presence. PAMPs induce strong innate immune responses, proportional to the amount that enters the body. Strong innate immune responses are required for strong adaptive immune responses. An effective vaccine contains an adjuvant, which includes PAMPs, that stimulates strong innate immune responses.

Radiation Attenuated Sporozoites (RAS) are made by irradiating the infectious stage of the malaria parasite and serve as a vaccine against the infection—likely the best available immunization against malaria to date. The RAS vaccine induces an abortive infection in humans, and induce the adaptive immune responses that help contain a subsequent challenge. Unfortunately, the RAS vaccine must be administered in frequent high doses and generates only short-term protective immunity against malaria. We surmised that this is because the RAS vaccine does not sufficiently stimulate the innate immune system. Previous work with the protozoan parasite *Trypanosoma cruzi* has suggested that conjugating PAMPs to its surface elicits greater adaptive responses after infection.

**Hypothesis:** Conjugation of a PAMP to radiation attenuated sporozoites will stimulate stronger adaptive immune responses in the murine model.

**Aims:** Examine adaptive immune responses following injection of a RAS-PAMP conjugate. Test different ways of optimizing the conjugation protocol.

**Method:** We used two procedurally distinct methods to conjugate CpG Oligodeoxynucleotide (ODN), a bacterial DNA motif and a strong PAMP, to the surface of RAS through synthetic glycolipid glycoprophatidylinositol (GPI) anchors, and injected them into BL/6 mice. The “pre-assembly” method assembles the PAMP complex separately and adds it to RAS just before inoculation, whereas the more time consuming “sequential assembly” assembles the complex on RAS in a step-by-step manner. Blood was collected and CD8+ T cell response to GAP50, a representative *Plasmodium*-specific antigen, was analyzed as a surrogate for adaptive immune responses.

**Results:** The “sequential assembly” method induced greater (p≤0.05) CD8 T cell responses at days 6, 9 and 12 post infection. The “preassembly” method did not induce greater CD8 T cell responses at any of these time points.

**Conclusion/Discussion:** Conjugation of a strong PAMP to the surface of parasites used in a malaria vaccine improved the adaptive immune response induced by the vaccine. Of the two methods tested, only the sequential assembly method improved the immune response. Further optimization should be done to discover more efficient ways of constructing the vaccine. Adjustable factors include dose, the kind of PAMP molecule, and the route of inoculation.
The Role of Inflammasomes in Bacterial Co-Infection in Cutaneous Leishmaniasis
Tiffany Lim, Gwendolyn Clay, Fayyaz Sutterwala, and Mary Wilson

Leishmaniasis is a collection of human protozoan diseases that are introduced into mammalian hosts through the bite of a sand fly carrying *Leishmania* spp. parasites. Cutaneous leishmaniasis (CL), the most common form of leishmaniasis, causes a localized skin lesion often followed by ulceration, characterized by a vigorous inflammatory response. There is evidence that ulceration is essential for response to treatment and cure, suggesting that factors leading to or resulting from ulceration are necessary for CL healing. However, these ulceration-associated factors are unexplored. Ulceration introduces skin microbiota into subdermal layers, and secondary bacterial infections are common CL complications. This led us to hypothesize that bacterial effects are critical determinants of CL outcome.

Inflammasomes are multi-protein complexes that form within innate immune cells in response to intracellular “danger” signals, leading to the secretion of pro-inflammatory cytokines IL-1β and IL-18. Inflammasomes respond to skin microbiota and have been implicated in the pathology of many inflammatory diseases. Inflammasome formation requires association of an NLR protein with the adaptor ASC protein as part of the inflammasome complex. Although our initial studies suggest *Leishmania major* does not directly activate inflammasomes, skin microbiota, such as *Staphylococcus aureus*, activate the NLRP3 inflammasome. *S. aureus* is often found in CL lesions, but it is unknown how skin microbiota might alter cutaneous *L. major* infection. Therefore, we hypothesized that bacteria present during *L. major* parasite inoculation and lesion ulceration activate inflammasomes, which augment local inflammation and contribute to host control of the parasitic infection.

To examine this hypothesis, we performed intradermal injections of *L. major, S. aureus*, or both in the ears of ASC−/− or wild-type (WT) C57BL/6 mice. We monitored ear inflammation and microbial burden by lesion area, histology, limiting dilution (Sa) and qPCR (Lm). Our data showed that: (1) lack of ASC showed few differences in *L. major* lesions; (2) lack of ASC showed differences in pathology during *S. aureus* infection compared to WT; (3) co-infection increased the size of 11-day *S. aureus* lesions in ASC−/− mice compared to WT; and (4) co-infection decreased parasite burden by 11-days post-infection. Overall, these data suggest that co-infection with *S. aureus* increases inflammation, which contributes to host control of *L. major* infection in WT and ASC−/− mice. Identifying a role for inflammasomes activated by skin microbiota in the development of leishmaniasis pathology could lead to novel therapeutic approaches for parasitic infection.
Acute effects of THC on the Brain Reward System of Healthy and Schizophrenic Individuals
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Background
It is well known that there is a high incidence of cannabis use among schizophrenics, and it has also been demonstrated that there are abnormalities in the reward system of schizophrenics. The effects of tetrahydrocannabinol (THC), the psychoactive constituent of cannabis, on the brain reward system of schizophrenics, however, have not been well studied. This study aims to obtain preliminary data on the effects of acute cannabis use on region cerebral blood flow (rCBF) and brain activation while performing a reward task which is widely used to assess reward and punishment, in 10 healthy and 10 schizophrenic individuals.

Hypothesis
We predicted that we would observe significant differences in rCBF and brain activation in areas associated with the brain reward system (ventral striatum, nucleus accumbens and prefrontal cortex) between healthy and schizophrenic unintoxicated subjects, and these differences would be more pronounced while under the influence of cannabis.

Methods.
Subjects with a diagnosis of schizophrenia or schizoaffective disorder who were chronic cannabis users (defined as using cannabis at least 4 times a month for 2 years) were recruited first. Healthy controls were then recruited to match on the basis of age, gender and current and past use of cannabis. rCBF was measured using Positron Emission Tomography (PET) with oxygen[15] water. The imaging session consisted of 8 PET image acquisition conditions performed during a monetary reward task where subjects were able to win real money (between $0.20 and $5.00 per trial). The first four conditions were done after smoking a placebo cannabis cigarette (cannabis without THC) and the second four were done after smoking a cannabis cigarette containing a calibrated amount of THC (24.9mg or 44.7mg) based on the subjects’ current use. After smoking each cigarette, the first conditions were a reading baseline, followed by two consecutive reward tasks and then another reading baseline task. All baseline and reward conditions were averaged and the baselines were subtracted from the rewards for analysis. Brain activation while performing a version of the same reward task, was measured using functional magnetic resonance imaging (fMRI) on a different day, when subjects were asked to abstain from cannabis use overnight. All images were analyzed using AFNI software.

Results
The PET rCBF imaging data showed a difference in activation patterns between control and schizophrenic subjects under both the placebo and THC treatments. Brain regions typically associated with the reward system, however, were not activated in either group. The fMRI imaging data showed activation in the ventral striatum, as expected, in control subjects only. Several areas in the cerebellum were activated in the schizophrenic group but not in the control group.

Conclusions
While the results observed during PET imaging were not what we had predicted, the fMRI results showed the expected pattern of reward activations in the non-schizophrenic subjects. If this protocol is to be used in future PET studies, the reward magnitude, reward intervals, or image acquisition time points may need to be adjusted to obtain images that will be useful in assessing the acute effects of THC on the reward system. Another constraining factor in this study is the small sample size; perhaps with a larger sample size the expected results would have been obtained. The observed differences in the fMRI data, however, lend some insight into potential areas of interest for future studies.
The efficacy of propofol as a replacement for amobarbital in intracarotid Wada testing

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ABSTRACT

BACKGROUND: The intracarotid sodium amytal procedure (more commonly known as the Wada test) has for many years been considered the gold standard for language and memory lateralization. It remains an important part of the pre-surgical workup for patients with medically intractable seizures. In this procedure, one hemisphere of the brain is temporarily sedated, while the patient undergoes cognitive testing to determine capacities of the non-sedated hemisphere. After a period of drug washout the procedure is repeated in the other hemisphere. Due to recent worldwide shortages in the key sedative (amobarbital), neuropsychologists and neurologists have turned to alternatives, such as propofol. Several small studies have supported the use of propofol in the Wada, although a few reported troubling adverse effects associated with using propofol.

AIMS: There were two aims to our study: (1) to investigate the efficacy of propofol relative to amobarbital with respect to identifying language and memory lateralization; (2) to investigate the rate of complications with propofol relative to amobarbital in the Wada examination.

METHODS: We performed a retrospective review of all Wada procedures done at University of Iowa Hospitals and Clinics from 2007 through mid-2015. Of 97 total cases, the first 49 cases received amytal, and the subsequent 48 cases received propofol. No patients were excluded from analysis and all were candidates for resective surgery. All data were analyzed using SPSS statistical software. The major variables of interest were whether language and memory were able to be confidently lateralized by the examining neuropsychologist, and whether the patient experienced complications during the test. Complications (e.g., obtundation, inadequate drug effect) were graded 0-3 depending on severity.

RESULTS: There were no demographic differences between groups. Findings indicated that successful lateralization rates were statistically similar for language (amytal=93.9%; propofol=93.8%) as well as memory (amytal=73.5%; propofol=66.7%). Regarding complications, rates of experiencing a significant (grade 2-3) complication were similar between groups (amytal=30.6%, propofol=33.3%), and similar rates of patients in each group went on the have the resective surgery (amytal=73.5%; propofol=68.7%).

CONCLUSIONS: These findings support previous studies indicating that propofol is as efficacious as amobarbital, and can continue to be used in Wada procedures with confidence.
An In-Depth Review of 368 Errors in Radiology

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ABSTRACT

BACKGROUND: Errors in medicine have become more notable in recent years, specifically in diagnostic radiology. The role of a diagnostic radiologist is to completely detect all abnormalities in an imaging examination along with an accurate diagnosis. An Error occurs when the observer fails to reach the same conclusion that would be reached by a group of expert observers with no dispute on the correct interpretation. Determining the errors that occur in diagnostic radiology at the University of Iowa Hospitals and Clinics can provide us with an opportunity to build protocols to correct for these types of error. Implementing these changes will likely increase the efficacy of the health care system by improving patient care.

HYPOTHESIS: Types of specific error rates in radiology have changed in the past 20 years.

METHODS: This project reviewed 368 errors in radiology that were reported at morbidity and mortality conferences at the University of Iowa Hospitals and Clinics Department of Radiology from 2009-2014. The errors were classified based on a scheme developed to separate the errors into 4 major error groups; perceptual/decision making, communication, misinterpretation/classification, and technique/procedure. Each of these major groups has multiple subsets of errors. The errors were then compared to a similar study done by Renfrew in 1992 at the University of Iowa Department of radiology to observe the changes in incidences of different types of errors.

RESULTS: It was found that perceptual/decision making errors are statistically more common type of error now than they were in the past; specifically false negative errors resulting from lack of knowledge or under-reading; however, false positives and no specific/other causes of false negatives were found to be more common type of error in 1992. Misinterpretation/classification errors also tended to occur more often in 1992.

CONCLUSIONS: The differences in the rates of specific errors were potentially triggered by the improvement in imaging technology. This likely resulted in the most common error being due to observer error, since the brain and eye are not able to improve to the same extent as the imaging technology. This project brought about a better understanding of what errors are taking place at the University of Iowa Department of Radiology and potential ways to improve the protocol to prevent them from reoccurring.
Injuries in Youth Tackle Football Are More Severe Than Flag Football
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Background: Around 2.8 million children from ages 7 to 14 years play youth football. Sports-related injuries are the leading cause of injury in adolescents. The literature on injury risk and associated missed playing time in youth tackle and flag football leagues is scant and inconsistent. Previous studies have shown a range of injury rates from 8.5/1000 to 43/1000 athletic exposures (a game or practice). Many of these studies have funding conflicts of interest. Little data is available regarding time to return to play (RTP) after injury. Recent research has found a similar concussion rate and maximal force head impact in youth football as high school and college levels. There is not currently sufficient evidence that flag football is a safer alternative to tackle.

Hypothesis: Youth flag football players return from injuries sooner than players in tackle football leagues. Concussions, compared to other injuries, occur more often in games than practice and to older players.

Methods: In this prospective cohort study, the research team has provided two tackle football leagues and one flag league with an electronic attendance and injury reporting form and database to replace their previous attendance and injury tracking methods. The leagues have access to player-specific data, but data is de-identified before research team members view it. The form tracks many factors including: attendance, player age and grade, type of league (tackle or flag), diagnosis or suspected diagnosis of injury, player position, setting of injury (game or practice), and playing time lost before RTP. Factors impacting RTP were analyzed using Kaplan-Meier survival curves. Likelihood of concussion was analyzed with conditional probability.

Results: Flag football players returned to play much more quickly than tackle players, with 50% of flag players returning from injury the first day after, whereas 50% of tackle players did not return until 7 days after injury. All flag football players returned in 2 days or less, while nearly 10% of tackle players had not returned 50 days post injury. Over 80% of players suffering severe injuries (projected loss of time over 7 days, concussions and fractures) missed more time than those with minor injuries, though the last 15% to return from both injury types returned after similar time. Players with concussions returned on par or sooner than players with other severe injuries, but had longer RTP than those with minor injuries. Given a player was injured, those in 6th grade and above had a higher likelihood of that injury being a concussion than younger players, additionally injuries suffered in games were more likely to be concussions than those that occurred in practices. There was a trend towards more concussions in flag football, but this trend did not reach statistical significance.

Conclusions: This project is the largest ever cohort study of youth football injuries. A second season of data collection is ongoing. Although another branch of this study found a higher overall rate of injury in flag football, tackle football has more severe injuries that cause players to return to play later. Older players and players in games were more likely for an injury they suffered to be a concussion, compared to younger players and players in practices, possibly due to stronger impacts. These results represent a dilemma for parents choosing flag football as a safer alternative, as injuries are more common in flag football, but are less likely to be severe.
Service Referral Rates and Their Determinants in Trauma-Exposed Children and Their Caretakers
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Background
Adverse childhood experiences (ACEs) have been identified as important potential predictors of a person’s lifetime health trajectory. The negative outcomes for individuals exposed to ACEs include behavioral problems, difficulties in interpersonal relationships, and impaired cognitive capacities as children, as well as adoption of health risk and poor parenting behaviors and the development of both chronic and acute diseases as adults. Adverse childhood experiences are highly prevalent in the United States, with multiple large analyses finding that between 55% and 59% of respondents had experienced one or more ACE. In order to prevent the reported negative outcomes, a specialized model of assessment and service provision called trauma-informed care (TIC) has been developed. A major function of TIC is the ability to accurately assess the need for and make appropriate referrals to services for individuals exposed to trauma.

Hypothesis/Aims
Thus far, there have been few studies examining the best way to provide TIC. In this study, we hoped to determine which factors influence the successful provision of TIC in terms of making appropriate and necessary referrals to services. We hypothesized that practitioners will be better able to make proper referrals to services for individuals seen in the outpatient Child Assessment Clinic (CAC) as compared to those seen as inpatients, and will also be better able to make referrals for those seen as outpatients in 2015 as compared to outpatients seen in 2014. Demographic factors, such as race and gender, should not influence referral rates.

Method
This is a retrospective chart review of 165 children evaluated for child abuse and/or neglect during a 1.5-year period from January 1, 2014 to June 5th, 2015 at a large tertiary hospital’s inpatient child abuse consultation service and associated Child Assessment Clinic (CAC). The following information was collected, when available: demographic information, trauma history of the child and of their caregiver(s), resiliency factors of the child’s caregiver(s), family psychosocial functioning information, and data on referrals to services. For 2015 outpatient CAC cases, this information was obtained via review of four questionnaires that were utilized as part of a full scope TIC implementation process. For 2014 outpatient CAC cases and 2014-2015 inpatient cases, trauma and psychosocial history and data on referrals to services were obtained via provider note review; these cases had little available information on resiliency factors and family psychosocial functioning. Data were selectively grouped and statistical analysis was performed using SPSS software.

Results
The average number of referrals for this population was 2.64 (SD=2.07). Of all patients evaluated, 86.1% (95.1% of outpatients and 72.1% of inpatients) received at least one referral. Group membership (outpatient=Group 1, inpatient=Group 2) was negatively correlated with referral category (categories were 0, 1-3, 4-6, or 7-9 referrals) by a factor of -.285 (p<0.01). Thus, in our sample, the referral rate for the inpatient group members was significantly lower than it was for the outpatient group members. An ANOVA was performed and was also significant at a p<0.01 level. The referral rates for outpatients from 2014 and from 2015 were not significantly different from each other. Similarly, referral rates did not differ significantly among races or genders.

Conclusions
Children evaluated for suspected abuse and neglect and their caregivers displayed psychosocial functional deficiencies in multiple domains, which required referrals to services at a high rate. The recognition of patient and caregiver needs for services and referrals to these services is currently more effective in the outpatient setting, likely due to the amount of staff time and human resources available to evaluate patients in the CAC. Race and gender had no impact on referrals to services in this patient population, meaning all races and both genders had similar needs for services when the common denominator is suspicion of abuse and neglect. Finally, the implementation of new trauma-informed care forms for outpatients seen in 2015 did not significantly change referral rates in the outpatient setting, which is consistent with staff perception that the CAC was already practicing TIC without labeling it as such.
Impaired Mucociliary Transport (MCT) in Newborn CF Pigs: pH Sensitivity and Interrogation of the Gland-Surface Interface
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INTRODUCTION: Cystic fibrosis (CF) is a life-shortening disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel that conducts chloride and bicarbonate. CF affects many organ systems, but airway disease is the leading cause of morbidity and mortality. CF is characterized by recurrent cycles of infection and inflammation, production of thick, viscous mucus and defective mucociliary transport (MCT). To study pathogenesis of CF airway disease, we generated CFTR knockout pigs that have normal lungs at birth but go on to develop an airway phenotype like that seen in humans with CF. Newborn CF pigs have acidified airway surface liquid (ASL) that impairs bacterial killing and also increases ASL viscosity, a function of the secreted airway mucus. In both humans and pigs with CF, the mucus is viscous and sticky. In newborn CF pigs, there is a MCT defect where strands of mucus fail to clear the airway and appear tethered to the ducts of submucosal glands. Mucus is a polymer gel with physical properties dictated by the molecular structure. Therefore, determining if there are differences in the structural conformation of CF and non-CF mucus and if the structure is modulated by pH will help in understanding the pathogenesis of disease and developing possible therapies.

