

## EFFECTS OF APELIN ON INSULIN-MEDIATED GLUCOSE UPTAKE AND LIPOLYSIS IN CULTURED 3T3L1 ADIPOCYTES

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A recently identified adipokine, apelin, is up-regulated in states of obesity and insulin resistance. It remains largely undetermined, however, whether the relationship between apelin and insulin resistance is causal or compensatory. Although apelin is secreted by adipocytes the effect of apelin in the release of free fatty acids (FFA) (lipolysis) is not known. In addition, its direct effects on insulin sensitivity have not yet been adequately quantified.

Using an *in vitro* model system, we used differentiated 3T3L1 adipocytes in culture. To study the direct effect of apelin on insulin-mediated glucose uptake we exposed 3T3L1 adipocytes to insulin  $\pm$  pGlu-apelin-13 pretreatment and measured [<sup>3</sup>H]-labeled 2-deoxyglucose uptake. By itself, a 30 minute apelin stimulation mildly increased glucose uptake in a dose dependent fashion (EC<sub>50</sub> = 152 nM). However, apelin also significantly augmented insulin-induced glucose uptake (EC<sub>50</sub> = 157 nM). Progressively longer apelin exposures also enhanced glucose uptake significantly, though when added to insulin, the increase in glucose uptake was noticeably accelerated. Finally, apelin-mediated potentiation of insulin-induced glucose uptake was inhibited by both TNF- $\alpha$  and the PI3K inhibitor LY294002. To examine the effects of apelin on lipolysis in 3T3L1 adipocytes, we investigated isoproterenol-induced FFA release and hormone-sensitive lipase (HSL) and acetyl CoA carboxylase (ACC). Apelin attenuated isoproterenol-induced FFA release. Apelin's inhibitory effect was reversed by pertussis toxin (PTX), the G<sub>q</sub> inhibitor Gp antagonist 2A, and the AMP-activated protein kinase (AMPK) inhibitor compound C, but not by the phosphoinositol 3-kinase inhibitor LY294002. Apelin increased HSL phosphorylation at the Ser-565 residue, and also abrogated isoproterenol-induced HSL phosphorylation at Ser-563. Notably, apelin also increased ACC phosphorylation, suggesting AMPK activation.

These findings suggest that apelin is involved in the regulation of insulin sensitivity at the cellular level. In addition apelin is important in the regulation of lipolysis. Its actions may be carried out through multiple pathways involving G<sub>q</sub>, G<sub>i</sub>, and AMPK. Given the prominence of type 2 diabetes and the metabolic syndrome in our society, these results could carry significant implications regarding the search for novel therapies for insulin resistance and the metabolic syndrome.

## **A COMPARISON OF PSYCHOLOGICAL DISTRESS SCREENING TOOLS FROM A CROSS-SECTIONAL SURVEY OF WOMEN AT TWO ANTENATAL CLINICS IN CHITUNGWIZA, ZIMBABWE**

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Previous research in the United States (U.S.) suggests that psychological distress is associated with HIV risk behavior. While much research has been done in the U.S. on these topics, very little to date has occurred in the region of the world where most of the HIV cases occur – sub-Saharan Africa. In 2007 we conducted a study to examine psychological distress (depression and post-traumatic stress disorder), and HIV risk behavior in a cross-sectional assessment of 200 women attending the Seke North and St. Mary's antenatal clinics in Chitungwiza, Zimbabwe. Psychological distress was assessed using two main scales: the PTSD Checklist Civilian Version (PCLC) a 17-item U.S. based screening tool for PTSD based on DSM IV criteria, and the Shona Symptom Questionnaire (SSQ) a 14-item survey developed and validated in Zimbabwe to screen for overall psychological morbidity. While the SSQ has been validated in Zimbabwe, the PCLC has not and it is in this study that the investigators wish to compare the two surveys, using cluster analysis to determine what aspects of psychological distress the SSQ might be assessing in comparison with the PCLC. The data will be analyzed to explore associations between these principal components of psychological distress within the SSQ and HIV risk behavior in women attending antenatal clinics in Zimbabwe for future screening uses of the SSQ in assessing psychiatric morbidity as it relates to high levels of HIV risk.

## COMPARISON OF CHRONIC REJECTION IN WORKING AND NON-WORKING MODELS OF HETEROTOPIC HEART TRANSPLANT

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Despite advances in the treatment of acute rejection and substantial improvement in 1yr survival, chronic rejection remains an important factor in long-term mortality of cardiac transplant patients with minimal improvement over recent decades. The two most common heterotopic models of heart transplant used to study chronic rejection include a non-working model first published in 1969 by Ono and Lindsey wherein blood flow circumvents the left ventricle and a working model published in 1995 by Yokoyama et al where blood flow through the left ventricle is preserved. This study directly compares the histological and functional features of these two models.

Twenty-five rats were separated into the working and non-working groups and then further separated into isogenic (ACI to ACI) and allogenic (PVG to ACI) transplants. Echocardiography was used to assess the transplanted hearts over the 90 day course at which time the animals were sacrificed and the hearts were examined using organ weight, Langendorff apparatus, and histology. Histological rejection observed by luminal obliteration on Elastin-van Gieson stain indicated increased obliteration in the allogenic transplants compared to isogenic as expected but no differences between the working and non-working models. Comparison of the groups taking into account the size of the affected vessels also failed to reveal any differences. Echocardiographic assessment demonstrated increased left ventricular diameter in the working group (0.5cm vs. 0.25cm,  $p < 0.05$ ) as well as decreased left ventricular wall thickness (0.125cm vs. 0.23cm,  $p < 0.05$ ) reaching statistical significance at 90 days. The isolated hearts and spleens from the working model also weighed more than those of the non-working model (1.3g vs. 0.52g and 0.77g vs. 0.59g, respectively,  $p < 0.05$ ). Decreased pulse pressures and  $dP/dT$  (20.7mmHg vs. 53.8mmHg and 750.9 vs. 3292.9, respectively,  $p < 0.05$ ) were also seen in explanted working hearts when compared to the non-working model using the Langendorff apparatus. There were no significant differences between isogenic and allogenic transplants in weight, left ventricular diameter or thickness, or functional measures.

Together these results suggest that there are no significant differences in chronic rejection between the working and non-working models as assessed by luminal obliteration on histology. However, significant functional and morphological changes occur whereby the ventricles enlarge, thin, and have reduced function in the working model. The cause of these changes is unclear and its relevance to use of this model for the study of chronic rejection is unknown. Further study will be needed to examine these processes.

## **DESIGNING A NOVEL WAY TO REDUCE STROKE RISK IN ATRIAL FIBRILLATION PATIENTS**

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In nonrheumatic, nonvalvular atrial fibrillation patients, 91-98% of the thrombi are located in the left atrial appendage. Previous attempts to control thrombi formation in the left atrial appendage have centered on ways to close the appendage, both surgically and percutaneously, in order to prevent clot formation and thus reduce the risk of stroke. There continues to be a need for a device which achieves complete left atrial appendage closure while minimizing complications. Potential users of such a novel system include: atrial fibrillation patients who prefer not to take lifelong anticoagulation, those for whom anticoagulation is contraindicated, and patients who have developed stroke despite being on anticoagulation.

To solve this clinical need, our multidisciplinary team used principles of the Stanford biodesign innovation process to develop our device. Current surgical closure solutions result in incomplete closure, while existing percutaneous device closure methods involve leaving a space occupying foreign body in the left atrium and are complicated by device embolization and pericardial effusion. As a result of our systematic analysis of this problem, we created a range of concepts, resulting in several designs. Our approach has been to develop devices that 1) do not require leaving a space-occupying device, 2) may be achieved using minimally invasive approaches. Our designs may be divided into three major groups based on access approach: combined epicardial-endocardial, epicardial only, and endocardial only. For example, the endocardial-epicardial device uses an endocardial balloon to keep the epicardial ligature around the base of the atrial appendage. The endocardial device is the most complex and involves closure of the atrial appendage from the left atrial wall surrounding the left atrial appendage ostium. Future steps will be to prototype these designs and perform preliminary ex vivo and in vivo tests.

## CHARACTERISTICS AND OUTCOMES OF PREHOSPITAL CHEST PAIN PATIENTS IN INDIA

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India has the world's largest burden of cardiovascular diseases (CVD); however, no studies to date have examined Indian patients with cardiac-related complaints in the prehospital setting. The Emergency Management and Research Institute (EMRI) launched in 2005 to provide prehospital care to all 76 million residents in the state of Andhra Pradesh, India. This study aims to describe patients transported by EMRI with possible cardiac-related emergencies. In this prospective cohort study, all patients transported by EMRI for the chief complaints of "cardiac" or "chest pain" over seven continuous days in July 2009 were included. Patients who were not found at the scene, refused service, or denied chest pain were excluded. Using a standardized questionnaire, patient demographic and clinical data were collected by phone in real-time from prehospital care providers. Follow-up information was collected at 48 hours, 14 days and 30 days.

585 patients were enrolled and the follow-up rate was 84.3% at 30 days. The mean patient age was 48.5 years (17.6, SD) and 61% were male. Seventy-four percent of patients were from rural or tribal areas, 90% were from lower socioeconomic strata, and 56% had CVD risk factors. Average call-to scene time was 20.6 minutes (15.5, SD) and scene-to-hospital time was 30.8 minutes (17.2, SD). Mortality ratios prior to hospital arrival, at 48 hours, and at 30 days were 13.8%, 19.3%, and 23.2%, respectively. Male gender ( $p < 0.0001$ ), older age ( $p = 0.0005$ ) and smoking history ( $p = 0.026$ ) were significant independent predictors of 30-day mortality.

This initial study of chest pain patients in the prehospital setting in India has revealed alarming early mortality ratios. Given the prevalence of CVD risk factors and significant prehospital mortality, educating the at-risk population and earlier medical intervention could improve survival.

## **OP-1 ENHANCES OSTEOGENESIS IN OSTEOPROGENITOR CULTURES CHALLENGED WITH POLYMETHYLMETHACRYLATE PARTICLES**

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Osteolysis is a major clinical complication of total joint replacements. Wear and friction between implant components give rise to micron- and submicron-sized particles that induce inflammation and bone degradation, causing the implant to fail. Particles of polymethylmethacrylate (PMMA) cement have recently been shown to suppress bone formation by inhibiting the differentiation of MC3T3-E1 osteoprogenitor cells. OP-1, also known as BMP-7, an osteogenic growth factor used clinically to promote bone growth, has been conjectured to stimulate osteogenesis among osteoprogenitors that have been inhibited by PMMA particles. In this study, we determine whether OP-1/BMP-7 can stimulate osteogenesis in osteoprogenitor cultures exposed to PMMA particles.

MC3T3-E1 osteoprogenitor cells (ATCC) were induced to differentiate into osteoblasts by addition of osteogenic factors dexamethasone (0.1  $\mu$ M), ascorbic acid (50  $\mu$ g/mL), and  $\beta$ -glycerophosphate (10 mM) to medium. PMMA particles (Polysciences) 1-10  $\mu$ m in diameter were added to culture on this first day of differentiation (day 1) at doses of 0.000, 0.075, 0.150, and 0.300% v/v. Cells were also treated with OP-1 (Stryker) at a dose of 200 ng/mL during the following time periods of culture: (1) days 1-20, (2) days 4-20, (3) days 1-4. In a fourth condition, cells were exposed to OP-1 through days 1-20, but were treated with particles on day 4 rather than on day 1. Mineralization and alkaline phosphatase production were assessed after 20 days of culture by von Kossa staining (1 hr exposure to UV light in 5% silver nitrate solution) and spectrophotometric measure of cell lysate reaction with p-nitrophenylphosphate respectively.

The addition of PMMA particles to osteoprogenitor cultures caused a dose-dependent decrease in mineralization and alkaline phosphatase activity in all experimental groups. The addition of OP-1 to cultures from days 1-20, 4-20, and 1-4 all resulted in significant increases in mineralization (50-200% increase) and alkaline phosphatase production (10-40% increase) in all particle-treated groups compared to corresponding groups in the control not treated with OP-1. Cells treated with PMMA particles on day 4 of culture showed similar increases in mineralization and alkaline phosphatase production when exposed to OP-1 compared to cells not exposed to OP-1. In summary, this study has shown that OP-1 enhances osteogenesis in osteoprogenitor cultures challenged with PMMA particles regardless of the time period of treatment. Exposure of cells to OP-1 during the first 4 days of culture/particle treatment is sufficient to improve the osteogenic response. Clinically, infusing OP-1 to areas of osteolysis in joint replacement may mitigate the inhibitory effects of particles on osteoprogenitor cells and diminish the rate of implant loosening.

# POLYMETHYLMETHACRYLATE PARTICLES INHIBIT HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION INTO OSTEOBLASTS

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Orthopedic wear debris generated from total joint replacements causes implant failure by inducing inflammation and osteolysis. These particulate materials have also been implicated to inhibit bone formation by suppressing stem cell osteogenesis. Given that the stability of an implant depends on adequate bone formation, it is crucial to determine whether wear debris particles affect the osteogenic differentiation, proliferation, and survival of stem cells. In this study, we exposed human mesenchymal stem cells (hMSCs) to particles of polymethylmethacrylate (PMMA) bone cement and examined the effects of these materials on the ability of hMSCs to differentiate into osteoblasts.