HYPOTHESIS: We hypothesize that CF mucus will have a different structural conformation than non-CF mucus and that the mucus will be pH sensitive.

METHOD: Mucus was collected from sedated CF and non-CF pigs maintained in a humidified chamber. After stimulation of tracheal secretion with acetyl-β-methylcholine chloride (Sigma), we extracted mucus through a small tracheal incision using a polyester swab. Samples were diluted 1:100 into HEPES buffered saline solution at either pH 6 or 8. Hydrodynamic radius ($R_n$) measurements were made using a Malvern Zetasizer Nano ZS instrument. Additionally, we used two treatments in vivo, hypertonic saline and tromethamine (THAM) that disrupt mucus in CF patients. Non-CF pigs were administered normal saline, hypertonic saline or THAM intratracheally. Then, the collected mucus was diluted 1:100 and $R_n$ was measured. To increase our confidence that we were studying mucus, we used microscopy to confirm the identity of material collected. We performed transmission electron microscopy (TEM) using a JEOL JEM 1230 electron microscope and prepared our sample by coating onto a carbon film and treating with negative stain. We also stained tracheal secretions with fluorescent lectins, wheat germ agglutinin-rhodamine (Life Technologies) and jacalin-FITC (Vector Labs). Lectins bind to the heavily glycosylated mucins in mucus. Images were acquired on an Olympus Fluoview FV1000 confocal microscope.

RESULTS: Mucus from CF pig airways shows a significant increase in hydrodynamic radius compared to mucus from non-CF pigs at baseline ($p<0.05$). Mucus from non-CF pigs that were treated with hypertonic saline had significantly larger $R_n$ ($p<0.05$) than untreated pigs. Pigs treated with THAM showed a trend for increased $R_n$. Altering the pH of the mucus ex vivo showed no significant difference. TEM of newborn pig tracheal secretions agrees with previously published TEM of mucus samples. Experiments on the secreted and cleared airway mucus also led to investigation of the retained mucus on the airway surface. CF piglet tracheas have substantial mucus retention in the submucosal gland ducts that can extend as strands from the glands to the luminal surface of the trachea. This phenotype can be partially produced in a non-CF piglet by removing ion transport by treating the trachea with bumetanide to block the NKCC channel in a bicarbonate free solution at pH 6.

CONCLUSIONS: Mucus is an essential component of normal airway defense. The size differences between CF and non-CF mucus at baseline could suggest that CF mucus has different physical properties that could include electrostatic interactions, hydrogen bonding and disulfide bonding. The increase in $R_n$ in hypertonic saline administration and the trend of increasing $R_n$ in THAM administration could suggest that formation of the mucus polymer has temporal sensitivity as it enters the airway surface. This may also suggest that electrostatic interactions play a role in the MCT defect. The ability to detect retained mucus at the gland duct and into the gland structure could provide a new assay to further understand the mechanism of impaired MCT. Our studies provide a basis to investigate and improve current therapies such as hypertonic saline, THAM and others.
Determining the Prevalence and Costs of Unnecessary Referrals in Adolescent Idiopathic Scoliosis

Thomas Meirick B.A., Apurva Shah M.D., Lori Dolan Ph.D., and Stuart Weinstein M.D.

Background

Adolescent Idiopathic Scoliosis (AIS) is a condition that has been associated with many unnecessary referrals to pediatric orthopaedic surgeons (1). In 2004, the U.S. Preventative Services Task Force advised against school screening due to a lack of evidence that preventative measures stopped AIS curves from reaching a surgical threshold, in addition to the harms and costs of unnecessary referrals associated with screening (2). Since that time, the effectiveness of bracing has been proven and leaders in the field have called for the USPTF to revisit their recommendation (3,4). With increasing evidence in favor of bracing as a preventative treatment, the cost-effectiveness of screening will likely be reexamined. However, in order to do so, the costs of unnecessary referrals must be known but they have yet to be determined.

Aims

The purpose of this study was to determine the prevalence of unnecessary referrals as well as the associated costs to both the family and the clinic.

Methods

We retrospectively reviewed the records of all newly referred patients with suspected AIS to a pediatric orthopaedic clinic during 2013 and 2014. We also performed a cross-sectional survey to obtain data not retrospectively available, and used time-driven activity based costing (TDABC) to determine the capacity costs of the providers. The retrospective review included 337 patients, and 24 patients completed the cross-sectional survey (none declined participation). A referral was deemed unnecessary if the patient’s Cobb angle was less than 20 degrees (4). Driving costs were determined using an online mapping program along with the Internal Revenue Services’ standard mileage rates. Lost wages were determined using time data available in the chart along with an estimate of yearly income provided by the census bureau for a given parent’s sex, educational attainment, and county of residence. Estimates of overhead costs and radiography costs were provided by the hospitals accounting offices.

Results

16.6% of the patients had a Cobb angle of less than 10 degrees and 38.9% of the patients had a Cobb angle of less than 20 degrees, which according to expert opinion could be adequately cared for in the community setting (5). A referral cost families between $1.34 and $335.61 (average $98.34) in travel costs and between $34.26 and $512.31 (average $132.73) in lost wages. The average capacity cost of the healthcare providers’ time was $116.74. Overhead costs for the clinic visit were $193.11, and the cost for unnecessary radiography was $239.00. The total average cost to a family and clinic was $779.92.

Conclusion

Unnecessary referral rates were found to be lower than those previously reported, but the cost to both families and providers was concerning. Going forward, an increased focus on the education of primary care providers with respect to AIS and specifically Cobb angles could lower the unnecessary referral rate, and e-referral programs could be used to keep travel and lost wage costs low.
Background & Significance: Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by the production of IgG autoantibodies targeting hemidesmosomal proteins. The immunodominant epitope in BP is the NC16A domain of collagen XVII (BP180). Antibody binding near the basement membrane zone (BMZ) leads to complement fixation comparable to other type II hypersensitivity reactions, causing blistering and erythema. Parkinson’s disease (PD) is a neurodegenerative disease characterized by the loss of midbrain dopaminergic neurons in the substantia nigra. This leads to hallmark clinical features including resting tremor, bradykinesia and muscle rigidity. Several epidemiological studies have identified PD as an independent risk factor for the development of BP. Preliminary data from Dr. Fairley’s lab indicate approximately one-third of patients with PD have antibodies targeting collagen XVII, primarily non-pathogenic epitopes outside of the NC16A domain. Crucially, collagen XVII is expressed in several regions of the brain, including midbrain dopaminergic neurons of the substantia nigra. However, it is not known what role, if any, these autoantibodies play in the progression of PD.

Hypothesis: Collagen XVII antibodies isolated from rabbit serum will bind to and alter the function of dopaminergic neurons in the rat substantia nigra.

Methods: New Zealand White rabbits were immunized with gel-purified sec180 (extracellular domain of collagen XVII) in conjunction with the shared Hybridoma Core Facility located at Iowa State University. Sera collected from these rabbits were screened by immunoblot and indirect IF for reactivity with cryosections of human skin and rat brain. IgG was purified using Thermo Scientific Pierce Protein A/G spin column. Twenty-five mg of purified IgG was then injected into the rat substantia nigra of 8 Long Evans rats (anteroposterior: -5.4, mediolateral: -1.8, dorsoventral: -7.6). Four control rats were injected in the same manner with control rabbit IgG. Two rats per group were sacrificed at 24 hours post-injection to confirm delivery of IgG to the substantia nigra by indirect IF. Two rats per group were sacrificed 7 days post-injection. Prior to perfusion of the 7-day group, rats underwent behavioral testing to assess motor deficits. Rats were placed in an open-top plexiglass cylinder (diameter: 15cm) and their behavior recorded for 5 minutes. Total number of rearing and paw bias was assessed. Additionally, rats received an intraperitoneal injection of (+)-methamphetamine hydrochloride (3 mg/kg); thirty minutes post-injection they were placed in a clean open field container (45×45 cm) surrounded by plexiglass walls and recorded for 5 minutes to assess ipsiversive rotation bias.

Results: Sera from two rabbits (R12 & R13) were seropositive based on immunoblot against sec180 and indirect IF on human foreskin sections. Additionally, both sera stained rat brain sections by indirect IF. This staining colocalized with tyrosine hydroxylase antibodies in the substantia nigra. Pre-immune rabbit serum was used as a negative control. Serum from R12 was negative while serum from R13 positively stained rat brain sections. For this reason, we moved forward with R12 serum for IgG purification and intracranial injection. Rat brains sections from the 24 hours post-injection group were positive for rabbit IgG by indirect IF. This staining colocalized with tyrosine hydroxylase antibody in the substantia nigra. No differences were observed in the behavior testing at 7 days post-injection between experimental and control groups.

Discussion and Future Directions: These findings validate our method for generation of rabbit polyclonal IgG against sec180 and confirm that we can successfully deliver this IgG to the dopaminergic neurons in the substantia nigra of rats. The negative behavioral findings 7 days post-injection do not contradict our original hypothesis. It is possible that antibody binding was toxic but the lesion was not enough to cause motor deficits. Patients with PD often do not present with symptoms until approximately 70% of dopamine has been lost. To assess this, we will use indirect IF to compare dopamine neurons in the injected and uninjected sides. Additionally, it is possible that the antibodies may act to exacerbate pre-existing disease. To assess this, purified IgG will be injected into the substantia nigra in PD disease models. This could include co-injection with a suboptimal dose of the dopaminergic neurotoxin 6-OHDA and transgenic mice over-expressing alpha-synuclein.
Selective spinal immobilization protocol for prehospital providers: Effects on practice and patient outcomes

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Background: In January 2015 a new Selective Spinal Immobilization Protocol for prehospital providers was adopted for use throughout the state of Iowa. The previous protocol proscribed near uniform application of a cervical collar and long backboard to all trauma patients with a concerning mechanism of injury. The updated protocol describes specific criteria under which a patient should receive spinal immobilization after traumatic injury. For both EMT and Paramedic level providers, the new protocol calls for the use of a long backboard or similar device only if the patient complains of midline back pain or tenderness. A cervical collar alone can be used after a traumatic injury in the presence of neck pain, neck tenderness, altered mental status, impairment, communications barriers preventing accurate assessment, or other severe or painful injuries. Mechanism of injury is no longer a factor in determining whether the spinal immobilization procedure is indicated. The primary goal of the protocol change was to reduce the rate of unnecessary spinal immobilization can lead to increased pain, tissue ischemia, restriction of breathing, decubiti formation, aspiration, treatment delays, and costs.

Hypothesis: Prehospital providers will adopt the new Selective Spinal Immobilization Protocol resulting in a reduced rate of prehospital spinal immobilization.

Methods: We conducted a retrospective chart review of all 239 patients arriving at the University of Iowa Hospitals and Clinics by ground or air ambulance during April 2014 (pre-implementation of protocol) and April 2015 (post-implementation of protocol) who met National Trauma Data Standard Patient Inclusion Criteria. All demographics, injury severity, and visit characteristic data were collected from the Iowa Trauma Registry database. Spinal immobilization status was identified using EMS patient care records, nursing flowsheets, and clinician notes.

Results: From April 2014 to April 2015, the data shows a statistically significant decrease ($P=0.0001$) in the percentage of patients with any spinal immobilization in place upon arrival to our emergency department. There was also a significant decrease in the use of the cervical collar and backboard together as a method of spinal immobilization. Even after controlling for patient age and injury severity, the adjusted odds ratio for spinal immobilization in April 2015 was 0.38 (95% CI 0.19-0.75). There was an increase in the percentage of patients receiving collar only immobilization, however, the observed change was not statistically significant. After controlling for patient age, head abbreviated injury score (AIS) and if a patient had spinal immobilization, there was no difference in the odds of spinal cord or vertebral injury, $aOR = 0.71$ (95% CI 0.31-1.62).

Conclusion/Discussion: The data demonstrates that a selective spinal immobilization protocol allows prehospital providers to correctly identify patients at risk for spinal injury. With both the decrease in spinal immobilization rate and the finding that there was no difference in the odds of spinal cord or vertebral injury after implementation, the 2015 Selective Spinal Immobilization Protocol is able to reduce the rate of inappropriate spinal immobilization.
Relapses after clubfoot correction by the Ponseti method: evaluating patient adherence and contributory obstacles in Vietnam
Norah Nguyen, Nhi Manh Huynh, M.D., Jose Morcuende, M.D, Ph.D.

BACKGROUND: Clubfoot, also known as congenital talipes equinovarus, is a common musculoskeletal birth defect that occurs in an estimated 1 in 1000 births in Vietnam. Although initial correction of the deformity by the Ponseti method is quite successful (>95% of cases), a major challenge to sustained treatment is preventing relapses. Lack of adherence to the bracing protocol has been considered the main factor leading to a relapse and it can increase its risk by up to seventeen fold. Understanding the extent of clubfoot relapses and factors that may be contributing to the problem in Vietnam is critical in designing and introducing culturally appropriate interventions that could yield long-term improvements to the management of clubfoot.

HYPOTHESIS: Relapses are associated with failure to adhere to bracing protocols following correction by the Ponseti method in Vietnam.

AIMS: The aim of this study was to assess a rate of relapse following Ponseti method correction of clubfoot and to evaluate contributory factors to relapses in Vietnam. Commonalities among patients that have a relapse will be evaluated that may indicate barriers to proper bracing.

METHODS: This was a survey study that evaluated frequency of relapse among Vietnamese clubfoot patients based on bracing time and other factors that affect brace use. Study participants were collected from Dr. Nhi Manh Huynh’s clubfoot patient list at the Hospital for Trauma and Orthopedics in Ho Chi Minh City, Vietnam. Potential participants met the criteria if they were treated by the Ponseti method for clubfoot, had proceeded to bracing for some period of time, and are of at least walking age (set at 14 months). Patients' parents/caretaker were contacted and asked in for a follow-up appointment if they consented to be in the study. There, the patient was assessed for relapse and took part in a brief questionnaire-based interview to collect data on bracing and other potentially related factors. Home and phone interviews were utilized when needed. 99 clubfoot patients agreed to participate, with four exclusions. One case was excluded due to the patient’s clubfoot being connected to arthrogryposis. Another was excluded due to mild nature of the clubfoot that did not require any bracing. A third exclusion was a case that turned out to not be treated by the Ponseti method. A fourth case was excluded due to not meeting the age criteria. Final count includes 95 data sets, which include health info like relapse status, but 70 interviews cases.

RESULTS: Of the 95 cases, 37% of the patients showed relapse requiring further correction. When focusing on the data for the 70 interview cases and accounting for for duration of bracing and whether a patient stopped bracing prematurely within two years, we see that 61% of patients that stopped bracing relapsed versus 24% in patients that continued bracing over two years. Relative risk will be addressed further within the poster. Of the factors that may be associated with unsuccessful long-term clubfoot correction and proper bracing, the more prominent issues included: distance and long travel times (greater than 2 h) to get to Ho Chi Minh City for treatment, cost and availability of transport, having older or younger siblings with less than a two year age difference, and the primary care taker being a family member other than those who were involved in the initial treatment. Chi-square analysis of each of these factors and the significance of each factor to bracing are addressed further on.

CONCLUSION: From the data, we can see a clear association between lack of proper bracing and relapse. This finding supports many previous country-based studies on relapse being strongly dependent on duration and quality of bracing. Through the parent interviews, we were able to determine commonalities between clubfoot patients and how these factors predispose a patient to successful long-term treatment or relapse.
Establishment of a Protocol for Generating Definitive Hematopoietic Progenitor Cells from Human Induced Pluripotent Stem Cells

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Background:
The advent of induced pluripotent stem (iPS) cells by the Yamanaka group introduced a new age in stem cell research. Past issues surrounding the use of embryonic stem cells, particularly ethical issues revolving around the destruction of embryos, were eliminated through the development of iPS cells. The theoretical applications of these cells are numerous. One clinical area that could likely benefit from the use of these cells is the realm of blood malignancies. Hematopoietic stem cell (HSC) transplantation is the most frequent stem cell transplantation in the world. The use of patient specific iPS cells to derive HSCs could eliminate many of the problems associated with transplanting allogeneic stem cells, such as rigorous tissue matching and the necessity of a large donor base. Currently there is no clear protocol for the efficient derivation of definitive HSCs from iPS cells.

Recently, research has indicated that the source of iPS stem cells influences their ability to form new progenitor cells. This is defined as epigenetic memory. Our group focused on utilizing small molecule inhibitors, which have been demonstrated to reverse certain epigenetic changes, to aid in the development of an efficient protocol for the generation of definitive HSCs.

Hypothesis:
We hypothesized that methylation of certain genes in iPS cells could inhibit iPS cells from differentiating into specific lineages. Treatment with small molecule inhibitors that reverse these epigenetic changes could increase the efficiency of iPS differentiation into HSCs.

Methods:
We studied the demethylation effects of 5 candidate small molecule inhibitors, including Valproic Acid, RG108, 5Aza-2’-deoxycytidine, Hydralazine hydrochloride, and Zebularine through the use of an Imprint Methylation Kit. From these 5 candidate molecules, RG108 and 5Aza-2’-deoxycytidine were selected to be further tested based on their low cytotoxicity and demethylating efficacy, respectively. Human iPS cells were differentiated into HSCs using a 2 phase process lasting from 10-14 days. In the first phase of the differentiation (Days 0-4) iPS cells were treated with a combination of cytokines to generate mesodermal precursors and exposed to the small molecule inhibitors, either RG108 or 5Aza-2’-deoxycytidine. RG108 was supplemented for the entire duration of the first phase (4 days) and analyzed at concentrations of 20μM, 100μM, and 500μM. The treatment with 5Aza-2’-deoxycytidine lasted 24 hours and was at a concentration of 1nM to avoid cell death. During the second phase of the differentiation (Day 4-Harvest), the cells were differentiated into HSCs using a separate combination of cytokines. Cells were harvested either on Day 10 or Day 14. Flow cytometry was performed on differentiated cells to determine the presence of HSCs.

Results:
We discovered differentiated cells expressing CD34, CD43, and CD45, cell markers that represent HSCs. This population of cells was shown to be markedly increased in the samples that were treated with the small molecule inhibitors RG108, and 5Aza-2’-deoxycytidine.

Conclusion/Discussion:
These results indicate that the use of small molecule inhibitors, specifically RG108 and 5Aza-2’-deoxycytidine, can significantly increase the effectiveness of hematopoietic differentiation of iPS cells. Although, the yield of HSCs from this protocol is still not optimal and far from having clinical potential it lays the foundation for the process upon which improvements can be made. Efforts should be directed towards increasing the efficiency of the protocol. Our group believes that future projects should focus on quantifying the number of mesodermal progenitors that are generated and ensuring their population is maximized. In addition, transplantation of fully differentiated cells expressing CD34, CD43, and CD45 will show whether these cells are truly definitive hematopoietic cells. Lastly, this experiment also indicates the potential inclusion of small molecule inhibitors in the differentiation of other cell lines such as β cells from the pancreas.
Title: Differential Vasopressin Receptor Expression and Response in Preeclampsia

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**Background/Rationale:** Preeclampsia is a major hypertensive disorder in pregnancy that is a leading cause of maternal and fetal morbidity and mortality, as well as a risk factor for future metabolic and cardiovascular events in both mother and child. Clinical improvements in the management of preeclampsia have been challenged by its heterogenous pathogenesis involving multiple molecular pathways including early immunologic dysregulation, angiogenesis, placental dysfunction, and renal abnormalities. Recently, we have demonstrated that the arginine vasopressin (AVP) pathway is a robust predictor of the development of human preeclampsia. Furthermore, we have demonstrated that mice infused with AVP throughout pregnancy recapitulate all the hallmark features of human preeclampsia. Based on our data and others, we hypothesize that AVP is a key initiator of the early immunologic and placental pathogenesis of preeclampsia.

**Objective:** To address this hypothesis, the objective of this study was to determine if CD4+ T cells (a major effector cell type in preeclampsia) from pregnant human controls and preeclamptics and HTR8/SvNeo cells (a human trophoblastic cell line) are able to respond to AVP stimulation by examining AVP receptor types. In addition, we tested the ability of the trophoblast cell line to secrete cytokines in response to AVP exposure.

**Methods:** HTR8/SvNeo cells were cultured for 24, 48 and 72 hours and exposed to increasing doses of AVP (50-200ng/mL). Quantitative real-time PCR was performed on RNA isolated from the cells for the following AVP receptors: AVPR1a, AVPR1b, AVPR2, CUL5 and LNPEP (enzyme that degrades AVP) and OXTR (oxytocin receptor) as well as 18S for normalization. Additionally, sFlt-1, IL17A, and IFNγ were measured in the cell culture media from these cells by ELISA (eBioscience). To further investigate the role AVP in modulating human immune cells, CD4+ T cells from preeclamptic pregnancies (N=26) and control pregnancies (N=27) were isolated from circulating mononuclear cells (Maternal Fetal Tissue Bank, IRB#200910784) using immunomagnetic negative selection (Stem Cell Technologies). Quantitative real-time PCR for AVPR1a, AVPR1b, AVPR2, CUL5, LNPEP, OXTR, and 18S was then performed on reverse-transcribed RNA from the isolated CD4+ cells.

**Results:** In the HTR8 cells, RNA for receptors AVPR1a, AVPR2, CUL5, LNPEP, and OXTR were found; AVPR1b was minimally detectable. Treatment of HTR8 cells with AVP across all time points and doses did not result in a differential expression of any of the AVP receptors. Additionally, AVP was not sufficient to stimulate the HTR8 cells to secrete measurable levels of sFlt-1, IL17A, or IFNγ. Yet, human CD4+ T cells also have RNA for the 5 AVP receptors with AVPR1b being minimally detectable. Furthermore, AVPR2 and AVPR1a are the most highly expressed in both controls and preeclamptics. Additionally, AVP1a is downregulated (p=0.009) and LNPEP is upregulated (p=0.001) in the CD4+ T cells of preeclamptic women.

**Conclusion/Discussion:** Given these results, we conclude that HTR8/SvNeo cells and maternal CD4+ T cells have the potential to respond to AVP given the presence of RNA for almost all of the receptors. There are cell specific distributions of all these receptors as CD4+T cells most highly express AVP2 and AVPR1a. Although no significant secretion of sFLT-1, IFNg, and IL-17A was observed in HTR8/SvNeo cells, this does not rule out the ability of AVP to stimulate human trophoblastic cells. Future experiments will address other immunologic and vascular molecular output from these cells relevant to preeclampsia. These novel data suggest a differential role of AVP in different cell types, which will lead to cell specific investigation of the early molecular pathogenesis of preeclampsia in the future.
**How Accurately Do Resident Case Presentations Reflect the Content of Clinical Interviews? – A Comparative Analysis**

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**Background** - The development of core communication skills is essential to providing effective patient care [1]. Residency training programs provide an environment where young physicians can practice and refine these vital skills [2]. Being able to elicit relevant information during clinical interviews is critical in order for residents to make accurate diagnoses and build therapeutic physician-patient relationships. However, residents are rarely observed by attending physicians during the patient encounter. As such, often the only insight that a clinical teacher has into learner communication skills comes from the content and quality of the oral case presentation given by the learner after the patient interview [2]. Very little is known about how accurately the information presented by the resident physician reflects the content of the clinical encounter. Gaining insight into this has the potential to improve how we teach residents to effectively communicate with patients.

**Objectives** - The aims of this study were two-fold: 1) to determine the extent to which resident case presentations reflect the content of the clinical interviews on which they are based, identifying trends in the types of information found to be most frequently incongruent between the two contexts, and 2) to preliminarily assess select resident communication skills employed in the patient interview that are not conveyed by the case presentation.

**Methods** - This observational study utilized three subject populations (family medicine residents, family medicine faculty physicians, and patients of the family medicine clinic.) Within UIHC’s Family Medicine Clinic, 15 resident physicians and 11 faculty physicians participated. A total of 25 patient subjects took part in the study. Patient interviews conducted by the resident physicians were video-recorded. Corresponding oral case presentations given by the residents to their attending physicians were then audio-recorded. The videotapes and audiotapes were transcribed verbatim to allow for analysis of content. Transcripts were systematically coded by content category, enabling discrete comparison of the content present in the interview to that found in the case presentation. Compiling the key incongruence(s) from each of the 25 cases revealed trends in the types of information most frequently not communicated by residents in case presentations. The transcripts were also utilized to assess the resident communication skills present during patient encounters.

**Results** - Analysis of the data revealed that content pertaining to the Chief Complaint(s) and corresponding HPI(s), as well as Assessment and Diagnosis, was congruent between the case presentation and patient interview in nearly all cases. Additionally, content in the categories of the patient’s Medications and Allergies, Past Medical and Surgical History, Family History, Relevant ROS, Physical Exam Findings, and Lab/Test Results was also largely congruent among the cases.

Major incongruences, however, were found with information related to the patient’s Social History, Patient’s Perspective (Ideas, Concerns, Expectations, Effect), Planning, Patient Education, and additional Patient Complaints (i.e. other than the Chief Complaint.) In several cases, content from the patient interview was missing from the case presentation. Patient Education information was found to be most frequently missing from the case presentation, and in a few instances, the information given to the patient by the resident during the interview was not factual.

Preliminary analysis of communication skills revealed that residents appropriately used an adequate number of open-ended questions in approximately one-half of the cases in the study sample. Similarly, in cases where Patient Perspective information was discussed, roughly one-half of residents elicited this information directly from the patient, as opposed to relying on the patient to bring it up on their own. Of note, case presentations were unable to accurately convey the nature of the relationship between the resident and patient, including specific aspects of rapport building and appropriate expression of empathy, which varied across cases.

**Conclusion** - While this study found that medical information was largely congruent between oral case presentations and the patient interviews on which they are based, certain types of information discussed in clinical encounters were frequently missing from case presentations. Because attending physicians regularly depend solely on the content and quality of the resident’s case presentation to assess resident performance in the clinical setting, this finding has noteworthy implications. Case presentations cannot paint a clear picture of what specifically happened during the patient encounter, so further investigation into the trends in the types of content most frequently absent from case presentations (i.e. elements of Social History, Perspective (ICEE), Planning, Patient Education, and other Patient Complaints) will function to highlight where valuable teaching opportunities may commonly be missed in residency training programs.

Epidemiology and Geospatial Analysis of All-Terrain Vehicle Crashes in Iowa

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Background: Each year in the United States, there are over 700 deaths, more than 400,000 injuries, and in excess of $22 billion dollars in costs related to all-terrain vehicle (ATV) crashes. In previous studies of Iowa ATV crashes, 80% of those injured were males, and one-third were children under the age of 16. Previous studies also showed that one out of three crashes occurred on the road, despite the fact that ATVs are manufactured for off-road use only. To our knowledge, only a single study in Texas has used geographic information system (GIS) mapping to characterize ATV crashes. The long-term goal of our research is to promote evidence-based public policy for ATV injury prevention using multiple approaches, including geospatial analysis to increase public knowledge and awareness of ATV-related deaths and injuries.

Objective: Describe the epidemiology and geographic pattern of ATV crashes in Iowa using a statewide ATV injury surveillance database and geographic information system (GIS) software.

Methods: A retrospective cross sectional study was performed using a unique statewide ATV injury surveillance database (2002-2013) that included records from the IA Department of Transportation (DOT), IA State Trauma Registry (STR), and IA Department of Natural Resources (DNR). SPSS statistical software was used to perform descriptive and comparative analysis, including multivariable logistic regression analysis. A geographic information systems (GIS) program called ArcGIS was used to map the location of ATV deaths and injuries in the state.

Results: The statewide database included a total of 1,951 crashes, 2,308 vehicles, and 2,330 riders in Iowa. Crash victims were 79% males, 30% under 16 years of age, and 4% of crashes resulted in fatalities. Thirty-six percent of all crashes were on the road. The vast majority (91%) of all crashes were non-collision events (i.e. vehicle rollovers). GIS mapping demonstrated that ATV crashes occurred in both rural and urban areas, including downtown Des Moines. Mapping also provided striking visual patterns demonstrating the following: the proportion of fatal crashes was twice as high on the road than off; of the roadway crashes, a majority were not traffic-related; ATV crashes are occurring in remote rural areas with highly limited access to emergency medical services.

Conclusion: Similar to previous studies, the majority of crash victims were male, and children under the age of 16 were disproportionately represented (1 in 3). Epidemiology and GIS mapping also indicated that crashes occurred both on and off the road, and that this public health issue is relevant to both rural and urban communities. The results also provided additional evidence that, contrary to what supporters of roadway riding believe, roadway crashes are more likely to be fatal and remote roads with low traffic volume are not “safe” places for ATV use. GIS represents a powerful tool for ATV safety education and public awareness, as well as for research. Results from this study will be used in our safety programming, as well as to inform public health and public policy makers, in order to promote evidence-based ATV safety laws.
Relating Fracture Severity to Post-Traumatic Osteoarthritis Risk after Intra-Articular Calcaneal Fractures

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INTRODUCTION: Patients with high-energy intra-articular fractures face a poor prognosis and an increased risk of developing disabling post-traumatic osteoarthritis (PTOA). Objective CT-based measures of fracture energy have been used to link fracture severity to PTOA risk following intra-articular fractures of the distal tibia [1-3]. This relationship has not been studied in other intra-articular fractures, such as of the calcaneus, where the Sanders classification scheme is used as a prognostic marker for long-term clinical outcomes [4] but has not been correlated with fracture energy. The purpose of this study was to establish the relationship between fracture energy, the Sanders classification, and PTOA development in patients with intra-articular calcaneal fractures.

METHODS: Ten patients with eleven intra-articular calcaneal fractures were consented with IRB approval for this study. The patients were selected from a series of 120 cases based on having a follow-up time > 18 months. Standard of care pre-operative CT scans were used to classify the fractures according to the Sanders scheme and to assess their severity. Fracture severity was quantified by fracture energy, which is proportional to the liberated inter-fragmentary surface area [1, 6]. Custom MATLAB code was used to identify and measure the inter-fragmentary surface area (Figure 1). Fracture energy was calculated by multiplying the liberated surface area by the energy release rate, scaled by CT intensities to account for variation in bone density [2, 6]. All patients were treated with percutaneous reduction and screw fixation. PTOA development was graded using the Kellgren-Lawrence (KL) scale from radiographs of the calcaneus taken between 20 and 74 months post-injury. Three experts independently measured the articular step-off, another potential predictor of PTOA. Because the measures to be compared mix ordinal and continuous values, agreement was assessed using concordance—the probability that the fracture energies correctly discriminate between pairs of Sanders classification and/or KL scores.

RESULTS: Calcaneal fracture energies ranged from 14.4 to 24.5 J (mean ± standard deviation = 18.7 ± 3 J). A concordance of 0.75 was observed between Sanders classification and fracture energy (Figure 2). There was a complex relationship observed between fracture energy, articular step-off, and KL grade (Figure 3).

DISCUSSION: The positive association of the Sanders classification with fracture energy has prognostic implications for intra-articular calcaneal fractures. However, no significant correlation was found between fracture energy and KL grade for calcaneal fractures, with the residual articular step-off a likely confounder influencing PTOA risk.

ACKNOWLEDGEMENTS: Saran Tantavisut, MD; Brian Westerlind.

Patient Satisfaction with Immediate Post-delivery Long Acting Reversible Contraception Placement

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BACKGROUND. Postpartum contraception is routinely initiated at the six-week office visit, however, only 50% of women attend these visits and many others have had intercourse prior. Long-acting reversible contraceptive (LARC) devices include intrauterine contraceptives (IUC) and the sub-dermal implant. These can be placed immediately following delivery, offering the advantage of highly effective contraception prior to discharge. IUCs and implants have very different side effect profiles including different bleeding profiles, and interval placement of an IUC has an increased risk of expulsion over an interval placement.

HYPOTHESIS. Patient satisfaction rates will be similar after immediate placement of IUCs versus subdermal implants. Secondarily, there will be increased reporting of complications in the IUC group.

METHODS. Non-incarcerated, English-speaking women over the age of 18 who had LARCs placed immediately after vaginal or cesarean delivery from 9/2013 to 6/2015 at University of Iowa Hospital were selected as potential participants. IRB-approved anonymous telephone surveys were given to women who met the inclusion criteria. Questions were asked regarding satisfaction with timing of contraception placement, pain scores for device placement, and complications or issues with their chosen LARC.

RESULTS. 167 of the 323 qualified women with immediately placed LARC were reachable by phone and 161 completed surveys. This included 83 immediate IUC and 78 immediate implant placements. IUC expulsion rate in this study was 16.9%, consistent with prior studies. 74.2% of all respondents were satisfied or highly satisfied with the timing of their device placement and 75.9% would recommend the timing of placement to a friend. There was no significant difference between rates of being satisfied/highly satisfied between IUC and implant (70.4% vs 78.2%, p=0.34) or in those who would recommend their procedure to a friend (70.3% vs 81.7%, p=0.16). More women in the IUC group wished they had their device placed at delayed interval (33.3% vs 16.7%, p=0.03). Overall pain scores were low, however, fewer women in the implant group reported low pain scores (91.6% vs 84.6%, p=0.09). Significantly more women reported significant complications after immediate IUC insertion than implant (11.0% vs 0%, p<0.001).

CONCLUSION
In addition to the contraceptive benefits, immediate placement of LARC, whether IUC or implant, is correlated with high satisfaction rates, with the majority of users recommending the procedure to a friend. The most notable difference between IUC and implant users is that implant users were found to have more issues after placement resulting in removal, likely due to abnormal bleeding, but IUC users had more reported complications, most commonly malposition of the device. These findings indicate that immediate LARC placement is a satisfying and low-pain experience for women regardless of device. Women should be adequately counseled about possible complications and bleeding profiles of LARC devices prior to delivery.
Correlating Dynamic SSEP and MRI in Cervical Myelopathy Treatment
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Background
The most common cause of spinal cord dysfunction is cervical spondylotic myelopathy. This condition is the result of progressive narrowing of the spinal canal due to degeneration. The spinal cord compression produces symptoms of numbness, clumsiness, weakness, or stiffness in the arms, legs, or chest depending on where the compression is occurring. Due to the degenerative nature of this disorder, it is most often seen in older adults. The conventional method of diagnosing cervical myelopathy was to analyze the spinal cord and the spinal canal with computed tomography (CT), myelogram, and magnetic resonance imaging (MRI). Recently, dynamic MRI has been added to the diagnostic tests to allow for images to be taken with the patient in different positions that may result in increased compression of the spinal cord. However, when the patients are examined in the positions demonstrating increased compression they do not present with different neurologic findings. This inconsistency has raised the question of whether cervical myelopathy is a result of static or dynamic compression.

This study uses dynamic MRI combined with somatosensory evoked potentials (SSEP) to investigate the effect of dynamic compression on the conductivity of the spinal cord. SSEP has been used as a reliable electrophysiological tool to measure the function of the spinal cord in a highly sensitive and objective manner for over 50 years. SSEP uses a stimulator attached to the median nerve and electrodes placed on the scalp to record amplitude and latency of the stimulatory signal travelling towards the brain. In patients with lesions, the conductivity of the signal is decreased resulting in latency as compared to healthy controls. In this study we plan to place subjects in neutral, flexion, and extension positions for the dynamic MRI and then record SSEP measurements with patients positioning their cervical spine at the same angles. We want to determine if the SSEP measurements are altered when patients are placed in these positions and correlate the changes with the degree of spinal cord compression seen in dynamic MRI.

Aims
The aims of this study are to first investigate whether the increased compression seen in MRI correlates to increased latency in SSEP measurements. A second aim is to determine if the dynamic movements performed during dynamic MRI correlate to changes in SSEP. The final aim of this study is to analyze the changes in the spinal cord that occur during dynamic motion.

Methods
This study has undergone and has received University of Iowa institutional review board (IRB # 201411728). Subjects were identified in the Neurosurgical clinic. Inclusion criteria included those ages 18-75 with a diagnosis of cervical myelopathy and evidence of worsening compression on dynamic MRI. Using a 3T MRI, T2 images were obtained from each subject in the neutral, flexion, and extension position with a goniometer recording the position angles. The subjects were then connected to SSEP electrodes with the stimulator over the median nerve to create a steady thumb twitch and data was collected for each of the three positions used in the MRI images. The subjects will undergo surgical treatment and then the dynamic MRI and SSEP data will be collected again to analyze surgical outcomes.

Results
The dynamic MRI measurements demonstrated that in the neutral position, cervical myelopathy patients had smaller diameter spinal cords and canal diameters than controls. We also found the cord diameter does not change in a statistically significant manner when a patient’s is moved from a neutral position to a flexed or extended position. This aligns with the SSEP data that revealed there is no significant change in conduction velocity as one person moves from a neutral position to the flexed or extended position. However, in the extended position, cervical myelopathy patients had a statistically significant increase in latency as measured by SSEP compared to the controls.