Primary hMSCs were obtained from Lonza. These cells were purified from human bone marrow by positive selection for mesenchymal markers CD105, 166, 29, and 44 and negative selection for hematopoietic markers CD14, 34, and 45. hMSCs were induced to differentiate in culture by addition of osteogenic factors dexamethasone (0.1  $\mu$ M), ascorbic acid (50  $\mu$ g/mL), and  $\beta$ -glycerophosphate (10 mM). Cells were treated with PMMA particles 1-10  $\mu$ m in size (Polysciences) on this first day of osteogenesis at doses of 0.000, 0.075, 0.150, 0.300% v/v (equivalent to 0, 400, 800, 1600 particles per cell). Proliferation, alkaline phosphatase (ALP) production, and mRNA expression of ALP and type 1 collagen were assessed at 2-day intervals throughout the first 10 days of differentiation. ALP production was assessed by spectrophotometric measure of cell lysate reaction with p-nitrophenylphosphate; mRNA expression was assessed by qRT-PCR (primers, Applied Biosystems). Mineralization on day 28 of culture was assessed by spectrophotometric measure of acid-extracted matrix calcium with o-cresolphthalein. hMSC viability was assessed by Annexin V staining with visualization of fluorescently labeled dead cells at 24, 48, and 72 hrs after particle exposure.

hMSCs treated with PMMA particles showed a significant dose-dependent decrease in proliferation, ALP production, and mRNA expression of ALP and type 1 collagen. These outcomes were continuously suppressed throughout the 10-day culture period. Mineralization as represented by matrix calcium content on day 28 of culture was also significantly reduced at all particle doses tested. On microscopy, hMSCs showed evidence of particle phagocytosis. Annexin staining of these cells for viability, however, did not reveal greater numbers of dead cells compared to control. These results indicate that PMMA particles inhibit the proliferation and osteogenic differentiation of hMSCs. Clinically, the exposure of mesenchymal stem cells in the bone marrow to wear debris particles inhibits their osteogenesis and accelerates osteolysis and implant loosening.

## **CCL2-CCR2 SIGNALING IS NEEDED FOR TRANSENDOTHELIAL MIGRATION AND SUBSEQUENT FUNCTIONAL RECOVERY AFTER INTRA-ARTERIAL DELIVERY OF NEURAL PROGENITOR CELLS IN EXPERIMENTAL STROKE**

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Most central nervous system pathologies are accompanied by an inflammatory response, leading to a chemoattractant gradient for recruitment of leukocytes to the site of injury. Although this physiological response carries important consequences for further brain damage, it also offers a unique therapeutic opportunity. It has been suggested that the same mechanisms which guide immune cell trafficking after brain damage may also stimulate homing of neural progenitor cells (NPCs) into the brain parenchyma after intra-arterial transplantation. However, the mechanisms underlying transendothelial recruitment of NPCs to the injured brain remain largely uncharacterized. Here we showed that the chemokine receptor CCR2 is not only necessary for transendothelial migration, but CCR2 expressing neural stem cells also improve functional recovery in the injured brain.

First we characterized and confirmed that we in fact used NPCs used in our experiments, and also performed in vitro experiments to show that our NPCs can migrate in response to the chemokine, CCL2. Next, we performed two necessary experiments to test our hypothesis. First, we induced stroke and delivered wild type NPCs labeled with a fluorescently tagged protein into the internal carotid artery of mice which did not have the chemokine, CCL2. We then performed the same experiment on mice that had CCL2 present, and found that there were more cells in the brain with the wild type group by confocal microscopy. Next we induced stroke on two groups of wild type mice, but this time one group of stroked mice were intra-arterially injected with NPCs that were prepared from mice knocked out for the chemokine receptor, CCR2, and the other group with wild type NPCs. We found that the mice which received NPCs with the chemokine receptor, CCR2, had more cells in the brain. This suggests that without the chemokine, CCL2, and the chemokine receptor, CCR2, NPCs transmigrate less efficiently into the brain after stroke. Finally, to investigate the contribution of CCL2-CCR2 signaling to the therapeutic ability of NPCs in mice, the performance of mice on a ladder behavioral test was measured before stroke, after stroke, and after NPC administration. We found a trend for wild-type NPCs to promote the greatest functional recovery (fastest decrease in score), followed by CCR2 <sup>-/-</sup> NPCs.

We therefore conclude that the CCL2-CCR2 interaction plays a central role for transendothelial recruitment of NPCs in response to ischemic stroke. Furthermore, this critical mechanism may be important for the therapeutic application of NSCs to improve functional recovery in the injured brain.



## **THE EFFECT OF ACUPUNCTURE OF NASOPHARYNGEAL CANCER PATIENTS UNDERGOING RADIATION THERAPY IN SHANGHAI, CHINA**

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Xerostomia is an important and common problem among cancer patients who have received radiation treatment to the head and neck area as it severely impairs patients' quality of life. In China, head and neck cancers are particularly important due to the high prevalence of smoking. In addition, there is no accepted standard for care for xerostomia; and current treatments, at best, are palliative for symptom relief.

Through a randomized control trial, this study aimed to investigate acupuncture's efficacy in preventing or reducing radiation-induced xerostomia among cancer patients at Shanghai's Fudan University Cancer Hospital who received radiation treatment to the head and neck area. A minimal set of standardized acupoints for the study were chosen based on previous published studies showing successful results of reversing xerostomia, and indications according to classical theory of Traditional Chinese Medicine integrated with biomedicine. Patients underwent initial assessments to include demographic data, exposure history, medical history, cancer history and family history. Patients who qualified received weekly acupuncture treatments for 7 weeks with follow-up after 1 and 4 months. Through the administration of a set of subjective questionnaires including a patient benefit questionnaire, and self-assessed quality of life measured by the M.D. Anderson Symptom Inventory for Head and Neck Cancer.

The questions pertaining to physical wellbeing and emotional wellbeing were selected out of the large set of questions and measured on a weekly basis and during followup. There were significant improvements in the physical and emotional wellbeing of patients who received acupuncture compared to those who did not, with the biggest differences being noted in the 3rd-6th week of the study, and less difference during the 1 and 4-month followup. Follow-up studies comparing an acupuncture group to a sham-acupuncture group are currently underway.

## COMPREHENSIVE ANALYSIS OF GENE EXPRESSION IN THE OTOCYST

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Progressive hearing loss affects over 30 million people in the US. It is caused by the irreversible loss of sensory hair cells, which are mechanoreceptors located in specialized sensory epithelia of the inner ear. Unlike humans, birds have the ability to regenerate hair cells and restore hearing, though the factors that allow for regeneration in birds are unknown. Our lab's ultimate goal is to restore hair cell function using inner ear stem cells. The goal of this study is to identify relevant transcription factors and cell surface markers necessary for hair cell formation in the avian ear.

### Objectives:

- 1) Identify a comprehensive set of genes that are expressed in otic progenitors.
- 2) Identify transcription factors critical for cochlear hair cell development.
- 3) Identify CD markers selective for cochlear hair cell progenitor populations.