Conclusion
In this study, cervical myelopathy patients were analyzed with dynamic MRI and SSEP to correlate spinal cord compression to spinal cord dysfunction. We found that as one person changes their position, the amount of compression on their spinal cord is not significantly altered and this correlates to the SSEP findings of no alterations in conduction velocity from position to position. The significant increase in latency seen in cervical myelopathy patients compared to controls is likely due to static compression causing an insignificantly elevated latency at the neutral position that is then exacerbated when the same patient moves to the extended position. These results seem to indicate that there is both a static and a dynamic compression aspect to the spinal cord dysfunction seen in cervical spondylotic myelopathy. These patients will continue to be followed after surgical treatment.
The Face Doesn’t Lie: A Novel Approach to Diagnose and Treat Light Sensitivity

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**Background:** Light sensitivity is one of the most prevalent and debilitating problems reported by military and civilian personnel following traumatic brain injury (TBI). It is also very prevalent among patients with migraine headache, even between attacks. In addition to being a common and often debilitating outcome of these conditions, light sensitivity may also be an important phenotypic marker. Currently, there is a critical need for an objective means to evaluate light sensitivity and to monitor the effectiveness of treatment. One of the best methods for objectively measuring physiologic data is by measuring a reflex response. The photic blink reflex is a natural brain reflex that relays light stimuli from the eye to facial muscles via the thalamus and central sensory trigeminal pain center of the brainstem. The reflex elicits a blink and squint reaction in response to light stimuli. The electrical activation of the subject’s eyelids and surrounding muscles triggered by this response can be measured by a photic-electromyogram (EMG). The facial muscles involved in the process include the procerus muscle (brow muscle involved in squinting) and the orbicularis muscles of both eyes (eyelid muscles). Dr. Kardon’s research team established that facial muscle movements could also be quantified from video frames of a digital recording using image analysis. The extraction of the facial features and their dynamic changes in response to light of increasing intensity appears to match the EMG response in the same proportion, and thus has the potential to be used as a valid surrogate biomarker of light sensitivity. Another brain reflex that assesses visual function is the pupillary light reflex. This reflex is used routinely in clinical applications to evaluate optic and oculomotor nerve function and pupil contraction is proportional to transduction of light by the eye to the brain. Dr. Kardon’s lab has previously used pupillometry to demonstrate that the pupillary light reflex in response to alternating red and blue light stimuli can be used to determine the functional state of the retina and optic nerve in each eye independent of the other. This approach controls for variations in pupil response due to CNS factors because it is a within eye comparison. Thus, the pupillary light reflex can be used in conjunction with the photic blink reflex to better determine if the location affected in light sensitive patients is in the eye or recipient neural areas in the brainstem.

**Purpose:** It is hypothesized that the relationship between light intensity and facial muscle contractions is abnormally sensitive in patients with various forms of photosensitivity. The aim of this ongoing study is to objectively assess light sensitivity by analyzing the relationship between light intensity and facial muscle contractions in patients with subjective complaints of light sensitivity compared to control subjects. Further, it is necessary to find the most robust diagnostic marker of light sensitivity to distinguish light sensitive and normal patients. Once this testing protocol has been optimized and analyzed thoroughly in the clinic through the use of photic-EMG and pupilometer recordings, it is desirable to progress to a less intrusive, more mobile platform for testing patients, namely using image analysis from digital recordings.

**Methods:** EMG and pupilometer data from 16 controls and 14 migraine/ TBI patients was collected. The photic-EMG electrodes were placed to measure the procerus muscle and the left and right orbicularis muscles. Arrington video pupilometer eyeframes were used to track the change in pupil size in response to increasing light intensities. EMG and pupil data collected were time-stamped and measured simultaneously. Subjects were exposed to a total of 20 epochs of alternating red (640 nm) and blue (485 nm) light stimuli of increasing intensity from -3 [log] to 2.6 [log] cd/m² for a one second duration followed by nine seconds of darkness. Collected data was visualized via a self-created Matlab application that contained a Data Viewer to see a subject’s video feed along with the change in pupil size and the photic-EMG tracings. The data was exported to excel and analyzed using GraphPad Prism. A 2-way repeated measure ANOVA was used to analyze the difference in patients compared to controls at each corresponding red and blue light intensity, matching each subject to their own data at all light intensities.

**Results:** The difference in percent contraction of the pupil in response to both red (p = 0.9740) and blue (p=0.3543) light was determined insignificant between patients and controls. The procerus photic-EMG response in light sensitive patients was significantly higher in both red (p = 0.0286) and blue (p = 0.0078) light compared to controls but more significant with blue light.

**Conclusion:** A lack of significant difference in the pupil light reflex between light sensitive patients and controls, but an exaggerated facial EMG response in light sensitive patients points toward recipient areas of the brainstem as being abnormally sensitive to the photic-EMG reflex. We conclude that the photic-EMG response can be used as an objective marker of light sensitivity. Facial features extracted from video recorded simultaneously with the photic-EMG is presently being evaluated as a rapid, novel method of evaluating traumatic brain injury patients and migraine patients for photosensitivity. This method could potentially be used for monitoring response to treatment.
Evaluation of Focal Neurologic Lesions in Refractory Epilepsy Utilizing
7T Magnetic Resonance Imaging
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Introduction
For patients suffering from refractory epilepsy, surgical resection of the epileptogenic region of the brain can either reduce or completely eliminate symptoms. The single most important variable in determining the success of surgical treatment is the ability to define then fully excise the entire epileptogenic region. This process hinges on the identification, characterization, and careful delineation of the lesion through EEG and MRI assessment. For a significant percentage of individuals with refractory epilepsy, no focal lesions can be identified based upon the MR images, which precludes them from surgical treatment. Our ability to delineate focal lesions in many of these subjects is in part limited by the spatial resolution and contrast provided by the MR imaging at clinically available field strengths (i.e. 1.5T and 3T). Recently, the University of Iowa has acquired a whole body 7T MRI scanner. The increased field strength coupled with the increased sensitivity of the receiver coils significantly increases the spatial resolution of the images acquired in this patient population. In addition, new state-of-art imaging pulse sequences are available on this scanner providing new contrasts that are currently not yet available on the clinical systems.

Our project aim was to first develop an 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
The imaging protocol was optimized in control subjects to acquire three-dimensional 0.6mm isotropic resolution images for T1 and T2 weighted images along with T2-weighted fluid attenuated inversion recovery (FLAIR). These sequences served as the primary images for screening subjects. Two additional sequences were acquired through the medial temporal lobe and/or through regions that were suggestive of the epileptogenic region based on whole brain T1 and T2 weighted images. The additional imaging sequences included tissue border enhancement (TBE) and susceptibility weighted angiography (SWAN) sequences. TBE applies RF waves such that there is a uniform appearance within homogenous tissue and a hypointensity at the junction between gray and white matter. This sequence was added for its ability to detect cortical dysplasias, polymicrogyria, heterotopias, and other similar abnormalities. The SWAN sequence provided the ability to assess the cerebral venous system that surrounds a lesion or area of hypo or hypermetabolic focus. All control participants were recruited from the Iowa City community and provided written informed consent in accordance with the local institutional review board (IRB).

The participants with epilepsy were recruited from the outpatient Neurology clinic at the University of Iowa. Inclusion criteria included a current diagnosis of refractory epilepsy and negative previous clinical 3T MR imaging. Participants provided written informed consent in accordance with the local IRB. Subjects were then imaged using the optimized 7T protocol. Finally, the images collected at both 3T and 7T were reviewed by a neuroradiologist to determine if 7T images were able to identify focal lesions not visible on the 3T images collected as part of standard clinical study. To date, five studies have been completed and three studies have been reviewed.

Results
Of the three reviewed studies, one was positive for a focal lesion and two were negative. The positive study was able to show a loss of normal architecture within the left hippocampus not visible on 3T imaging. An ipsilateral choroid fissure was visualized subtly indicating hippocampal volume loss. These findings support a diagnosis of hippocampal sclerosis. The remaining brain parenchyma was appropriate for the patient’s age showing no coexisting or dual pathology. The remaining two studies were negative for definite signs of focal lesions. One of the two studies was interpreted as a normal brain MRI while the other showed signs of abnormality (right sided mammillary body volume loss suggestive of an existing ipsilateral hippocampal pathology). When the hippocampi were examined there were no definite features to support a diagnosis of hippocampal sclerosis. Since no focal lesion was identified in either case, both studies were read as negative.

Discussion
It is promising that in our small sample size we have been able identified a focal lesion and seen abnormalities not visible on 3T images. To determine the significance of these findings we must increase our sample size.

Conclusion
High resolution 7T imaging has allowed us to identify a focal neurologic lesion 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.
ABSTRACT

Recombinant Trypanosoma cruzi antigens in enzyme-linked immunosorbent assay yield diagnostic confirmation for blood donors in Northeast Brazil

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BACKGROUND

The parasites Trypanosoma cruzi and Leishmaniasis infantum are digenetic flagellated kinetoplastid protozoa, which are the causative agents of visceral leishmaniasis (VL) and Chagas’ disease, respectively. In these diseases, which are endemic to Brazil, the parasitic load remains very low, and most diagnoses are made using clinical indicators and serological testing. Enzyme-linked immunosorbent assay (ELISA) is often used as a primary method of serological testing; unfortunately, this method has shown a high prevalence of cross-reactivity between Trypanosoma cruzi membrane antigens and serum with high levels of anti-Leishmania infantum antibodies, especially when using antigens from T. cruzi epimastigote extracts. This cross-reactivity frustrates efforts by those wishing to screen for T. cruzi infection. The Institute of Tropical Medicine of Rio Grande do Norte (IMT) collected blood samples from the blood bank of Natal, Brazil which screened positive for T. cruzi infection using the commercially-available ELISA Chagas III test. This and many other commercial kits have been known to have a high degree of sensitivity, but low specificity, especially when sera from VL are tested. However, the development of recombinant T. cruzi antigens for use in serological testing promises to be highly specific, allowing for the differentiation of Chagas’ from VL, while retaining high sensitivity for Chagas’ positive sera.

HYPOTHESIS

An ELISA protocol utilizing recombinant anti-T. cruzi antigens will yield higher specificity for sera of Chagas’ patients compared to serological tests using whole T. cruzi epimastigote extracts.

METHODS

In-house ELISA assays utilizing novel antigens TcF26 and TcF43 (developed by the Infectious Disease Research Institute in Seattle, WA) were completed with a total of 108 serum samples from clinically-confirmed Chagas’ cases from various municipalities in the state of Rio Grande do Norte in Northeast Brazil and 75 clinically-confirmed VL cases to test for cross-reactivity with anti-L. infantum antibodies. Positive results were indicated by an optical density (OD) above the mean of the negative controls plus 3 SDs. The 108 Chagas’ sera were also tested for L. infantum coinfection using an in-house SLA/rK39 ELISA assay with a predetermined cutoff, and for comparison they were tested using the Chagas III kit, with a plate cutoff calculated as (mean of positive control + mean of negative control)*0.42.

RESULTS

For T. cruzi infection, we found that the sensitivity and specificity of ELISA using TcF26 were 82.4% (95% CI 73.6-88.8%) and 89.3% (79.5%-95.0%), respectively; sensitivity and specificity using TcF43 were 86.1% (77.8-91.8%) and 96.0% (88.0-99.0%), respectively. The Chagas III assay showed sensitivity and specificity of 88.0% (80.0-93.2%) and 26.9% (12.4-48.1%), respectively. 32.4% of the Chagas’ patients tested positive using ELISA-SLA, with only 8.33% testing positive using ELISA rK39.

CONCLUSION

Due to cost limitations of more advanced confirmatory diagnostic tests, an assay is needed in this setting as a secondary confirmation to diagnose blood donors who screen positive for T. cruzi infection. We suggest that ELISA-based testing with recombinant antigens TcF26 and TcF43 provides a much-needed opportunity to differentiate between T. cruzi-infected blood products and cross-reactive blood products in Northeast Brazil and similar settings.
A New, Low Frequency Gene Predisposing to Juvenile Polyposis

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Introduction: Juvenile polyposis (JP) is a disease characterized by hamartomatous polyps and a predisposition to upper and lower gastrointestinal cancer. The genes known to be responsible for JP are both involved in the bone morphogenetic protein (BMP) signaling pathway. Fifty percent of all JP cases are due to germline mutations or deletions in the genes BMPR1A and SMAD4. The causative genes responsible for the remaining fifty percent are yet to be identified and several other genes in the BMP pathway have been ruled out by sequencing, including SMAD1, SMAD2, SMAD3, SMAD5, and SMAD7. However, SMAD9, a gene also involved in the BMP pathway has not been thoroughly explored. The purpose of this study was to determine whether germline SMAD9 mutations are present in JP patients without mutations or deletions of BMPR1A or SMAD4.

Methods: Exome sequencing was performed in 15 members of 11 different JP families/sporadic cases found to not have mutations of BMPR1A or SMAD4. Sequence data was aligned to the human reference genome sequence then analyzed using the gene analysis toolkit for single nucleotide variants, insertions, and deletions. Synonymous variants, intronic variants, those with minor allele frequency >1%, and those predicted to be non-damaging by SIFT and PolyPhen2 were discarded. Further analysis of candidate variants was carried out using DNA from 30 JP probands (without germline mutations in BMPR1A or SMAD4) for PCR amplification of each exon and intron-exon boundary of genes. PCR products were sequenced, and chromatograms were analyzed against the wild-type sequence using Lasergene software.

Results: Exome sequencing revealed a germline heterozygous mutation of SMAD9 (314A>G; H105R) in 3 affected siblings of a JP family and their father. This mutation was predicted to be damaging by PolyPhen2 and was not seen in the dbSNP or exome variant server databases. In the additional 30 probands, no substitutions or indels were found in the SMAD9 exons.

Discussion: We have found a germline mutation in SMAD9 shared by a father and 3 children of a JP family. Although this was not found in the 30 additional probands, it was also not seen in genome databases and the missense change was predicted to be damaging. These results, plus a new report now in press describing another JP family with a germline SMAD9 mutation suggests that SMAD9 may also be a predisposing gene for JP, albeit only accounting for a minority of cases.
The Role of N-AgrD Polymerization in a Novel Form of Bacterial Motility
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Background: *Staphylococcus aureus* is a gram-positive coccus that transiently colonizes humans and causes tremendous mortality and morbidity worldwide. It is well appreciated that *S. aureus* is capable of alternatively regulating gene expression through the agr system when a high local concentration of a signaling molecule called auto-inducing peptide (AIP) is reached. Many of the genes regulated by this system encode virulence factors, such as the phenol soluble modulins and alpha toxin, and agr activation plays a major role in disease progression as highlighted by increased basal expression in hyper-virulent antibiotic resistant strains such as USA300. Importantly, a peptide fragment (N-AgrD) generated by cleavage of agrD to form AIP has been shown to have amyloid genic properties and is capable of forming elegant fibers when polymerized. Additionally, previous work in the lab has shown that many of these agr regulated gene products, including N-AgrD, may be contributing to a novel form of bacterial motility in a canonically non-motile organism. Given this knowledge our aim was to screen FDA approved drugs in the search for compounds that would inhibit the polymerization of N-AgrD to better understand its contribution to this novel motility phenotype.

Hypothesis: We hypothesize that inhibition of N-AgrD polymerization will reduce or inhibit *Staphylococcus aureus* motility on low nutrient, low agar TSB plates.

Methods: Motility assays were performed by adding 10ul of bacteria (some with fluorescent beads/drug) to 10% tryptic soy broth (TSB) plates made with 0.24% Noble Difco Agar and incubated for 24 hours at 37° C. Thioflavin T (ThT) assays were performed by adding ThT to sterile water with purified N-AgrD and measuring fluorescence at 495 nm every 5 minutes for 20 hours. Drug screening was accomplished by adding 1ul of 10mM candidate drug from the Screen-Well FDA Approved Drug Library V2 (Enzo Life Sciences) in addition to the normal ThT assay reagents and observing fluorescence.

Results: Fiber formation from purified N-AgrD was observed in a dose dependent manner by the ThT assay and then visually confirmed via transmission electron microscopy (TEM). After screening the 780 compound drug library we identified Amiodarone as a strong candidate for inhibiting N-AgrD polymerization and thus formation of amyloid fibers. Additionally Amiodarone did not inhibit growth of *S. aureus* and fluorescent microscopy showed it did not inhibit expression of the P3 promoter. We also observe that amyloid fibers are capable of generating enough force to move fluorescent beads and again confirm these findings by TEM.

Conclusions: Based on these observations we can conclude that N-AgrD polymerization plays an important role in the novel motility of *Staphylococcus aureus* on low nutrient/lower agar plates. Additional work on N-AgrD will be important in establishing this as a biologically, and clinically, relevant phenomenon.
Dynamic needle tip positioning with ultrasound versus palpation technique for radial artery cannulation. A prospective randomized controlled trial.
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ABSTRACT

Background: Most commonly, blood pressure is measured using a non-invasive blood pressure cuff. However, in the operating room and critical care settings where closer monitoring of blood pressure is needed, invasive blood pressure monitoring is required. The radial artery is the preferred site for monitoring due to the dual blood supply to the hand as well as the superficial position of the radial artery. Radial arterial cannulation is often performed by palpation; however, the addition of ultrasound has been shown to increase the success rate of radial arterial line placement. A modified technique where the needle tip is advanced in a stepwise fashion under ultrasound guidance (dynamic needle tip positioning) has been used for peripheral vein cannulation. To date, no studies have been performed to evaluate this novel ultrasound technique during radial artery cannulation. This new technique could increase the first pass success rate and decrease the risk of complications associated with the palpation technique of radial artery cannulation.

Hypothesis: The use of dynamic needle tip positioning with ultrasound guidance will lead to a higher first-pass success rate in comparison to palpation when placing a radial artery catheter.

Methods: Patients who were having an elective surgical procedure that required the use of a radial artery catheter, as determined by the attending anesthesiologist, were enrolled in this study. Patients were excluded if they were minors, incarcerated, pregnant, in shock, non-English speaking, had a negative modified Allen’s test or had a radial artery cannulation in the previous 30 days. Enrolled patients were randomized into either the ultrasound or palpation group, and the specified technique was used to place the radial artery catheter for a maximum of five minutes, at which point the study ceased. All radial artery catheters were placed by an anesthesia faculty member or resident, both of whom were trained on each technique. Data that was collected included: first pass success rate of radial arterial line placement, number of total attempts to place the radial arterial line, number of catheters used, time to achieve successful cannulation, and the number of skin perforations.

Results: A total of 183 patients have been enrolled and randomized into the study, split between the ultrasound group (n=94) and the palpation group (n=89). The dynamic needle tip positioning using ultrasound group was shown to be more effective for both the first pass success rate (82.99% vs. 41.57%) and the overall success rate of radial artery cannulation (89.36% vs. 62.92%) in comparison to the palpation group.

Conclusions: This data demonstrates that the use of dynamic needle tip positioning with ultrasound is more effective than using palpation to place a radial artery catheter for this patient population. With further analysis of more patients, this project could promote for the increased use of the dynamic needle tip positioning technique with ultrasound for radial artery cannulation.
Dynamic serial casting and physical therapy to prevent hip dislocation in children with cerebral palsy
Caroline Sanderson, BA; Jose Morcuende, MD, PhD; and Maria Teresa Ferrer, PT, DPT, C/NDT, C/TBI

ABSTRACT

BACKGROUND: Hip dysplasia is common among children with cerebral palsy due to spasticity in the lower extremity muscles. Hypertonia in the adductors and hamstrings combined with weakness in the abductors and lateral rotators leads to deformity of the hip joint. Pediatric orthopedic surgeons often recommend surgical procedures—tenotomies, myotomies, femoral osteotomies, and acetabuloplasties—to promote correct hip positioning. But recently dynamic serial casting—applying a flexible cast each week for 3 to 5 weeks—in combination with physical therapy to strengthen the abductors and lateral rotators has been used to prevent hip dislocation in children with cerebral palsy. If started at an early age, this new application of established conservative treatments could prevent progression of hip subluxation and promote correct lower extremity alignment, thus avoiding surgical intervention. This study is the first documentation of this procedure and the first attempt to evaluate its efficacy.

PURPOSE: This pilot study has two purposes. First, to describe the indications and procedure of a new conservative treatment for preventing hip subluxation in children with cerebral palsy. Second, to compare cases of children who underwent surgery to those who underwent the conservative treatment. We hypothesized that there would be no statistical difference in the degree of hip subluxation between the two groups.

METHOD: This was a retrospective chart review that evaluated hip development among a surgery group and a casting with therapy group. We reviewed charts from January 2011 through May 2015 at Genesis Pediatric Therapy Center in Coralville, IA. 36 children were identified to have a diagnosis of cerebral palsy and 17 also had a hip deformity. Inclusion criteria were diagnosis of cerebral palsy and existing hip pathology for which they had received treatment. Patients were excluded from the study if they had a diagnosis of idiopathic scoliosis, if it had been less than 2 years since the hip-correcting procedure, or if they did not receive physical therapy after the hip-correcting procedure. 8 patients met the study criteria (4 in each group). I performed detailed chart reviews and recorded the following variables for each patient: age, gender, date of birth, gestational age at time of birth, administration of botox injections in the lower extremity muscles, ambulatory status before and after treatment, concomitant diagnosis, type and date of surgical procedures, type of cerebral palsy (hemiplegia, diplegia, or quadriplegia), and age at the time of intervention. Pre- and post-treatment radiographs were analyzed and the following measurements were taken: neck-shaft angle, center-edge angle of Wiberg, Reimers’ migration percentage (MP), and acetabular index (AI), and Shenton line. Change in these parameters were analyzed with a paired student’s t-test due to the small sample size.

RESULTS: The study findings revealed that there is not a statistically significant difference in the degree of hip subluxation between the two groups. All patients demonstrated the coxa valgus deformity as measured by the neck-shaft angle being greater than 140 degrees. The change in neck-shaft angle between groups was not significant (p=0.072, CI 3.19-41.7) and ranged from -27.0-0.0 degrees in the surgery group and -20.0-9.0 in the therapy group. The change in the center-edge angle of Wiberg (normal >20 degrees) between groups was not significant (p=.2638, CI: -57.3-22.8) and ranged from -6.0-31.0 in the surgery group and -9.0-19.0 in the therapy group. The change in MP (normal <30 degrees) between groups was not significant (p=0.7113, CI: -51.4-39.7) and ranged from -11.2-37.6 in the surgery group and -20.6-25.0 in the therapy group. The change in acetabular angle (normal >25 degrees) between groups was also not significant (p=0.848, CI: -20.3-17.9) and ranged from -13.0-0.0 in the surgery group and -17.0-4.0 in the therapy group. 75% of patients in the surgery group were able to ambulate before surgery; this decreased to 25% after surgery. 75% of patients in the surgery group were able to ambulate before therapy; this increased to 100% after therapy.

CONCLUSION/DISCUSSION: The findings supported the hypothesis, but because this treatment is only offered at one clinic, this study is limited by a small sample size. More research is needed to further compare the outcome of casting and therapy to surgery. The first step is to train more physical therapists in the casting procedure and therapy methods. Next, a multi-site longitudinal study would be necessary to reach enough patients. Additionally, future studies should include more thorough assessments of pre- and post-treatment functional status, such as GMFCS. If future studies continue to support the hypothesis, serial casting with therapy may achieve the surgery’s benefits without its risks, improve gross motor function, and decrease overall medical costs.
Glycerol Monolaurate (GML) Inhibits T Cell Activation by Simultaneously Decreasing Oxidative Phosphorylation and Increasing Glycolysis

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Background. Glycerol monolaurate (GML) is a natural fatty acid monooester found in breast milk and used widely in foods, personal items, and industrial products. It powerfully inhibits the growth of bacterial, viral, and fungal pathogens and is on the FDA’s Generally Recognized as Safe (GRAS) list. For these reasons, GML is currently being tested to treat Toxic Shock Syndrome, HIV transmission, and other topical and systemic microbial infections. Interestingly, select studies hint that GML may also act as an immunosuppressant. GML-treated T cells exhibit decreased superantigen-induced cellular proliferation and inositol triphosphate generation, suggesting that GML inhibits T cell signaling and function. Preliminary data from our lab show that GML inhibits T cell receptor (TCR)-mediated cytokine production and the SLP-76-AKT signaling axis. AKT function is critical for regulating T cell energy metabolism. Furthermore, T cell metabolism is intimately linked with T cell activation and effector functions. Thus, GML may significantly modulate the metabolism of human T cells that suppresses T cell functions. The purpose of this study was to characterize the effects of GML on T cell glycolysis and oxidative phosphorylation as a possible mechanism for GML-induced T cell suppression.

Hypothesis. GML alters the metabolism of pre-activated T cells by attenuating glycolysis and/or enhancing oxidative phosphorylation.

Methods. Peripheral blood mononuclear cells (PBMCs) were freshly isolated from the whole blood of healthy donors using leukocyte reduction system cones. T cells were selectively pre-activated and expanded from PBMCs using anti-CD3/CD28 coated beads and human IL-2 for 5 days. The resulting activated peripheral blood T cells (APBTs) were rested in fresh media without stimulation for 24 hours and treated with either GML or ethanol as control. Cellular metabolism was assessed by measuring basal oxygen consumption rate (OCR) for oxidative phosphorylation and extracellular acidification rate (ECAR) for glycolysis using the XF-96 Extracellular Flux Analyzer (Seahorse Bioscience).

Results. We found that pre-activated T cells treated with GML exhibited significantly decreased oxidative phosphorylation at maximal respiration with a trending decrease in basal respiration in a dose-dependent manner. These same cells also exhibited increased basal glycolysis, possibly as a compensatory energy production mechanism for decreased oxidative phosphorylation. Further time-course experiments revealed an immediate (within 5 minutes) decrease in OCR following the direct addition of GML to the cells, accompanied by a simultaneous jump in glycolysis trending upwards over time.

Conclusion and Discussion. GML-treated cells have altered metabolism characterized by decreased oxidative phosphorylation and increased glycolysis. This metabolic change could be caused either by direct physical inhibition of mitochondrial function by GML or by indirect GML-mediated disruption of mitochondrial localization. Regarding the direct mechanism, preliminary data show that GML alters membrane dynamics by increasing lipid disarray in the plasma membrane. Mitochondrial function depends critically on membrane composition for the proper execution of respiratory processes and may be similarly affected by GML. Regarding the indirect mechanism, mitochondria are transported along cytoskeletal tracks to the TCR upon T cell activation, where they play key roles in calcium signaling and ATP production. Preliminary data show that GML treatment drastically reduces cytosolic calcium influx and disrupts actin polymerization. We believe that the GML-induced metabolic phenotype is caused either by altered mitochondrial membrane composition or by decreased cytoskeletal trafficking of mitochondria to the TCR that is ultimately caused by GML-mediated disruption of actin polymerization.
Title: The LPO/DUOX/Halide Airway Host Defense System Has Antiviral Properties Against Influenza A.

Names: Rachel Schenkel, Anthony Fischer, Sateesh Krishnamurthy, Jennifer Bartlett, Paul B. McCray

Background: Lactoperoxidase (LPO) is secreted by submucosal glands in the conducting airways and catalyzes an oxidative reaction between hydrogen peroxide produced by dual oxidase (DUOX) enzymes DUOX1 and DUOX2 and SCN⁻ and I⁻ present in airway secretions. The resulting hypohalides (HOI and OSCN⁻) have been shown previously to have antimicrobial and antiviral properties. In particular, an earlier study in our lab indicated that HOI can inactivate respiratory viral pathogens including the respiratory syncytial virus (RSV) and adenovirus.

Hypothesis: Influenza A (IAV) is inactivated by the LPO/DUOX system in a manner similar to RSV and adenovirus.

Methods: We first tested whether the LPO/DUOX system could inactivate influenza A (strain PR8) under cell-free conditions. H1N1 PR8 IAV expressing green fluorescent protein (PR8-GFP) was incubated for five minutes in test tubes with components of the LPO/DUOX/halide system in various combinations. Viral inactivation was then assessed by applying each inoculum to MDCK cells and visualizing fluorescence 24 hours later. Next, we tested for cell-dependent viral inactivation by infecting well-differentiated human airway epithelia with PR8-GFP in the presence of these components and visualizing fluorescence 24 hours later.

Results: We observed that the LPO/DUOX/halide system successfully inactivated IAV PR8-GFP under cell-free conditions, as well as in the more physiologically relevant context of cultured well-differentiated primary human airway epithelial cells.

Conclusion/Discussion: Our findings suggest that the LPO/DUOX/halid system effectively reduces the infectivity of the influenza A virus. Interestingly, we also observed that HOI may display more effective viral inactivation than OSCN⁻ within the LPO/DUOX system, and that OSCN⁻ may be more effective at inactivating influenza than it is at inactivating RSV or adenovirus. Future studies with large animals will address the relative biological significance of this antiviral mechanism.
Evaluation of the efficacy, effectiveness, and cost-effectiveness of telemedicine for urologic care in the state of Iowa’s prison population

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ABSTRACT

Background: Access to specialist physicians such as urologists is limited in rural areas, and often requires long-distance travel by patients and/or physicians. Telemedicine offers a potential solution to this problem, though its use has yet to be adequately evaluated for efficacy in urology. The UIHC Department of Urology has been using telemedicine to consult with Iowa’s prison population for the last decade.

Purpose of the Study: The purpose of this study was to describe the use of telemedicine in Iowa’s prison population from 2007 to 2014 and to evaluate its effectiveness.

Methods: We retrospectively reviewed the medical records of all prisoners in the state of Iowa who were evaluated by the Department of Urology at UIHC via telemedicine from Jan 2007 – Jul 2014 after obtaining IRB approval. During this time, a total of 376 unique and 154 repeat telemedicine encounters occurred, of which 100% were reviewed for the type of urologic disease, the initial management, and the effectiveness of the telemedicine visit which was determined by comparison to an actual clinic visit. We counted a telemedicine visit as saving a trip to the clinic if it was completely handled by telemedicine, if radiology was performed before the clinic visit, if medication was taken before the clinic visit, or if the patient had more than one telemedicine visit before the clinic visit.

Results: The most common chief complaints were voiding/storage complaints (53%), pain in a genitourinary organ (40%), hematuria (20%), a symptomatic urologic lesion (19%), and an asymptomatic urologic lesion (11%) resulting in the most common primary diagnoses of benign testicular lesion (23%), lower urinary tract symptoms/BPH (14%), and hematuria (11%). Telemedicine management included medication prescription (40%), radiologic testing (47%), follow-up to UIHC urology for cystourethroscopy (26%), and lab work (urine: 24%, blood: 22%). Return UIHC urology visits were scheduled for 48% of patients, and 37% had telemedicine visits rescheduled. Of patients returning for a UIHC urology visit, 60% were asked to obtain a pre-visit radiologic test, of which, 88% were completed and 36% were prescribed medication of which 91% were compliant.

The telemedicine diagnosis exactly matched the clinic visit diagnosis 79% of the time and 9% of the time it was part of the differential provided by the telemedicine provider. Only 9% of the time did the UIHC urology diagnosis differ significantly and 3% did not have a specific diagnosis mentioned at the telemedicine encounter. Telemedicine effectively prevented the need for an in-person visit in a minimum of 76% of our patient population. In total, we estimate that telemedicine led to a direct cost savings of approximately $75,000 in transportation and personnel costs, or an average of $100 per patient per trip. On average, each patient who was saved a trip saved 3 hours 50 minutes of travel time per trip.

Conclusions: We demonstrated that telemedicine was an effective and efficient method to increase access to urologic care for our state’s prisoners. At least 76% of telemedicine visits saved the patients an in-person visit leading to significant direct cost savings. Importantly, telemedicine visits effectively diagnosed the urologic disorder 88% of the time. We believe these data show that telemedicine may be an effective tool to expand urology care to patients in rural areas and simultaneously decrease costs. Future directions will include identification of risk-factors for incorrect telemedicine diagnoses and the need for earlier in-person visits so to continue to improve the effectiveness of the program and to expand our program to non-prisoner populations.
Relationship between hippocampal volume, spatial memory, and cardiorespiratory fitness in older adults

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¹M2, ²Neuroscience PhD Candidate, ³PhD, Assistant Professor of Psychological and Brain Sciences

Background
As the elderly population in the United States continues to rise, the reality of age-related declines in cognitive abilities is becoming a significant public health concern. Particularly during Alzheimer’s Disease and other dementias, individuals may lose abilities that are essential for independent daily living, such as navigating through the world and forming and consolidating memories. One region of the brain that is especially vulnerable to age and responsible for many of these critical functions is the hippocampus. Previous research has shown that the hippocampus atrophies during normal aging. Fortunately, researchers have found that the hippocampus demonstrates plasticity, adapting structurally and functionally in response to various forms of mental and physical engagement. Evidence has shown that higher physical fitness is related to lower rates of hippocampal atrophy in aging adults, as well as better performance on memory tasks. One particular task that utilizes the hippocampus focuses on evaluating spatial memory. Watson and colleagues discovered that amnesic patients, individuals with damaged hippocampi, had a marked error rate in a particular aspect of a spatial reconstruction task (swaps). Therefore, it may follow that hippocampal volume may predict performance on a spatial reconstruction task and could also be related to fitness level. Demonstrating this relationship would provide compelling support in developing a task that could be used as a functional assay of hippocampal function, as well as further stress the importance of fitness to our mental health.

Hypothesis
Hipocampal volume in healthy older adults will be positively correlated with 1.) performance on spatial reconstruction task, with swap errors demonstrating the strongest relationship and 2.) cardiorespiratory fitness.

Methods
Data were obtained from 11 older adults (7F/4M), aged 56-80 years old (M: 64.6 yrs, SD: 3.34). Participants had an average of 20 years of education (SD: 2.26). Aerobic fitness was assessed using a graded maximal exercise test, during which participants pedaled on a stationary bike with incremental increases in resistance every 2 minutes. The test concluded when participants could no longer continue, providing a measure of maximal oxygen usage (max VO₂ ml/kg/min). Spatial reconstruction ability was tested using a laboratory task administered on a touch screen device. Performance was measured using four error metrics (misplacement, edge deflection, edge resizing, and swaps). High-resolution (1mm³) T1-weighted structural scans were acquired using an MPRAGE protocol in a 3T Siemens MRI scanner. Hippocampal volume was determined using FSL’s Integrated Registration and Segmentation Tool (FIRST) and adjusted on an individual bases for brain size.

Results
Several error metrics showed negative correlations with respect to right hippocampal volume, including average misplacement ($r = -.488, p = .076$), average resizing ($r = -.594, p = .035$), and average deflection ($r = -.468, p = .086$). It was noted that average misplacement and average deflection were only trending towards significance. Average swaps had a negative, but non-significant correlation with right hippocampal volume ($r = -.395, p = .129$). None of the error metrics showed significant correlation to left hippocampal volume. Absolute VO₂ showed moderate positive correlation to left hippocampal volume ($r = .650, p = .021$), and a positive, though non-significant, correlation with right hippocampal volume ($r = .474, p = .083$).

Conclusion
We observed that left hippocampal volume was positively correlated with physical fitness. Additionally, right hippocampal volume correlated with performance on the spatial reconstruction task. Both of these results partially support our initial hypothesis, as we did not specify laterality. Interestingly, our results showed that of the four error metrics, swaps was the only metric not to demonstrate a relationship with hippocampal volume. Based on previous studies, the swap error metric was predicted to have the strongest negative correlation with hippocampal volume. One possible explanation for this finding was that the literature evaluated amnestic, while we evaluated healthy elderly adults. We suggest that swapping is not an error that healthy adults often commit, therefore explaining this discrepancy. We also found it interesting that the hippocampi seemed to demonstrate laterality with regards to the correlation with cognition and physical fitness. Based on our results and current understandings in the field of neuroscience, we believe that maintaining a higher level of physical fitness may contribute to larger hippocampi in the elderly and promote better spatial reconstruction performance.
Cardioprotective Effects of Leptin Supplementation in the Midst of Neonatal Growth Restriction
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BACKGROUND:
During the critical third trimester of human gestation, a vast majority of cardiomyocytes enter the terminal phase of proliferation and then become binucleated. Intrauterine growth restriction (GR) is associated with reduced cardiac growth and increased cardiovascular mortality. In both sheep and rat models, GR delays cardiomyocyte maturation and suppresses proliferation, resulting in substantially depressed cardiomyocyte endowment and adult cardiac size. We sought to develop a mouse model of environmentally constrained cardiac growth and our prior investigations suggested a potential therapeutic role for leptin. Plasma leptin levels are dramatically reduced in the midst of GR, and in developing cardiomyocytes, leptin exerts pro-proliferative effects.

PURPOSE OF THE STUDY:
The specific aims of the study were to determine the effect of leptin on cardiac growth by (i) assessing cardiomyocyte fate with neonatal leptin administration, (ii) testing the ability of leptin to normalize the cardiac morphology of GR mice.