Serial Analysis of Gene Expression (SAGE) provides an unbiased, comprehensive and quantitative readout of gene expression, which is unequaled by any gene array currently available. 110 HH stage 18 (E3) chicken otocysts were dissected, and their total RNA was subjected to SAGE. SAGE library content was analyzed with three reference libraries (Ensembl 52, RefSeq, and Unigene) and resulting matches were annotated using Ingenuity Pathway Analysis software. Gene expression library content was validated using in situ hybridization.

39,326 17-mer tags were sequenced comprising 16,008 unique sequences. These sequences corresponded to 4,153 annotated genes and 8,054 non-annotated sequences. Our analysis identified 355 transcriptional regulators of which 303 are previously unknown in the context of the inner ear. In addition, 8 out of 14 growth factors detected were previously not linked to the inner ear. 41 cell surface marker CD molecules were identified.

Comprehensive gene expression in the otocyst was identified. Several novel transcription regulators, growth factors and cell surface marker molecules in the context of inner ear were revealed. These findings provide a foundation for further studies concerning inner ear and hair cell development and hair cell regeneration. CD molecules may be used to identify inner ear stem cells.

## **THE COST AND FEASIBILITY OF PROVIDING BASIC HEALTH SERVICES FOR THE MANAGEMENT OF SICKLE CELL ANEMIA IN LOW-INCOME SETTINGS: A TANZANIAN CASE STUDY**

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On June 19, 2009, Secretary-General of the United Nations Ban Ki-moon issued a statement encouraging governments, civil society, and other partners to engage in efforts to improve the quality and length of lives of those affected by sickle cell anemia (SCA). SCA currently contributes to at least 5% of under-five mortality in Africa. Despite SCA's significance on the continent, there are very few health service programs aimed at managing the disease. One reason for this is the perception that effective care for patients is unaffordable in low-income settings.

A micro-costing study of the direct and indirect costs of providing outpatient clinical services, penicillin prophylaxis, pneumococcal vaccination, and newborn screening at Muhimbili National Hospital (MNH), a tertiary health center in Tanzania, was undertaken from the perspective of the hospital, program administrators, and patients. Current program funding streams were also analyzed to assess feasible financing mechanisms.

The incremental cost of running an outpatient SCA clinic at MNH is US\$ 117 per patient treated. A penicillin prophylaxis program would cost approximately US\$10 per child each year. Pneumococcal vaccination would cost US\$ 15.18 per fully vaccinated child if the program received support from the Global Alliance for Vaccines and Immunisations (GAVI). Otherwise, vaccinations would cost up to US\$ 254.13 per child. A newborn screening program could cost as much as US\$ 9751 per child identified. Attending clinic would cost patients and their families US\$ 4.16 per visit. Assessment of funding provided by the Ministry of Health, research and program grants, and donor assistance indicates that current funding could not support the implementation of all four programs. Further, donor assistance currently provides the least amount of funding for the outpatient clinic at MNH.

In conclusion, an outpatient clinic and penicillin prophylaxis can be provided at a relatively low cost and may be easy to adopt when incorporated into the existing infrastructure of a tertiary hospital setting. However, pneumococcal vaccination would require heavy subsidization to be feasible. It is highly likely that donor assistance would be required to provide a more comprehensive set of SCA services on a wider scale in Tanzania. Historically, though, international donor funding for chronic diseases has been almost non-existent in sub-Saharan Africa. Thus, considerable advocacy efforts on behalf of those affected with SCA may be necessary to improve service development and implementation.

## PROSOPAMNESIA: A RARE DISORDER OF FACE-FORGETTING

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Prosopamnesia is a rare neurologic disorder wherein those afflicted have a severely impaired ability to remember faces. Crucially, individuals with *prosopamnesia* are still able to “see” a face, a characteristic which differentiates this condition from the closely-related *prosopagnosia*; however, their memory for a face is transient and significantly shorter than control subjects. Further, the fusiform face area – an area of the brain specialized for face analysis and recognition- remains intact in prosopamnesics, implying a selective deficit in face-specific memory encoding or recall processes. We used magnetoencephalographic (MEG) methodology to investigate the neurophysiologic underpinnings of this disorder by examining differences in evoked high-frequency (gamma) oscillations for later-remembered vs. later-forgotten pictures of faces and places.

Whereas the temporal-frequency MEG data are still being analyzed at this time, behavioral data are consistent with prosopamnesics performing poorly at face-specific recall, with no statistical difference in recall of places or scenes. Psychosocial consequences of prosopamnesia are discussed, along with the relevant recent literature (2010) regarding likely genetic involvement and differences between face-recognition disorders.

## NEUROPILINS ARE ESSENTIAL POSITIVE REGULATORS OF MAMMALIAN HEDGEHOG SIGNALING

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The Hedgehog (Hh) signaling pathway is a phylogenetically durable mode of cell-cell communication. Defects in this signaling pathway cause abnormalities in neural tube patterning, limb digit identity, and midline separation. Mutations causing excessive Hedgehog pathway activation can lead to cancer. Neuropilins are single-pass transmembrane receptors with well-characterized roles in neuronal growth cone chemotaxis and VEGF signaling. During mammalian development, neuropilins are found in many tissues undergoing active Hedgehog signaling. We identified neuropilin-1 as a positive regulator of Hh signaling in a high-throughput RNAi screen. We subsequently found that neuropilin-1 and neuropilin-2 have partially redundant roles in Hh signaling, as the combined loss of both gene products results in a near-complete inhibition of Hh signaling in cultured cells. We found that neuropilin loss-of-function does not affect formation of the primary cilium, a cellular protrusion thought to be critical for Hh signaling to occur. Using genetic and chemical methods of modulating Hh pathway activity we have determined that neuropilins act between Smoothed and Suppressor of fused (Sufu). We also found that both neuropilins are regulated by Hh signaling, suggesting a heretofore unidentified positive feedback relationship. Taken together, these data describe two new critical members of the mammalian Hh signaling pathway. This discovery provides a novel vista on the poorly understood signaling events that lie downstream of Smoothed activation.

## **POSSIBLE DYSFUCTION OF FIBROBLAST POSITIONAL IDENTITY IN SCLERODERMA PATIENTS**

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Scleroderma is a disease characterized by progressive fibrosis of the skin and internal organs and has mortality of approximately 5-8 times that of the general population. There is some evidence that perturbations in the proximal distal axis via the WNT and or HOX signaling systems may play a role in the fibrosis seen in Scleroderma. Scleroderma often starts at specific anatomic sites that are sites of expression of WNT5A and excessive activation of WNT signaling can cause aggressive fibromatosis and fibroblast proliferation in response to wounding. In order to search for perturbations in HOX gene expression or objective is to compare the RNA expression profile of scleroderma patient skin to that of normal skin on HOX tiling arrays.