METHODS:
Given their relative developmental immaturity, neonatal mice model the critical third trimester of human cardiac development. At birth, C57/B6 pups were cross-fostered into litters of 6 or 12 to encourage variation in weaning weight. From postnatal day 4 to 8, pups received daily injections of saline or leptin at a dose shown to normalize plasma levels with altering neonatal growth (80 ng/g/d). On day 8, pups <3.6g were considered GR. For some day-old mice, hearts were mounted, sectioned and immunostained for Ki67, as a marker of proliferation. Immunoreactive cells were counted using the Aria slide scanner and then indexed to total cell counts with hematoxylin nuclear counterstain. Alternatively, cardiomyocytes were enzymatically dissociated, then stained with methylene blue (cell body) and hematoxylin (nuclei) followed by determination of nuclear count and surface area with ImageJ and Spot software. Additional male mice were raised into adulthood and those with a weaning weight < 10th percentile were considered GR. Echocardiography was performed in 4-month-old mice by an investigator blinded to group assignment using the Vevo 2100 High Resolution Imaging System (VisualSonics Inc.). Left ventricular structure and function was determined in accordance with the American Society for Echocardiography Guidelines. Statistical significance was assessed by two-tail two-way ANOVA factoring for GR and leptin administration.

RESULTS:
On postnatal day 8, cell cycle activity was detected by Ki67 staining in 48-49% of cardiac cells, independent of neonatal growth restriction or leptin administration. However, a significant interaction was present between GR and leptin influenced the percent of cardiomyocytes that were terminally differentiated or binucleated (Table 1). On average both mononucleated and binucleated cardiomyocytes from GR mice were 22% smaller than those from control mice, and cell size was normalized by neonatal leptin supplementation (Table 1).

Table 1. Effects of Neonatal Growth Restriction (GR) and Neonatal Leptin Supplementation on Day 8 Cardiomyocyte Morphology

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight (g)</th>
<th>Binucleated (%)</th>
<th>Mononuclear Cell Area (µm²)</th>
<th>Binuclear Cell Area (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline</td>
<td>4.8 ± 0.1</td>
<td>72 ± 2</td>
<td>285 ± 10</td>
</tr>
<tr>
<td>GR</td>
<td>Saline</td>
<td>3.1 ± 0.1 *</td>
<td>50 ± 1 *</td>
<td>224 ± 2 *</td>
</tr>
<tr>
<td>Control</td>
<td>Leptin</td>
<td>4.2 ± 0.1 †</td>
<td>64 ± 14</td>
<td>252 ± 3 †</td>
</tr>
<tr>
<td>GR</td>
<td>Leptin</td>
<td>3.0 ± 0.2 *</td>
<td>68 ± 3 †</td>
<td>268 ± 19 †</td>
</tr>
</tbody>
</table>

*P < 0.05 versus control, † P < 0.05 versus saline

As adults, GR mice had significant reductions in left ventricular volumes in diastole (GR 60+/−5 microliters; control 75+/−4 microliters) with corresponding increases in adult heart rate (GR 576+/−4 bpm, control 551+/−5 bpm, P<0.05). The changes in adult cardiac structure and heart rate were independent of neonatal leptin administration.

DISCUSSION:
Environmentally-induced perinatal growth restriction reduces or delays cardiomyocyte growth and differentiation, potentially leading to long-term morphologic and hemodynamic effects. Further studies are needed to identify the GR-related pathways and processes that limit neonatal cardiomyocyte growth and contribute to postnatal recovery.
Flow Differential as a Method of Predicting ECMO Oxygenator Failure

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ABSTRACT

BACKGROUND: Extracorporeal membrane oxygenation (ECMO) is prolonged partial heart-lung bypass used for patients with severe but reversible cardiac or respiratory failure. Complications related to the oxygenator affect 20% of patients carrying a mortality of 40-80%. Current practices for monitoring oxygenator function measures pressure differential, requiring in-line access, increasing the risk of infection, clot emboli, and air in the system. Furthermore, the function is only checked periodically, which can delay the recognition of decreased oxygenator function. Alternatively, measuring the flow differential across the oxygenator does not require in-line access and provides continuous data on oxygenator function.

HYPOTHESIS: The flow differential across the oxygenator is an accurate indicator of oxygenator failure.

METHODS: The experimental ECMO circuit was comprised of the same components as those used in clinical practice. Flow sensors were added immediately before and after the shunt, as well as an adjustable resistance component after the oxygenator. Saline was used as the experimental fluid. Flow rates were increased incrementally from 500 to 5000 ml/min, with obstruction increased from none to severe obstruction for each flow rate by varying degrees of circumferential narrowing of the circuit tubing. For each experimental setup, the following measurements were obtained: pressures before and after the oxygenator, flow out of the pump, and flow through the oxygenator. From these measurements, the pressure drop (ΔP) and flow differential (ΔQ) were calculated and compared. Data was analyzed using student-t tests comparing ΔQ at moderate and severe obstruction to baseline measures at no obstruction with statistical significance of two-tailed p<.05. This was repeated with ΔP for comparison.

RESULTS: Twelve experiments were performed on the ¼” circuit with flow rates of 500, 1000, and 1500 ml/min. Ten experiments were conducted on the ⅜” circuit with flow rates of 2000, 3000, 4000, and 5000 ml/min. For each experimental setup, mean ΔQ and ΔP were calculated, as well as the respective standard deviations. For all flow rates, ΔQ increased from none to moderate to severe obstruction with a p-value of <0.01. The current marker, ΔP, also increased from none to moderate to severe obstruction for all flow rates with a p-value of <0.01.

CONCLUSION: These results are consistent with the hypothesis that flow differential across the oxygenator is an accurate indicator of oxygenator obstruction. Flow differential has the potential to be an equally as accurate indicator of oxygenator function as pressure differential, enhances safety by avoiding in-line access, and minimizes delay by providing continuous data.
Analysis of Physician and Practice Baseline Surveys for The ICARE (Improved Cardiovascular Risk Reduction to Enhance Rural Primary Care) Study

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ABSTRACT

BACKGROUND: An AHA report states that “…more than 2,200 Americans die of cardiovascular disease (CVD) every day…1 death every 39 seconds.” In an effort to combat the high prevalence of CVD and its burden on the United States’ healthcare system, it is necessary to understand and enhance the role played by primary care providers in conjunction with pharmacists to manage CVD and multiple other associated chronic health conditions. The overall objective of this clinical trial is to determine whether a centralized, remote pharmacist-managed cardiovascular risk service (CVRS) can work with primary care physicians located in locations across the state of Iowa to decrease risk of CVD in high-risk patients. Another aim of this study, and the focus of this abstract, is to utilize validated surveys to evaluate physician openness to inter-professional collaboration with pharmacists.

HYPOTHESIS: At baseline, there should be no difference between the intervention and control arms regarding provider views towards collaboration with pharmacists.

METHODS: 59 healthcare providers at 12 family physician offices throughout Iowa participated in this portion of the study. These 59 healthcare providers are treating the 300 subjects recruited to the ICARE study. Prior to the initiation of the intervention, 34 providers at control sites and 25 providers at intervention sites filled out two validated surveys. The Physician Collaboration Survey asks all healthcare providers in each office to identify factors that influence the ability to develop a positive working relationship between physicians and pharmacists. The Managing Cardiovascular Disease States Questionnaire asks all providers in each clinic to quantitatively assess the physician/pharmacist collaborative model put into practice in their clinic. The survey results were entered into the study database and analyzed using SPSS statistical software.

RESULTS: Using a non-parametric independent sample Mann-Whitney test to analyze the data, analysis showed that for both surveys, the median provider scores were higher in the control group. This indicates that providers at the control clinics are more likely than counterparts in the intervention clinics to refer patients to pharmacists, and communicate, cooperate with, and trouble-shoot patient-related problems alongside pharmacists.

CONCLUSION & DISCUSSION: At baseline, the healthcare providers in the control arm had more positive views towards collaboration with pharmacists. 12 Month Follow-Up surveys will be sent out and analyzed to determine whether the 12-month intervention period plays a role in shifting the views of either or both arm of the studies. It is hypothesized that the 12-month follow-up survey should yield results indicating that the providers at intervention sites are more open to collaborating with pharmacists.
Discovery of differential HDAC4 methylation sites in anorexia nervosa
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1Roy J. and Lucille Carver College of Medicine, University of Iowa; 2Department of Psychiatry, University of Iowa

Background
Anorexia nervosa (AN) is a multifactorial disorder characterized by maintenance of low body weight and body image dissatisfaction. Many studies have shown a significant genetic contribution to AN, yet environmental factors also play a major role. Epigenetics, the study of changes in gene expression without alteration of DNA sequence, may mediate the impact of the environment on the brain in AN. DNA methylation is one main epigenetic mechanism, and it involves the addition of a methyl group to a cytosine-phosphate-guanine (CpG) nucleotide. Genome-wide DNA methylation studies have shown that several genes are hypermethylated in AN, and it is hypothesized that methylation may suppress gene expression, leading to pathophysiology in the disease. We therefore sought to discover DNA methylation patterns of the transcription corepressor histone deacetylase 4 (HDAC4) based on two lines of converging evidence: first, HDAC4 contains a CpG island that was significantly associated in a genome-wide DNA methylation study of AN. Second, whole exome sequencing demonstrated that a rare missense mutation in HDAC4 was implicated in AN.

Hypothesis
Targeted methylation sequencing will validate hypermethylation of the HDAC4 CpG site at chromosome 2: 240171748 in females with AN compared to females without AN.

Methods
We designed a case-control study using saliva-derived DNA. Cases were defined as females between 18-40 years of age with restrictive-type AN. Controls were age- and sex-matched to cases and included individuals without eating disorder history and excluded those on psychiatric medications at the time of saliva collection. DNA was obtained from the Lutter Lab and the Tissue Procurement Core at the University of Iowa for cases and controls, respectively. DNA was then bisulfite converted using the EZ DNA Methylation™ Kit. To amplify and sequence the HDAC4 CpG region of interest, we created primers for the DNA region 300 basepairs upstream and downstream of coordinate 240171748. We then performed a nested-PCR reaction for amplification and used PyroMark Q96 MD for methylation pyrosequencing of six CpG sites. Site-specific percent methylation was recorded for cases and controls and data were analyzed using Student's t-test.

Results
We assayed 27 cases and 27 controls for each sequencing primer. Our findings demonstrate that two out of the six HDAC4 CpG sites were nominally associated with restrictive-type AN and hypomethylated. At DNA coordinate 240171760, the average methylation was 85.4% (standard deviation ± 6.1) in cases and 88.4% (± 3.7) in controls (p=0.03, difference=3.0%); at location 240171772, the average methylation was 79.5% (± 8.6) in cases and 84.7% (± 7.5) in controls (p=0.02, 5.2%). We found no significant association between the CpG site at 240171748 and AN (p=0.2).

Conclusions
By using a targeted methylation sequencing approach, we were able to expand coverage of CpG sites over a small area and identify additional CpGs nominally associated with AN. However, it is not clear whether the small differences in methylation between cases and controls for associated sites are biologically meaningful. To gain a better understanding of our data and HDAC4 methylation patterns, we will first increase the sample size of our study; second, we will conduct similar analyses on peripheral blood-derived DNA; third, we will assess methylation patterns of other subphenotypes of AN, such as binge-eating/purging AN.
Discovery of proteins that interact with ATF4 in skeletal muscle

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Background: The basic leucine zipper transcription factor ATF4 plays an essential role in skeletal muscle weakness and atrophy during aging, malnutrition, and immobilization. However, the mechanism by which ATF4 promotes muscle weakness and atrophy remains poorly understood.

Hypothesis: ATF4 is a central component of a multiprotein complex that causes muscle weakness and atrophy.

Methods and Results: We initiated an unbiased search for proteins that interact with ATF4 in mouse skeletal muscle. For this purpose, we generated an ATF4 construct suitable for tandem affinity purification. This construct, which we termed ATF4-TAP, consists of full-length ATF4 with two sequential epitope tags (FLAG and S-tag) at the NH2 terminus. After verifying that ATF4-TAP retains the original capacity of ATF4 to activate a key target gene (Gadd45a) and cause muscle fiber atrophy, we performed a large scale experiment in which the tibialis anterior muscles of 11 C57BL/6 mice were transfected with plasmid encoding ATF4-TAP (one leg) or plasmid encoding the FLAG and S-tag epitopes without the ATF4 insert (contralateral leg). We then harvested bilateral muscles from each mouse, and conducted tandem affinity purification; briefly, we prepared and pooled protein extracts containing either ATF4-TAP or the control construct, and then subjected each of the two pooled protein extracts to sequential purification steps with anti-FLAG and S-tag affinity gels. Purified proteins were visualized by SDS-PAGE and silver staining. Silver staining revealed that multiple proteins were present in the sample containing ATF4-TAP and absent in the control sample lacking ATF4-TAP. We are now using mass spectrometry to determine the identity of proteins that specifically interact with ATF4 in skeletal muscle.

Conclusion: ATF4 is a component of a multiprotein complex in mouse skeletal muscle. Skeletal muscle proteins that interact with ATF4 represent novel potential mediators of ATF4 activity, weakness, and muscle atrophy during aging, malnutrition, and immobilization.
The Role of Effective Communication during the Postoperative Conversation with Family

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Background
Families often have an essential role in postoperative care of surgical patients. It has been found that families are able to support patients more effectively if they are well-informed about surgical outcomes. The conversation in the immediate postoperative period provides an opportunity for surgeons to communicate with the family regarding patient status and care. While surgeons and surgical residents acknowledge the value of postoperative communications with family members, there has been minimal research as to the content and effectiveness of such conversations. While a previous study of surgeons` self-reported perioperative communication practices with families revealed that content addressed in postoperative discussions was similar among surgeons, there is a lack of primary observational data of postoperative discussions between families and surgeons.

Purpose
The aim of this study was to broaden the understanding of surgeon-family postoperative conversations that occur immediately following surgery through the analysis of conversation content and structure.

Method
Postoperative conversations immediately following surgery between surgeons and patient families were audio recorded, after which surveys regarding communication effectiveness were administered to family members. The recordings were transcribed, coded, and analyzed using the QSR NVivo 10 program. An iterative process was utilized to identify and compare the content of these conversations (i.e. patient status, outcome, postoperative course, etc.) as well as aspects of the communication process (i.e. question elicitation, number and types of questions asked, etc.). Using a modified EPSCALE, conversations were evaluated regarding general communication skills in explanation and planning by two independent researchers. For each pre-defined category, a numerical scale was applied, with zero indicating poor performance and three indicating excellent performance. The survey data was analyzed using quantitative methods.

Results
Postoperative conversations from a total of 25 surgical cases were recorded and analyzed. Most of the cases required inpatient admission (84%) and included bariatric, colorectal, surgical oncology, and minimally invasive procedures. The average conversation length was four minutes (range 1-7, mode 3). Family members present for the discussion ranged from one to three (mode of one) with an average age of 51 years (range 17-72). Subject matter pertained to what happened during surgery, surgical outcome, and short-term postoperative course, which were addressed in nearly every discussion (100%, 96%, 92%, respectively). In 84% of the postoperative conversations, surgeons explicitly prompted family inquiry, largely at the closure of the conversation (88%) as opposed to throughout. Additionally, checking for family understanding was a notable component in 76% of conversations, predominantly in the midst of the discussion (63%). The average EPSCALE score for “Checks family’s understanding” was 1.1 (mode 1, range 1-2). Overall, families believed they understood what the surgeon said with a mean survey score of 4.875/5. The family’s baseline knowledge of the procedure was only assessed in two of 25 cases, and the mean EPSCALE rating for “Assesses the family’s starting point” was accordingly low (0.12). Family members asked an average of four questions per conversation, primarily related to the short-term postoperative course (29%), what happened during surgery (25%), and logistics on visiting the patient (16%). With an average satisfaction score of 4.95/5, families felt that all of their questions were answered. On the whole, patient families considered surgeon communication very effective with a mean survey rating of 4.9/5.

Conclusion
Surgeon-family communication is often a disregarded facet of surgical patient care, and up to this point, there has been no direct observational data of postoperative conversations. This study presented an initial assessment of the contextual and structural patterns of postoperative conversations with families. It was demonstrated that these conversations share common contextual themes but lack multiple elements of effective explanation and planning. Data collected from this investigation has potential to contribute to the development of educational training materials for medical students, residents, and surgeons. The long-term goal is to guide surgeons on how to communicate effectively with families, thus improving family, patient, and surgeon satisfaction. Future directions for this area of study include: evaluation of family comprehension, comparison between surgeon and family perceptions of the conversations, and juxtaposition of the pre and postoperative interactions.
Identifying differentially expressed genes following the knockdown of RGD1562963 in H4IIE hepatoma cells

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Abstract

Background: Metabolic Syndrome (MetS) is a complex disorder encompassing hypertension, obesity, dyslipidemia, and diabetes. This set of disorders affects 34% of Americans and costs the healthcare system 30 billion dollars per year. In previous studies, a novel candidate gene, RGD1562963, was identified as a putative regulatory gene contributing to the complex etiology of MetS. To better understand the genes regulated by RGD1562963, we used siRNA to knockdown RGD1562963 expression in the H4IIE rat hepatoma cell line. RNA-Sequencing was subsequently performed to assess which genes are regulated by RGD1562963.

Hypothesis: Silencing RGD1562963 in the H4IIE hepatoma cells would dysregulate downstream gene expression and lead to the traits associated with MetS.

Methods: The Galaxy interface was used to run TopHat2, Cufflinks, and featureCounts to convert the raw RNA-Sequence data into mapped reads on the genome. EdgeR was run on these results using the RNASeqGUI through R Studio to determine differential expression between cells that had been transfected with the siRGD1562963 and those transfected with a scrambled siRNA control. The results were run through DAVID to determine the functional annotations of the genes identified by EdgeR. qPCR was performed on a subset of the differentially expressed genes based on their significance, expression level, and DAVID results.

Results: The RNA-Seq analysis identified 24 differentially expressed genes (FDR <0.05). Multiple genes (Ahsg, Gpx1, Slc25a24, and Pon2) identified through DAVID have been shown to influence cardiac health and oxidative stress. This could indicate a possible mechanism connecting RGD1562963 to the phenotype associated with MetS.