We procured normal and diffuse scleroderma forearm skin RNA and/or tissue samples from two sources Dr. D Fiorentino and Dr. ML Whitfield. RNA was isolated from tissue biopsies, reverse transcribed into CDNA and then amplified using in vitro transcription. The RNA concentration, quality was checked via nanodrop and then it was run out on a gel to check for degradation.

In the future the scleroderma and normal skin RNA samples will be reverse transcribed into DNA, labeled with dye and hybridized to HOX tiling arrays in order to look for differences in HOX RNA expression. In addition it would also be beneficial to analyze the RNA expression profiles using human genome microarrays which would allow one to analyze whether there are connections between certain HOX perturbations and microarray gene signatures.

## **LONG-TERM EFFECT OF BILIARY ATRESIA AND LIVER TRANSPLANTATION: THE EFFECT OF SOCIAL AND MEDICAL VARIABLES ON IQ AND SCHOOL PERFORMANCE 6, 8, 10 YEARS POST-TRANSPLANT**

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As the survival rate for pediatric liver transplant has increased, the importance of long-term quality of life has increased concomitantly. Development is of particular concern because of the inability of the liver to breakdown chemicals that are toxic to the brain and the effect of ascites, severed abdominal muscles, frequent hospitalizations, and immunosuppressants. It has been demonstrated the pediatric liver transplant recipients are at risk for poor developmental outcomes. However, there are few studies that either chronicle the long-term effects of liver transplantation or delineate the risk factors for long term developmental delays. Our study looks at potential risk factors for long-term (6, 8, and 10 year post-transplant) low IQ scores and school performance problems in children who received a liver transplant before three.

Through a retrospective chart review, we have gathered data on medical variables, development, and school performance - IQ as measured by WPSSI-III or the WISC-4 and school performance as measured by the descriptive statistics. We have gathered data on 54 children who received liver transplants before the age of 3 who are now 10 and 15 years post-transplant. Of those that we have school performance information for, 35% require additional support in school. Our data is currently with a statistician to determine what the risk factors are for such poor school performance.

The better parents can understand the long-term effects of liver transplant, the better equipped they will be to support their children and get their children the support they need. Additionally, if we have more information about what the risk factors are for poor developmental outcome, studies could be done to determine if reducing the risk factors helps with developmental outcomes. The results will help providers better explain what the developmental prognosis is for such patients and help elucidate what treatments might better prevent long-term developmental problems.

## THE ROLE OF THE RETINOBLASTOMA/E2F1 TUMOR SUPPRESSOR PATHWAY IN THE DNA LESION RECOGNITION STEP OF NUCLEOTIDE EXCISION REPAIR

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The retinoblastoma Rb/E2F tumor suppressor pathway plays a major role in the regulation of mammalian cell cycle progression. The pRb protein, along with closely related proteins p107 and p130, exerts its anti-proliferative effects by binding to the E2F family of transcription factors known to regulate essential genes throughout the cell cycle. We sought to investigate the role of the Rb/E2F1 pathway in the lesion recognition step of nucleotide excision repair (NER) in mouse embryonic fibroblasts (MEFs). Rb<sup>-/-</sup>, p107<sup>-/-</sup>, p130<sup>-/-</sup> MEFs repaired both cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs) at higher efficiency than did wildtype cells following UV-C irradiation. The expression of damaged DNA binding gene DDB2 involved in the DNA lesion recognition step was elevated in the Rb family-deficient MEFs. To determine if the enhanced DNA repair in the absence of the Rb gene family is due to the derepression of E2F1, we assayed the ability of E2F1-deficient cells to repair damaged DNA and demonstrated that E2F1<sup>-/-</sup> MEFs are impaired for the removal of both CPDs and 6-4PPs. Furthermore, wildtype cells induced a higher expression of DDB2 and xeroderma pigmentosum gene XPC transcript levels than did E2F1<sup>-/-</sup> cells following UV -C irradiation. Using an E2F SiteScan algorithm, we uncovered a putative E2F-responsive element in the XPC promoter upstream of the transcription start site. We showed with chromatin immunoprecipitation assays the binding of E2F1 to the XPC promoter in a UV-dependent manner, suggesting that E2F1 is a transcriptional regulator of XPC. Our study identifies a novel E2F1 gene target and further supports the growing body of evidence that the Rb/E2F1 tumor suppressor pathway is involved in the regulation of the DNA lesion recognition step of nucleotide excision repair.



## **NEW INSIGHTS INTO PACEMAKER LEAD-INDUCED VENOUS OCCLUSION: SIMULATION-BASED INVESTIGATION OF ALTERATIONS IN VENOUS BIOMECHANICS**

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Venous obstruction is a major complication of transvenous pacemaker placement. Despite the increasing use of pacemakers and implantable cardiac defibrillators, a lack of understanding remains with regard to risk factors for the development of device-associated venous obstruction. We hypothesize that computational fluid dynamics simulations can reveal prothrombogenic locations and define thrombosis risk based on patient-specific anatomies.

Using anatomic data derived from computed tomography, computer models of the superior vena cava, subclavian, innominate, and internal jugular veins were constructed for three adult patients with transvenous pacemakers. These models were used to perform patient-specific simulations examining blood flow velocity, wall shear stress, and blood pressure, both with and without the presence of the pacing leads. To better quantify stasis, mean exposure time fields were computed from the venous blood flow data. In comparing simulations with leads to those without, evident increases in stasis at locations between the leads and along the surface of the vessels closest to the leads were found. These locations correspond to regions at known risk for thrombosis.

This work presents a novel application of computational methods to study blood flow changes induced by pacemaker leads and possible complications such as venous occlusion and thrombosis. This methodology may add to our understanding of the development of lead-induced thrombosis and occlusion in the clinical arena, and enable the development of new strategies to avoid such complications.

## **QUANTITATIVE ULTRASOUND RENAL PARENCHYMAL AREA CORRELATES WITH GRADE OF VESICoureTERAL REFLUX IN CHILDREN WITH FIRST FEBRILE URINARY TRACT INFECTION**

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In 1999 the American Academy of Pediatrics published recommendations for children 2 months - 2 years of age with febrile urinary tract infection (UTI). Recommendations included renal and bladder ultrasound (RBUS) and strong consideration of a voiding cystourethrogram (VCUG). VCUG is invasive and exposes children to radiation. Radiopaque dye is injected into the urethra and voiding is visualized fluoroscopically. Technicians look for vesicoureteral reflux (VUR), graded on a severity scale from I-V. VUR in the setting of a UTI is thought to predispose children to long term renal scarring and problems.