Conclusion: Decreasing expression of RGD1562963 in rat hepatoma cells appears to alter expression of a subset of genes, some of which are already related to MetS or its associated phenotypes.
Long Term Evaluation of Patients with Congenital Heart Disease and Congenital Arrhythmias with Pacemakers

Justin Vaverka, Joseph Turek, Mark Olson, Ian Law, Nicholas Von Bergen

Introduction: Congenital heart diseases (CHD) and congenital arrhythmia or bradycardia syndromes represent a common cause of both morbidity and mortality, and may be associated with both structural and electrophysiological concerns. Many CHD patients undergo pacemaker implantation for reasons including heart block, sinus node dysfunction, and arrhythmias and those with congenital arrhythmia substrate may have a surgical placement of a pacemaker at a young age. This study evaluates the longterm outcomes, need for repeat intervention, and longevity of pacemaker generators and leads in patients treated with pacemakers who were diagnosed with a congenital heart disease, congenital arrhythmia.

Methods: IRB approval was obtained. Surgical records, patient charts, pacemaker interrogations, and echocardiographic data were collected from the University of Iowa pacemaker, medical records and surgical databases. Patients were included if they had a pacemaker and were diagnosed CHD, congenital heart block, sinus node dysfunction or other congenital arrhythmia substrate. All patients who underwent pacemaker implantation before May 30th 2014 were included. A REDCap database utilized.

Results: The study population is made up of 239 patients, who combined have had 484 generators and 599 leads implanted since 31 March 1975. The median age at first pacemaker implant was 6 years. Of the 239 patients, 68.2% (163) utilized epicardial leads for their first pacemaker, 31% (74) endocardial leads, and 0.8% (2) a mixture of lead types. Patients with structural CHD represented 71.1% (170) of the population and included 10.9% (26) with dextro-TGA and 12.6% (30) with levo-TGA. Surgical heart block was an indication in 38.5% (92) patients, sinus node dysfunction in 15.5% (37) and for arrhythmia treatment in 14.6% (35) of patients. The most common indication for patients without structural heart disease was congenital complete heart block (24.3%). DDD (40.6%) and VVI (23.8%) were the most common pacing setting at the time of first implant. On average, the first generator was replaced 5.8 years after implantation (N=121). The mean longevities did not differ significantly for the first four generators in an individual (p=0.745). 15.1% of the population was still using their first generator 10 years after initial implantation by Kaplan-Meier. By this same time mark, approximately 70% had undergone a lead revision or lead addition. For patients requiring lead addition or revision, the mean time to this surgery was 5.78 years (N=100). Echocardiographic analysis revealed no substantial change in ejection fraction and a slight decrease in shortening fraction over a 30 year timeframe.

Discussion: This study evaluated pacemaker implants over the last four decades in the congenital heart disease and congenital arrhythmia patients. In this population, initial pacemaker implantation frequently occurs in childhood, and epicardial leads were consequently the most common initial lead type. Many patients required follow-up pacemaker surgeries within 10 years of implantation for generator depletion, lead revision, or device upgrade. Despite the need for lifelong care in this population, systolic function did not appear to suffer substantially in the long-term.
**Precision Medicine: Personalized Proteomics for Diagnosis and Treatment of Idiopathic Inflammatory Disease**

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**Abstract**

**Background:** Inflammation of the retina and choroid (termed posterior uveitis) accounts for approximately 10% of blindness in the US; and, in one in 4,500 people, it can be an early sign that heralds a debilitating and systemic autoimmunity. Nevertheless, the etiologies of more than 50% of posterior uveitis cases are undetermined, and so labeled as idiopathic. Misdiagnosis delays the prompt, aggressive treatment of posterior uveitis and allows immunologic attack, which is poorly tolerated by the neurosensory retina, to progress unabated, causing significant morbidity and blindness.

**Hypothesis:** The vitreous acts as a molecular catalogue of intra- and extracellular immune signaling events. In a patient with chronic, idiopathic posterior uveitis, we hypothesized that proteomic profiling of their vitreous biopsy might further define their disease.

**Methods:** A membrane-based, antibody array was used to analyze the presence of 200 different cytokines in the vitreous of 15 uveitis cases and five non-inflammatory control cases. Bioinformatics was used to analyze cytokine signatures and identify molecular pathways upregulated in uveitis.

**Results:** Unbiased cluster analysis identified a unique cytokine expression pattern that showed our patient’s biopsy fluid was highly similar to patients generating antiretinal antibodies. Subsequent laboratory testing verified serum expression of antibodies against S-arrestin, a known antigenic trigger of retinal inflammation, and appropriate therapy was initiated. In addition to disease-specific patterns, the analysis also identified a common cytokine expression signature for posterior uveitis, suggesting the use of specific anti-cytokine therapies that are otherwise typically not used for inflammatory eye disease.

**Conclusions:** Cytokine expression profiles of eye-fluid biopsies were characterized in a panel of uveitis patients and used to diagnose a patient that conventional testing failed. This case illustrates the power of proteomics to discriminate autoimmune retinopathy from other inflammatory causes and individualize therapy. Such an approach can be applied to fluid biopsies of other inflamed tissues.
CHOP expression in Schwann cells of CMT1B patients and morphometric analysis of cutaneous nerve fibers

David S Wang, Yunhong Bai, Xingyao Wu, Michael E Shy

**Background:** Charcot-Marie-Tooth Disease or CMT is an inherited, degenerative, peripheral neuropathy caused by irregularities in the myelin sheaths or neuronal axons; it occurs in around 1:2500 individuals. CMT type 1B (CMT1B) is dominantly inherited and in many cases are characterized by reduced motor nerve conduction velocities and segmental demyelination – this can manifest itself through foot deformities, muscle weakness, atrophy, and reduced sensation in the distal areas of limbs while also resulting in problems with balance and gait. Symptoms in many CMT cases are likely to progress with age. In CMT1B, mutations in the Myelin Protein Zero (MPZ) gene can negatively impact the folding of MPZ – an essential structural glycoprotein that constitutes a significant portion of all myelin protein. Misfolded MPZ can be retained in the endoplasmic reticulum (ER) of myelinating Schwann cells rather than transported to the myelin sheath; the continuous buildup of misfolded protein creates ER stress in the cell and activates the Unfolded Protein Response (UPR), a multi-cascade signaling pathway that functions to reduce the amount of misfolded protein, such as through attenuating protein translation and decreasing the rate of protein synthesis. C/EBP homologous protein (CHOP) transcription factor translocation to the nucleus in Schwann cells is a consequence of the PERK arm of UPR and promotes apoptosis or an altered phenotype in the stressed cell when other methods to reduce ER stress fails. Schwann cell stress, apoptosis, and altered phenotypes can all directly impact the morphology and characteristics of myelin.

**Methods:** Immunohistochemistry using a panel of different antibodies was performed on skin biopsies of CMT1B patients to study both the morphology of nerve and myelin in CMT1B patients as well as to identify CHOP in the nucleus of Schwann cells as a result of UPR response in CMT1B. 9 affected and 3 non-affected individuals generously provided skin biopsies for indirect immunofluorescence evaluation. Tissue samples were fixed in 4% paraformaldehyde for 30 minutes before being washed in 1X phosphate-buffered saline (PBS) three times, 10 min per wash. Specimens were then embedded using optimal cutting temperature (OCT) medium and frozen to form a solid block for sectioning. Tissue samples were sectioned 20µm thick and mounted on slides. Slides were incubated with primary antibodies overnight at 4°C, washed with 1X PBS 3 times, then incubated with secondary antibodies at room temperature for 2 hours before being washed with 1X PBS 3 times and ddH2O 3 times, then dried and coverslipped using anti-fade mounting medium with DAPI fluorescent stain. Slides were examined with Leica DMi6000 fluorescent microscope and Zeiss 710 confocal microscope. Image analysis was performed using Image J software (NIH, Bethesda, MD).

**Results:** CHOP expression was observed in the nuclei of some Schwann cells of CMT1B patients but not ubiquitously expressed in all Schwann cells. In regards to morphology, nerve fiber diameter to node diameter ratio comparisons between CMT1B and controls were lower in CMT1B but not statistically significant. Myelin basic protein (MBP) density comparisons between CMT1B and controls were lower in CMT1B but not statistically significant. Paranodal length comparisons between CMT1B and control revealed CMT1B paranodal lengths to be statistically shorter than in control paranodal lengths.

**Conclusions:** CHOP expression in Schwann cells of human CMT1B patients confirms UPR activation as a direct result of ER stress due to mutations in MPZ gene causing misfolding to occur. Myelin basic protein (MBP) density comparisons along with nerve fiber diameter to node diameter ratio comparisons between CMT1B and controls suggest possible decreases in nerve fiber diameter as well as possible reduced MBP in CMT1B patients, both of which are morphological changes that may partake in the clinically observed symptoms of CMT1B. Significant differences in paranodal lengths between CMT1B and control suggest that stress of Schwann cells may also negatively impact the health and morphology of axons around the nodes of Ranvier, which are vital to efficient and proper electrical signal conduction down an axon.

Student: David S. Wang
Mentor: Dr. Michael E. Shy
New Insights for Glaucomatous Visual Loss: The Outer Periphery

Rob Wanzek, M1; Eric Lee, M1; Trina Eden; Andrew Turpin, PhD; Luke Chong, PhD; Michael Wall, MD, Neurology, Ophthalmology and Visual Sciences

Background: Glaucoma is one of the leading causes of blindness and is monitored using visual field testing (perimetry). Visual field testing has a long history, with the earliest tests performed prior to Hippocrates. Goldmann perimetry, introduced in 1945, has been one of the most significant advances in visual field testing, allowing for skilled technicians to map visual field defects using moving light stimuli, or kinetic perimetry. With the advent of inexpensive computers and automated static perimetry in the last 35 years, the focus has been on the central 30 degrees of the visual field due to time constraints and unreliability of peripheral tests. Data from Dr. Wall’s lab suggests that Size V stimuli, rather than the clinical standard Size III, offer more reproducible results in the periphery, and a test has been developed in Dr. Wall’s Lab with Drs. Turpin and Chong which forgoes the standard step-wise method of light threshold determination to use a faster, Bayesian, strategy.

Purpose: The visual field outside 30 degrees has not been systematically explored using static automated perimetry. The aim of this study is to determine whether the peripheral visual field is useful in early identification of disease.

Methods: We tested 35 controls ages 18-78 with central visual field tests (30-2 size III 0.43° of visual angle, 30-2 size V 1.72°) and peripheral size V tests to determine limits of normality. We tested the subjects twice using the Octopus 900 perimeter running the OPI (Open Perimetry Interface) in a test our lab has developed. In addition, we collected these same tests twice from 9 early stage glaucoma patients ages 58-74. We organized the data by averaging eccentricity zones of the visual field with an effort to keep the number of presentation locations in each eccentricity zone equal. We averaged the threshold values for tests of the same visual field type and stimulus size. Since visual field sensitivity decays linearly with age, we standardized data for each subject to age 50 using linear regression and standardized the values of the glaucoma patients to account for the effects of ageing. For the glaucoma patients, difference between the threshold at each location and the expected value based on the controls was calculated and plotted against eccentricity. Statistical tests were performed to compare the slopes of best-fit lines for glaucoma patients and controls.

Results: The graph to the right shows the decrease in visual sensitivity with increasing eccentricity. This effect is more pronounced in the glaucoma patients (orange symbols and line). Due to unequal variances, a rank-sum test was performed and a statistically significant (P=.005) difference in the slopes between glaucoma patients and controls was found.

Conclusion: While all subjects show increasing visual field loss with increasing visual field eccentricity, our new finding is that glaucoma patients demonstrate a greater and linear decrease in sensitivity with eccentricity. This suggests the outer visual field periphery may be useful for clinical testing.
A Safer Place to Ride: Regulations and DNR enforcement in OHV Parks Increases Safety Behaviors

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Mentor: Karisa Harland
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Background: As Off-Highway Vehicles (OHVs), including all-terrain vehicles (ATVs), dirt bikes and side-by-sides (SxSs), have increased in popularity, so too have OHV-related deaths and injuries. For ATVs, carrying passengers, riding without a helmet; and children operating adult-sized vehicles are major independent risk factors for these deaths and injuries. Iowa OHV parks have regulations regarding helmet use and youth users that do not apply outside of parks, and have Department of Natural Resources (DNR) personnel who patrol the parks and provide enforcement. In previous studies, we found that a higher proportion of ATV crash victims were helmeted when crashes occurred in OHV parks as compared to off-road crashes outside the parks (91% vs. 24%). In addition, a significantly lower percentage of victims were passengers (2.5% vs. 13%) or youth (7% vs. 31%) inside vs. outside the parks.

Research Objective: To determine the demographics and prevalence of safety behaviors among OHV park users and how posted regulations and enforcement affect the rider safety behaviors.

Methods: Photos of users of Iowa’s eight OHV parks from May to September of 2014 were obtained using motion-activated cameras placed at the entrances of each park. Riders were coded by vehicle type, estimated age, sex, and safety behaviors. Descriptive and multivariable logistic regression analyses were performed.

Results: A total of 6,718 vehicles and 9,083 riders were analyzed. Riders on ATVs comprised 44% of OHV park users, 51% were on dirt bikes and 5% were on SxSs. Among ATV riders, 84% were male and 89% were adults. While only 11% of ATV park users were <16 years old and less than 1% were pre-schoolers, 96% of the child operators were on adult sized ATVs. Approximately 10% of single-rider ATVs in the parks had passengers. Among dirt bike riders, 96% were male, and 82% were adults. Of SxS riders, 79% were males and 12% were <16 years old. Multivariate modeling controlling for important covariates indicated that dirt bike operators were 87% more likely to use helmets than ATV riders. SxS riders were half as likely as ATV riders to use helmets. Female ATV operators were 50% more likely to use a helmet than male ATV operators. In comparison with young adults (16-39 years) on ATVs, children were nearly 6 times as likely to wear a helmet, whereas middle aged adults (40-60 years old) were half as likely to wear one. Patrol officer presence had a positive effect on multiple safety behaviors. For example, there was a 3.6 times higher likelihood of helmet use, and a 40% lower likelihood of passengers on dirt bikes and ATVs when officers were patrolling the parks.

Conclusions: High helmet use in the parks among ATV and dirt bike users suggests that park regulations have had a significant effect on rider safety behavior. There are no helmet requirements outside of the parks in Iowa and previous studies have shown far less helmet use than in this study. Other regulated safety behaviors such as riding without passengers were also highly practiced by OHV park users. However, there are no OHV park regulations regarding children only driving youth sized vehicles, nor requirements that SxS users must be restrained by a safety belt. These unregulated safety behaviors had low compliance by park users. Moreover, regulated safety behaviors, such as helmet use and no passengers on ATVs and dirt bikes, were significantly increased when the parks were patrolled by DNR officers. This study shows that a combination of regulation and enforcement is critical to ensure compliance with rider safety behaviors in OHV parks. These increased safety behaviors should lead to decreased injuries and deaths and make OHV parks a safer place to enjoy recreational riding. The establishment of similar safety regulation and enforcement efforts outside of parks would likely have a more far-reaching effect on decreasing the burden of OHV-related deaths and injuries.
Resident physician perceptions and practices toward integrating primary care in psychiatry practice

Lisa Wehr, Erik Vanderlip (collaborator), Patrick Gibbons (collaborator), Jess Fiedorowicz (mentor)

Background: Patients with mental illness have higher levels of comorbid chronic medical conditions than the general population with poorer health outcomes. Integrated care is a proposed solution to improve health outcomes in psychiatric patients. Due to their training and frequent contacts with patients, psychiatrists are in a unique position to provide medical care to this population.

Purpose of this study: This study sought to evaluate how psychiatry residents perceived psychiatry as a primary care specialty and how their opinions and training affected their practices and comfort level in providing basic primary care services to patients.

Method: Psychiatry residents were anonymously surveyed regarding their opinions toward primary care and their current practices and comfort level with providing primary care services.

Results: Of the 1,007 residents contacted, 214 (21.3%) completed surveys. Overall, 55% of residents considered psychiatry to be a primary care specialty, but there was a significant downward trend in the proportion of residents who held this opinion as they progressed through training (72% of PGY1 residents v. 33% of PGY5 residents; Z=3.15, p<.001). Those who viewed psychiatry as a primary care specialty were more likely to counsel their patients on alcohol/tobacco/drug use, work-related health risks, family planning/contraception, and immunizations (p<.05). They were also more likely to screen and treat patients for infectious diseases and feel more comfortable treating dyslipidemia and hypertension (p<.05).

Conclusions: Psychiatrists have the opportunity to take the lead in integrating medical and behavioral health care to decrease health disparities in the mentally ill population. However, current residency training curriculums do not foster a positive perception toward psychiatrists as primary care providers. Changes in the curriculum to increase relevant medical experiences may increase the percentage of psychiatry residents who desire and feel comfortable providing basic primary care services to their patients.
Pulse Wave Velocity as a Predictor for Postoperative Cardiac Events: Preliminary Results

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Abstract

Background: Vascular stiffness increases as a person ages, due to the repetitive stress that is put on the vascular system which causes changes in the elasticity of the vessel walls. The increased stiffness of the arteries puts added stress on the circulatory system. This rise in stiffness has been shown to be associated with an increased risk of cardiovascular events, in both presumably healthy patients, as well as elderly patients. Applanation tonometry is a non-invasive technique that has been shown to reliably provide indices of arterial stiffness. While the use of applanation tonometry has been widely studied in vascular physiology and general medicine, it is has not been studied for pre-operative risk assessment in surgical patients.

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

Methods: This study was conducted at the University of Iowa Hospital and Clinics and was approved by the University of Iowa Institutional Review Board. This study was conducted between June and August of 2015. A total of 97 patients (> 48 years old) who were having major surgery were enrolled. Patient’s carotid-femoral pulse wave velocity were measured using a SphygmoCor in the Day of Surgery Area, before the patients went to surgery. Patient’s medical history, intraoperative hemodynamics, and any postoperative cardiovascular events within 7 days, including, prolonged angina, MI, rhythm issues, heart failure, PE, DVT, stroke/TIA, respiratory failure, liver failure, AKI, and wound infections were recorded to determine significant correlations and relationships. Data was analyzed using SPSS. First, univariate associations of potential covariates including gender, age, pre-operative mean BP, PWV, BMI, surgical procedure, surgical duration, and the lowest intra-operative mean BP, were examined with the presence of cardiovascular related post-operative cardiovascular adverse events. Second, to work with a smaller subset of covariates that are most likely to be significant, those covariates with univariate p-values less than 0.2 were considered as potential covariates in the multiple regression models.