Recent work by our group showed that ultrasound renal parenchymal area (US-RPA) correlated with VUR grade in VUR+/UTI+ children (1). We aimed to further validate this by evaluating US-RPA in children with a first febrile UTI who do not have VUR. 2700 charts of children undergoing VCUG from 2005-2008 were reviewed to select this VUR-/UTI+ group. Children were included if they were referred for UTI and did not have VUR. On RBUS, children were excluded for renal abnormalities such as hydronephrosis or duplication. A general review of medical records was employed to exclude children with major known comorbidities predisposing to poor renal growth (biliary atresia, VACTERL, trisomy 21, etc). About 250 VUR-/UTI+ children met our criteria. US-RPAs were calculated. VUR-/UTI+ children's US-RPAs correlated well with previously published nomograms of renal growth in healthy children.

Pediatric urologists debate the role of VUR in UTI and renal scarring. Some believe that VUR is evidence of a developmentally impaired kidney. Others posit VUR represents a continuum, from poor development to a normal variant that will resolve. Although sterile reflux is thought more benign, the fear of scarring in any child with a UTI prompts careful evaluation for VUR. We feel that the US-RPA correlation with VUR grade will reduce VCUG-related fluoroscopy exposure in low-risk children with a first febrile UTI. The next step is a randomized controlled clinical trial testing RBUS detection of VUR.

### References:

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## **A TEXT MESSAGE-BASED COMMUNICATION NETWORK TO ADDRESS THE PATIENT-PHYSICIAN GAP IN RURAL MALAWI**

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Healthcare delivery in the rural developing world is limited by a severe shortage of health workers as well as profound communication and geographic barriers. Understaffed hospitals are forced to provide care for patients that reside at a great distance from the institutions themselves, sometimes more than 100 miles away. Community health workers (CHWs), volunteers from local villages, have been integral in bridging this patient-physician gap, but still lose enormous amounts of time in transit between hospital and village. We report the results of a retrospective mobile health (mHealth) pilot at St. Gabriel's Hospital in Malawi designed to eliminate many of these trips in favor of communication via text messages.

A group of 75 CHWs were supplied with cell phones and trained to utilize the texting network for a variety of usage cases, including patient adherence reporting, appointment reminders, and physician queries. During the first four months of the pilot, a total of 1,330 SMS messages were received at the hospital (central hub). The majority of these were patient adherence reports for TB or antiretroviral therapy (30.83%). Following this, requests for additional SMS units, symptoms reports, and requests for acute care were also relatively common (16.47%, 14.96%, and 8.05%, respectively). Over six months, the hospital reported approximately \$3,000 in fuel savings, compared to a network operational cost of approximately \$250 (2,945 SMS messages at 8.5 US cents each). Additionally, CHWs and coordinators estimated a savings of 2,048 hours of transportation time as a result of SMS messages sent during the pilot. Finally, the patient enrollment in the TB treatment program doubled from 100 to 200 patients as a result of increased operational efficiency.

We conclude that mHealth interventions can provide cost-effective solutions to communication barriers in the setting of rural hospitals in the developing world.

## PRE-CLINICAL VALIDATION OF A PORTABLE INFANT WARMER USING PHASE-CHANGE MATERIAL NOT DEPENDENT ON A CONTINUOUS EXTERNAL SUPPLY OF ENERGY

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Delivery of an optimal thermo-neutral environment for low-birth-weight infants at risk for environmental hypothermia is universally accepted. Recent advances in warming technologies have led to development of a low-cost reusable phase-change material (PCM) that can store and release sufficient heat for infants without the need for a continuous external heat supply. First, we determined and validated the physical and chemical properties of a non-toxic, wax-like PCM (latent heat capacity of approximately 200J/g and an ability to withstand >50,000 thermal cycles). Next, we developed a plastic pouch for the PCM and a novel sleeping bag design to position the pouch for dorsal surface conduction warming. Finally, we evaluated the PCM's ability to maintain a steady temperature by comparing the temperature-cooling curves between 400 grams of PCM and a 400 gram pure water control pouch, among 400 grams of PCM in varying clinically-relevant ambient air temperatures (20.0°C - 30.0°C), and among varying amounts of PCM (200 grams - 600 grams) at 25.0°C. For each test, the PCM was entirely melted, temperatures were measured using thermistors, and heat loss (in Celsius per minute) and duration of efficacy (time between 35.0°C to 38.0°C as advised by the WHO for a comparable heated water mattress) were calculated and compared with ANOVA.

Repeated testing showed that the steady-state heat loss of the PCM ( $0.0078 \pm 0.0014$  °C/min) was lower than that of pure water ( $0.0147 \pm 0.0029$  °C/min;  $p < 0.0001$ ). In addition, increasing ambient air temperature resulted in decreased steady-state PCM heat loss ( $0.0109 \pm 0.0023$  °C/min at 20C,  $0.0078 \pm 0.0014$  °C/min at 25C, and  $0.0051 \pm 0.0008$  °C/min at 30C;  $p = 0.0001$ ). Finally, increasing PCM mass also resulted in decreased steady-state PCM heat loss ( $0.0104 \pm 0.0038$  °C/min for 200 grams,  $0.0078 \pm 0.0014$  °C/min 400 grams, and  $0.0066 \pm 0.0011$  °C/min for 600 grams;  $p = 0.001$ ). Correspondingly, PCM had a longer duration of efficacy than water ( $367.5 \pm 64.7$  min vs.  $20.7 \pm 6.3$  min,  $p < 0.0001$ ), increasing ambient air temperatures have a longer duration ( $187.3 \pm 29.5$  vs.  $367.5 \pm 64.7$  vs.  $573.5 \pm 20.1$  min for 20°C, 25°C, and 30°C, respectively;  $p < 0.0001$ ), and increasing PCM masses have a longer duration ( $233.5 \pm 35.3$  vs.  $367.5 \pm 64.7$  vs.  $388.7 \pm 65.3$  min for 200, 400, and 600 grams, respectively;  $p = 0.001$ ).

We report a portable PCM based conduction-warming device that has the ability to maintain a surface temperature within WHO guidelines (35.0 – 38.0C) without continuous external supply of energy. Features include portability, low cost, simple maintenance. This newly designed and bench-tested device now requires prospective *in-vivo* evaluation. Planned testing on pre-term lambs will precede low-birth-weight human infant studies.

## **DEVELOPING A PROTOCOL TO ASSESS AND ALTER CHOLESTEROL CONTENT IN COCHLEAR HAIR CELL MEMBRANES**

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In the mammalian ear within the organ of Corti, hair cells detect and amplify sound. The “hairs” are actin-filled finger-like projections called stereocilia that are organized into rows of increasing height. Very small hair bundle deflections initiate hair cell mechanotransducer channel activation while a “tip-link” connects the tip of a shorter stereocilium to the lateral wall of its taller neighbor. This tip-link is thought to be tethered to mechanosensitive ion channels at either or both ends. Whether this connection between tip-link and ion channel is direct or indirect has not been determined. Our study aimed to change the membrane stiffness of the hair cell by altering cholesterol content with the ultimate goal of elucidating whether there is a direct or indirect connection between tip-link and ion channel.