Results: The results of this preliminary study found that PWV (p=.014), type of surgical procedure (p<.001), surgical duration (p=.001), and the lowest intra-operative blood pressure (p<.001) all were linked with a greater chance of post-operative cardiac complications. Multi-variate analysis demonstrated both average PWV OR=1.5, 95% CI 1.05-2.2 (p=.028) and lowest intra-operative blood pressure OR= 0.86, 95% CI 0.77-0.98 (p=.018) were significantly associated with higher rate of post-operative cardiac related events.

Discussion: Preliminary results suggest, that PWV can be an independent variable of postoperative cardiovascular adverse events following major surgery. Also the lower the patient’s blood pressure drops during the surgery, the higher the risk of complications after surgery. More subjects are warranted to confirm to the exact relationships.
Loss of TRAF3 increases BCR signaling and B cell proliferation with BCR stimulation
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Mentor: Gail Bishop, PhD
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Introduction
Tumor necrosis factor receptor associated factor 3 (TRAF3) is an adaptor protein that plays an important role in B cell homeostasis. TRAF3 deletions or inactivating mutations are common in human B cell malignancies. TRAF3 negatively regulates several receptors in human B cells (including the B cell activating factor receptor, CD40, and interleukin 6). B cell specific TRAF3 knockout mice have enlarged spleens and lymph nodes and develop autoimmune manifestations as they age. B cells from B-cell specific TRAF3 deficient mice have markedly increased survival and autoantibody production. The B cell receptor (BCR) is crucial in B cells for proliferation and antibody production. Defects in BCR signaling can lead to autoimmunity and B cell malignancy. The purpose of this study was to investigate the possible role that TRAF3 plays in regulating BCR signaling.

Hypothesis
We hypothesize TRAF3 negatively regulates BCR signaling. B cells deficient in TRAF3 will have increased early BCR signaling events, and certain BCR-influenced downstream B cell biological functions such as proliferation and survival with BCR stimulation. Additionally, phosphorylation of downstream BCR signaling targets will increase in the absence of TRAF3.

Methods
Splenic B cells were isolated from wild type and TRAF3 deficient mice using anti-mouse CD43 Ab negative selection. The B cells were stimulated using goat anti-mouse IgM antibody. Proliferation of the B cells was analyzed using Carboxyfluorescein succinimidyl ester (CFSE) dye and flow cytometry. Annexin V and propidium iodide staining was used to visualize cell death. Protein was isolated after 5, 10, and 15 min of anti-IgM stimulation and analyzed by Western blotting.

Results
B cells from TRAF3 deficient showed modestly increased proliferation after 72 hours of anti-IgM BCR stimulation. TRAF3 deficient B cells had increased survival with and without anti-IgM BCR stimulation compared to wild type, which was anticipated, as these B cells show enhanced survival that is independent of receptor-mediated activation. Downstream BCR signaling targets including Akt, ERK, p38 MAPK, and Jnk displayed increased phosphorylation in the absence of TRAF3 after 5, 10, and 15 min of anti-IgM stimulation.

Discussion
Our studies suggest that TRAF3 may play an important role as a negative regulator of BCR signaling. Future directions include analysis of more proximal BCR signaling targets to better understand the role of TRAF3 in BCR signaling. Additional experiments will test binding of TRAF3 to the BCR signaling complex. Greater understanding of TRAF3 and BCR signaling may provide potential treatment targets or disease biomarkers for autoimmunity and B cell malignancies.
Dignity Therapy: Narratives at End of Life. Emily C. White, Michelle T. Weckmann. Department of Family Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA 52242

**Background:** 90% of U.S. physicians believe that the concept of human dignity has “practical relevance for clinical medicine.” It has been shown that loss of dignity has profound implications at end of life and is the most common reason patients seek out physician-assisted death. Dignity Therapy is a brief therapeutic intervention shown to improve quality of life, suffering, and depression in patients nearing the end of life. Dignity related distress is commonly measured by the Patient Dignity Inventory (PDI) which explores a broad range of issues which have been shown to influence a patient’s sense of dignity. A study to investigate correlations between the patient’s narrative and a quantitative measurement (the PDI) of patient dignity has yet to be done.

**Hypothesis/Aims:** This study aimed to determine how specific qualitative themes discovered during Dignity Therapy relate to a quantitative measure of patient dignity (the PDI). It was hypothesized that the themes and subthemes of the Dignity Therapy Model identified within the patients’ narratives provide unique understandings of dignity at the end of life. Additionally, it was hypothesized that the PDI would demonstrate decreased dignity related distress following the Dignity Therapy intervention.

**Methods:** Patients enrolled in Iowa City Hospice were considered for inclusion. All patients who were not comatose, spoke English, and had a life expectancy greater than 2 weeks were eligible. There were 14 patients overall. Dignity Therapy interviews were conducted between January 2015 and August 2015 at the patient’s bedside. The PDI was administered before the therapy began and after the therapy was complete. Interviews were transcribed and analyzed for narrative themes and subthemes from the Dignity Therapy framework.

**Results:** 9 Patients completed at least one PDI questionnaire. 5 Patients did not complete any PDI. Other patients died (n=1) or declined (n=3) before the second PDI could be administered. 1 Patient completed 3 PDIs, and 4 patients completed 2 PDIs. Comparing the patient responses over time showed decreased dignity related distress in some areas, and increased distress in others. Dignity Therapy themes and subthemes were present in each patient’s narrative, but did not correlate with PDI responses.

**Discussion/Conclusion:** This study is the first to look at patient narratives to identify themes and subthemes of the Dignity Therapy Framework to further validate the use of Dignity Therapy and encourage the use of personalized therapy in end of life care. The types and number of themes and subthemes identified in the patients’ narratives are unique to that patient. This study determined that the PDI questionnaire was difficult for patients nearing end of life to understand. Frequently, patients would not be able to assign a number between 1 (indicating “no problem”) and 5 (indicating “an overwhelming problem”). These findings suggest that dignity is very individualized. Consequently, dignity related concepts are hard to quantify and are better understood through patient narratives.

**References:**

CaMKII Inhibition in Type II Pneumocytes Protects From Bleomycin-Induced Pulmonary Fibrosis

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Background: Pulmonary fibrosis occurs as an idiopathic disorder or as a result of inflammatory states or injuries, such as radiation or bleomycin administration. Experimental studies and in vivo data have established that type II pneumocytes are critical mediators of fibrosis development. In the bleomycin model of pulmonary fibrosis in mice, the number of Type II pneumocytes is strongly decreased within seven days after instillation of bleomycin, and fibrosis develops by day 14. Our preliminary data imply the Ca\(^{2+}\) and calmodulin-dependent kinase II (CaMKII) is as attractive candidate signal for promoting pulmonary fibrosis since it is well known to mediate apoptosis. In preliminary experiments, we demonstrated expression of CaMKII in type II pneumocytes and its activation after bleomycin exposure. Thus, we developed a transgenic mouse model, which specifically inhibited CaMKII in type II alveolar pneumocytes (Tg SPC-AC3-I mice). By exposing Tg AC3-I mice and WT littermate controls to bleomycin, we observed a significant reduction of lung collagen deposition via hydroxyproline assay and via Masson’s Trichrome Staining.

Hypothesis: CaMKII inhibition in type II pneumocytes reduces lung fibrosis by attenuating type II cell ER stress and inflammation.

Methods: MLE-12 cells were infected with an adenovirus expressing CaMKIIN for 48 hours. The cells were then exposed to bleomycin for either 0, 1, or 24 hours to induce ER stress. Western blots were then performed on cell lysates to analyze the abundance of the ER stress proteins transcription factor C/EBP homologous protein (CHOP), and the molecular chaperone BiP. To clarify the inflammatory mechanism, ELISA assays for macrophage inflammatory protein 2 (MiP 2), tissue necrosis factor alpha (TNF-\(\alpha\)), and interleukin 2 (IL-2) were performed on right lung homogenates from two cohorts of Tg SPC-AC3-I mice and WT littermates which were either exposed to bleomycin or saline for 3 or 14 days.

Results: Densitometric analysis of immunoblots for CHOP and BiP revealed an increase in protein levels after bleomycin exposure for 1 and 24 hours. The levels of BiP were decreased in CaMKII infected cells at 24 hours post bleomycin exposure, CHOP were decreased with CaMKIIN upon exposure to bleomycin for 1 and 24 hour. ELISA assays of lung homogenates at day 3 after bleomycin exposure revealed little to no induction of inflammatory proteins MiP 2, TNF-\(\alpha\) and IL-2 with bleomycin exposure. At day 14, all three inflammatory proteins were strongly increased in WT mice with bleomycin exposure. In lungs from Tg SPC-AC3-I mice, the induction of inflammatory proteins was significantly lower than in WT.

Conclusions: Our data demonstrate that CaMKII regulates ER stress in type II pneumocytes in response to bleomycin in vitro and mediates inflammation in vivo. These data provide mechanistic evidence for a role of CaMKII in bleomycin-induced pulmonary fibrosis.
Can We Use the Banana Slug to Study Mucus Expansion?

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Background

Cystic fibrosis (CF) begins as a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encodes an accordingly named ion channel conducting chloride and bicarbonate across the epithelium. The airway surface presents vastly different conditions in terms of pH and ion concentrations in the absence of CFTR. Mucociliary transport (MCT), an essential defense mechanism where pathogens are captured in mucus and expelled from the airway, also is compromised in advanced CF cases. Recent work demonstrates that defective MCT is present at the onset of the disease. Mucus, upon release from the submucosal glands (SMG), irreversibly ‘unfolds’ and forms strands. In CF cases, these mucus strands ‘tether’ to the SMG instead of detaching into the airway. Mucus is a hydrogel that consists primarily of mucins, a family of large glycoproteins. A hydrogel gains its properties from its structure: should the mucus unfold differently, the resulting polymer would be abnormally shaped and consequently have atypical properties, contributing to defective MCT. To study mucus unfolding, one must look at pre-unfolded mucus (stored in the SMG as granules). This poses a challenge because in established models of CF (i.e. porcine, murine), it is difficult to collect these granules. *Ariolimax columbianus* (banana slug) may be a promising model as it secretes copious amounts of mucus granules that can be collected intact.

**Purpose of the Study: Hypothesis and Specific Aims**

Our hypothesis is that the mucus itself is not different but rather a different airway surface environment in CF causes the mucus to unfold differently than it would in a non-CF airway. The aims for this project are the following:

1. Verify that the banana slug mucin sequence is similar to that of porcine and human
2. Identify an effective fluorescent label for the real-time visualization of mucus expansion
3. Test the effects of pH and ion concentration on mucus expansion

**Method**

The University of California Santa Cruz provided unassembled banana slug genomic sequence data. We utilized a stand-alone version of the NCBI BLAST tool on an Ubuntu virtual machine to conduct sequence alignments with human mucin sequences. We obtained banana slug DNA from Niles Biological and stored at 13°C. Slugs were euthanized and the foot (lower half of slug) was electrically stimulated at 10 V to collect granules. Granules were placed in differing ionic solutions buffered with HEPES to evaluate the effects of various ions (i.e. Ca2+) and pH on mucus volume. To image intact granules, we utilized the plasma membrane dye FM 1-43 (Life Technologies). To visualize the mucus, we used several labeling methods: 1) differential interference contrast (DIC) imaging 2) brightfield (BF) imaging 3) the autofluorescent properties of mucus and 4) sulforhodamine 101 (Sigma Aldrich). Afterwards, we imaged the samples with confocal microscopy.

**Results**

Homology was identified between human gel-forming airway mucins and certain sequences in the banana slug genome. Conserved mucin protein domains were located in the banana slug genome as well. FM 1-43 labeled the granule membrane while sulforhodamine 101 labeled the secreted mucus. DIC, BF, and autoflourescence were also identified as methods to visualize the mucus. Higher concentrations of calcium were associated with larger mucus aggregate volumes while magnesium seemed to disrupt mucus shape and size.

**Conclusion and Discussion**

The phylogenetic analysis of the terminal regions of MUCs in the literature suggests that these portions are highly conserved. Thus it is reasonable that slugs would also have these regions in their mucins. One limitation is that the identity of the substance within the granule still cannot be associated with the homologous sequences found in the banana slug genome. Proteomics work would be necessary to confirm this identity. DIC, BF, and autoflourescence are also methods utilized in the past to visualize mucus in other models. For future studies, these methods may be used to avoid any confounding effect the label molecule itself may have on mucus expansion. There were several autofluorescence wavelengths emitted from granules on the slug; this may imply that the granule contents are not homogenous. There is no known use of sulforhodamine 101 for labeling mucus so these results demonstrate a novel way to label mucus. The results suggest that calcium may facilitate the aggregation of mucus from several granules. In other words, it may be involved in the dimerization and/or polymerization process of mucins. Furthermore, the results may indicate that Ca2+ is necessary for mucus aggregation as other divalent cations (i.e. Mg2+) do not have the same effect. These results overall lay the foundation for future studies of ionic and pH effects on mucus expansion.
Consequences on balance and movement. Organisms to compensate behaviorally and overcome the higher risk of debilitating falls and subsequent health problems related to bone healing with a projected cost of ~100 billion by 2050. Understanding inner ear sensory organ deterioration to maintain balance could alleviate medical complications in the elderly. Thus, there is a need for the development of a model system mimicking the reduced inner ear sensory input characteristic of age-related disruptions. Vismodegib, a selective Shh antagonist that curtails proliferation, differentiation, and morphogenesis was used to pharmacologically reduce the size of the ear and its neurosensory parts to develop a new model of ear-related balance disorders to evaluate countermeasures.

Hypothesis: Shh inhibition by vismodegib diminishes ears in terms of the number and size of the sensory epithelia, generating reduced vestibular input that causes balance disorders to allow testing countermeasures.

Aims: This study had three primary purposes. The first objective was to quantify the reduction in ear size in *Xenopus laevis* following pharmacological treatment with the Shh inhibitor vismodegib mimicking the age-related ear deficits exhibited in the elderly. An additional objective was to characterize the neurosensory development (i.e. number of distinct sensory epithelia) occurring as a function of vismodegib concentration. The final objective was to implement a behavioral testing program on tadpoles with reduced vestibular input to assess the nature of the resultant motor output.

Methods: *Xenopus laevis* oocytes were acquired through induced ovulation of female frogs with an injection of human chorionic gonadotropin. Fertilized oocytes developed at 15 – 18 °C until the onset of gastrulation. At least six gastrulae were treated each with 6.25 µM, 12.5 µM, 18.75 µM, and 21.875 µM vismodegib (a Shh inhibitor), concentrations previously determined to disrupt ear development. Untreated embryos were kept at 15 - 18 °C until the onset of the swimming and feeding stage (stage 46). Animals were then introduced to an imaging apparatus that delivered a mechanical stimulus to the tadpoles sufficient to activate the C-startle response reflex and used a high-speed camera to render real-time, high-resolution images of tadpole movements. The angle between the head and tail of the animal during the onset of the C-startle response (maximum flexion) was measured using ImageJ. Following behavioral testing and once they reached stage 46, all animals (treated and controls) were anesthetized in 0.2% Benzocaine and immersed in the fixative 4% paraformaldehyde. Fixed animals were dissected and brains and heads were immunostained with a primary antibody against acetylated tubulin to label all neurons and a primary antibody against myosin VI to label inner ear hair cells and muscle fibers. Hoechst stain was applied to stain the nuclei. Secondary antibodies with distinct fluorophores were used that recognize the primary antibodies. Following immunohistochemistry, all animals were imaged using a Leica SP5 confocal microscope. Ear size for all animals was determined via full 3D volume rendering of serial confocal microscopy sections of the ear using Amira software. Deficits were assessed in terms of size and number of sensory epithelia in the inner ear as a function of Shh inhibitor concentration and significance was tested using ANOVA.

Results: We show that a reduction in Shh signaling via treatment with vismodegib results in smaller and incompletely differentiated ears. The severity of this reduction in ear size is most obvious along the anteroposterior and mediolateral axes of the ear in a dose-dependent fashion (p = 0.0005). The compromised ear formation also is manifested in the failure of the sensory epithelia of the ear to segregate into distinct patches, indicative of the incomplete differentiation occurring with Shh inhibition. Furthermore, we illustrate the caudal to rostral expansion of hypaxial somites (muscle fibers) that occurs with inhibition of Shh signaling. Finally, our results for the first time showcase a system to quantitatively analyze swimming behavior in *Xenopus* such as the C-startle reflex to assess for deficits in vestibular functioning and motor behavior. Treated animals that underwent behavioral testing showcase a statistically significant reduction in angle between the head and tail at maximum flexion during the onset of the C-startle response compared to controls (p = 0.002).

Conclusions and Discussion: The data provides proof of principle that Shh inhibitors can generate a model for disruption of vestibular function. However, the rostral expansion of muscle fibers in treated animals may confound with the aforementioned ear deficits in producing the aberrant motor output. Transplantations of reduced ears onto otherwise normal animals is needed to eliminate this confounding variable. By understanding how animals such as *Xenopus* generate controlled movements guided by reduced gravitational sensory input, it is possible to design a program that trains these organisms to compensate behaviorally and overcome the sensory deficits that otherwise would have deleterious consequences on balance and movement.
forestSV v2: Resolving structural variation from whole-genome short read sequencing data with statistical learning.

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Abstract

**Background:** Detecting structural variations in high-throughput sequencing data remains a non-trivial task. There are many tools that can identify regions of structural variation from next-generation sequencing data. However, due to intrinsic algorithmic differences, specific tools are often biased to detecting specific structural variations. Therefore, using only one tool can lead to poor sensitivity, i.e. the ratio of true positives to the sum of true positives and false negatives. The simplest way to overcome this challenge is to use multiple tools that utilize a spectrum of strategies and consolidate the results post-hoc. However, combining results from different tools brings its own set of challenges, e.g. deciding what to do with a variant candidate when one tool calls it but another misses it. The aim of this project is to develop a computational tool for detecting structural variants that nullifies the need for the pooling approach yet maintains high sensitivity.

**Methods:** forestSV v2 takes aligned Bam files and generates a report of identified structural variants. We train the classifier using data from six high coverage whole-genome sequencing experiments, from the 1000 genomes project, with microarray validated structural variation sites.

**Results:** forestSV v2 builds on forestSV [Nat. Methods, 9(8):819-21 (2012)] , a structural variant discovery tool based on statistical learning and shown to have high sensitivity. We improve upon the previous version by providing copy number estimates for structural variation calls, separate modes of analysis for haploid and diploid genomes, and a more robust segmentation algorithm.

**Conclusion:** We have developed a tool for detecting structural variations from whole-genome short read sequencing data, which is based the random forest learning algorithm.