We dissected organ of Corti from P0 to P8 rat cochlea and cultured them for 0-8 days. Using filipin staining and confocal microscopy, we attempted to quantify the baseline amount of cholesterol in a hair cell membrane. Through the addition of cyclodextrin and cholesterol we attempted to add cholesterol to the membranes. Through the addition of cyclodextrin alone we attempted to remove cholesterol from the membranes. We completed 92 total dissections in 46 animals. Fifty of the dissections were unusable for various reasons. Key discoveries in establishing our protocol to assess and alter cholesterol in the membranes were 1)use of an appropriate amount of Cell Tak, 2)filipin stain selection, and 3)timing of staining.

By altering cholesterol content, we are changing the membrane stiffness of the hair cell. Further studies that will be done include comparing mechanotransduction in cochlear hair cells with cell membranes containing 1)baseline cholesterol, 2)added cholesterol, or 3)subtracted cholesterol. The results of these studies will help elucidate whether membrane stiffness has an effect on mechanotransduction (indirect tip-link model) or not (direct tip-link model).

# A HIGH-PERFORMANCE CORTICALLY-CONTROLLED MOTOR PROSTHESIS ENABLED BY A FEEDBACK CONTROL PERSPECTIVE

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Neural prostheses, or brain-computer interfaces (BCIs), translate recorded neural signals into control signals that can guide a paralyzed arm, artificial limb, or computer cursor. Although current laboratory demonstrations provide a compelling proof-of-concept, clinical viability is hampered by limited performance. BCIs can be viewed from a feedback control perspective: the brain is the controller of a new plant, defined by the BCI. This perspective leads us to two advances that result in significant qualitative and quantitative performance improvements. We tested these advances in closed loop with two rhesus macaques. On each trial, subjects used a cursor, controlled by the native contralateral limb or a BCI, to acquire a 2D target within an allotted time period. Neural data were recorded from a 96-electrode array (Blackrock) implanted spanning PMd and M1.

By applying the assumptions of a simple feedback model, we augmented a position/velocity Kalman filter that is commonly used in the literature (e.g., Kim et al., 2008). All experiments used spike counts generated by a threshold detector without spike sorting. Such a system has clinical appeal, particularly for arrays with potentially decreased SNR (these experiments were 22-26 months and 4-7 months post implantation for monkeys L & J, respectively). In the first advance, using a standard Kalman filter, we fit neural data to a 'guess' of the desired volitional control signal, instead of observed or instructed kinematics. In the second advance, we developed a modified velocity-only Kalman filter, whose observation model incorporates cursor position as feedback. The new BCI appears more controllable and produces straighter reaches and crisper stops.

Compared to the standard Kalman BCI, mean time to target is reduced from 1200-1300ms to 650-700ms for both monkeys L & J. This system can run freely for hundreds to thousands of trials, making point-to-point reaches to targets randomly placed across the workspace without performance degradation. These feedback-perspective based algorithmic innovations, together with experimental verification, suggest significant performance advances are possible, thereby increasing clinical viability.

## CHONDROGENIC INDUCTION OF HUMAN ADIPOSE-DERIVED STEM CELLS USING PLATELET-RICH PLASMA

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Cartilage tissue engineering using adult stem cells represents a promising strategy for the treatment of articular cartilage damage. Adipose-derived stem cells (ADSCs) are a particularly well-suited source of adult stem cells, given their low donor morbidity, high cellular yield, and capacity for chondrogenic differentiation. Current techniques for the expansion and chondrogenic differentiation of ADSCs rely on the use of xenogenic products including fetal bovine serum (FBS) and exogenous growth factors. In the setting of potential infectious risks and documented immunogenic risks, the FDA is unlikely to approve stem cell techniques for clinical use that rely on these exogenous products. Platelet-rich plasma (PRP) is derived by simple techniques from whole blood, and represents a readily available autologous source of sera containing high concentrations of growth factors, including those routinely included in chondrogenic media. We hypothesize that PRP can supplant the need for exogenous growth factors and FBS in the expansion and chondrogenic induction of human ADSCs.

Chondrogenic induction of human ADSCs was attempted using a 6-week micromass culture technique and media containing 5% PRP. Chondrogenic differentiation was assessed using real-time PCR for standard markers of chondrogenesis including collagen type II, aggrecan, and SOX9. Also analyzed were markers of chondrocyte hypertrophy (collagen X) and fibrocartilage formation (Collagen I). Compared to induced controls (10ng TGF-B1 and 1% FBS), ADSCs induced using a 5% PRP media failed to express collagen type II at levels equivalent to induced controls or human chondrocytes. Statistically equivalent levels of aggrecan, collagen X, and Collagen I were expressed in the 5% PRP group vs. the induced control.

These findings represent a failure of chondrogenic induction using a 5% PRP media under the conditions analyzed, as collagen type II expression is a fundamental feature of successful chondrogenic induction. Given the myriad of growth factors and proteins contained in PRP, we hypothesize that the presence of certain inhibitory and/or interfering proteins may be to blame for this failure of chondrogenic induction. Prior research has revealed VEGF, Insulin-like Growth Factor Binding Protein 2 (IGFBP2), and IGFBP3 as potential inhibitory candidates. Our lab has demonstrated the ability to neutralize these three proteins from PRP using specific antibodies and trials are currently underway to evaluate the chondrogenic potential of this modified PRP-based media.

## **ACCURATE TRACKING OF RADIOLOGY RESIDENCY CONFERENCES USING A WEB BASED ATTENDANCE SYSTEM**

**Bhargav Raman, TS Desser**

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The Accreditation Council for Graduate Medical Education (ACGME) requires that residency programs record attendance at educational conferences. The traditional manual method of using sign-in sheets is time-consuming and inefficient. We aimed to solve this problem by implementing a completely automatic sign-up system that is web-based using either an inexpensive Bluetooth-based system, an SMS-based system, or an email-based system. The web interface is leveraged to allow residency program directors to not only track attendance but also to analyze trends and improve conference quality. The first part of this project was to investigate which type of system is best suited for attendance tracking, taking into account convenience, feasibility, practicality and resident preferences.

The user interface to the system was implemented as follows: After a thorough evaluation which included consultations with residents, faculty and support staff, a hybrid system using both a paper-like system as well as an email-based system was decided upon. Residents mark attendance by sending an email to the system with their rating for the conference and comments and their attendance is marked once the email is received. Alternatively, they can mark attendance on a computer in the room, which always shows the current conference, much like a paper-based system. Residency directors and support staff have administrative rights and can manage conferences as well as access reports and graphs analyzing conference attendance. Conference schedules can be downloaded in a format that can be easily tacked onto a wall. The system also stores current year in training, resident rotation and vacation schedules that enable the system to track whether residents are attending conferences that are mandatory for them to attend.

The current system received very favorable responses and has increasingly replaced the paper-based system as of January, 2010. Uptime has been more than 99% for the last 3 months and the system was used for 54 conferences with a total of 435 attendees thus far. We expect total replacement in the next 3 months. Residents and support staff are asked for feedback on the system on a regular basis, and many minor and major changes to the user-interface have been made. The system has thus achieved buy-in from administrators and residents of the Radiology Department. The future might see our system deployed to other departments or institutions.



## EXTENDING AND EVALUATING WARFARIN PHARMACOGENETIC ALGORITHMS USING CYP4F2 AND RARE VARIANTS IN CYP2C9

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Warfarin dosing remains challenging due to its narrow therapeutic window and large variability in dose response. We enrolled 108 patients on chronic warfarin therapy to analyze factors involved in its dosing and evaluate eight dosing algorithms, including two developed by the International Warfarin Pharmacogenetics Consortium (IWPC). Complete clinical and pharmacy records were obtained and patients were genotyped at SNPs relevant to the *VKORC1*, *CYP2C9*, and *CYP4F2* genes using integrated fluidic circuits made by Fluidigm.

When applying the IWPC pharmacogenetic algorithm to our cohort of patients, the percentage of patients within 1 mg/day of the therapeutic warfarin dose increases to 63% from 54% using clinical factors only, or from 38% using a fixed-dose approach. The *CYP4F2* gene adds approximately 4% to the fraction of the variability in dose ( $R^2$ ) explained by the IWPC pharmacogenetic algorithm ( $p < 0.05$ ). Importantly, we show that pooling rare and common variants substantially increases the  $R^2$  for *CYP2C9* (rare variants alone:  $p = 0.0065$ ,  $R^2 = 6\%$ ; common variants alone:  $p = 0.0034$ ,  $R^2 = 7\%$ ; rare and common variants:  $p = 0.00018$ ;  $R^2 = 12\%$ ), indicating that relatively rare variants not routinely assessed in genome-wide association studies may be significant. When comparing different algorithms in the literature, the IWPC pharmacogenetic equation and the Gage (2008) equation have the best performance (IWPC:  $R^2 = 50\%$ ; Gage:  $R^2 = 49\%$ ), and all pharmacogenetic algorithms outperform the IWPC clinical equation ( $R^2 = 22\%$ ). *VKORC1* and *CYP2C9* genotypes did not affect long-term variability in dose. Finally, the Fluidigm platform, a novel warfarin genotyping technology, was effective with 99.65% concordance.

The IWPC's pharmacogenetic dosing algorithm performs well on this independent cohort of patients on stable anticoagulation, and additional factors such as *CYP4F2* and rare *CYP2C9* variants can potentially increase its performance.

## **INTEGRATIVE GENOMIC ANALYSES IDENTIFY *CDX2* AS A LINEAGE SURVIVAL ONCOGENE AMPLIFIED IN COLORECTAL CANCER**

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Lineage-survival oncogenes are activated by somatic DNA alteration, and give rise to cancers derived from the cell lineages normally regulated by these genes. Here, we surveyed a collection of 29 colorectal cancer cell lines and 442 primary colorectal tumors for DNA copy number alterations and identified in a subset of samples amplification of chromosome 13q12.2, containing the caudal type homeobox transcription factor gene *CDX2*. *CDX2* is a known transcriptional regulator of normal gut development and is required for intestinal epithelial cell differentiation and maintenance. However, in the context of genomic amplification, RNA interference experiments here show *CDX2* is required for proliferation and anchorage-independent growth of colorectal cancer cells. Gene expression profiling revealed that *CDX2*-driven tumors express markers of intestinal differentiation, and that the oncogenic function of *CDX2* may be mediated by deregulation of Wnt signaling and *HOX* family gene expression. Taken together, these data characterize *CDX2* as a novel lineage-survival oncogene deregulated in colorectal cancer.

## ESTIMATING THE DEAD: MORTALITY MODELS IN THE GLOBAL BURDEN OF DISEASE REPORTS

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Department of Medicine

The Global Burden of Disease (GBD) is a series of reports produced by the World Health Organization since 1996, with a new edition to be published this fall, that attempt to estimate the mortality and Disability-Adjusted Life Year (DALY) burdens of human disease worldwide. The findings of these reports are commonly used to guide global health efforts by aid agencies and not-for-profits. For most developing countries, where vital registration data is unavailable, estimates for mortality and disease burden are derived from models developed by the report's authors; these models, however, have never been suitably validated in the literature.

Using count-based population and mortality data obtained from EuroStat for 27 European countries in 2008, we compared actual survival curves versus estimates produced using GBD models and appropriate model inputs. Estimated and actual survival values were in close agreement until age sixty. After this point, estimated survival was dramatically lower than actual survival for both males and females, with decreasing agreement with increasing age. Least dramatic disagreement occurred for Azerbaijani women; estimated 20-year survival after age sixty was 31%, as opposed to an actual value of 47%. Most dramatic disagreement was that of Finnish women, with survival estimated at only 6%, as opposed to an actual survival of 76%.

These findings suggest that the GBD estimates may substantially overstate the mortality burden for persons over age 60. However, by overestimating mortality the models may also underestimate the socioeconomic and health care burdens of chronic diseases, which tend to affect people later in life. Refinement of these models to more accurately estimate survival at older ages is essential to properly understand the rising global burden of chronic diseases.

## FUNCTIONAL CONNECTIVITY IN PATIENTS WITH CHRONIC NEUROPATHIC PAIN

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Studies estimate that 75 million Americans suffer from chronic pain conditions annually with staggering personal and financial costs. Because chronic pain is difficult to treat clinically, treatments outside of traditional pharmacological interventions have been explored including the use of cognitive strategies such as distraction from pain. Another novel treatment modality for chronic pain is called real time functional MRI (rt-fMRI), which gives patients feedback of blood-oxygen-level dependent (BOLD) signal from brain regions involved in pain processing in real time. Rt-fMRI was shown to reduce pain scores in chronic pain patients and functional activity in regions related to pain.

The present pilot study seeks to determine regions of interest (ROIs) that alter their connectivity between the conditions of distraction and attention to pain. To do this, we recruited five female patients with chronic neuropathic pain in a single limb and trained them over one week to use the strategies of attention and distraction to modulate their pain. Each subject then completed three functional scans: one scan using each strategy and one resting scan. An ROI map was constructed using local maxima obtained from an fMRI study of patients in pain, which was then used to extract and compare the time courses from each subject's scan (Coghill, 1999). Functional connectivity analysis for each subject was calculated as the pair-wise correlation between average ROI time-courses. Then, the average connectivity was determined by averaging across subjects. Finally, connectivity in different scans was compared using Fisher's Z transformation.

The following results were significant at  $p < 0.05$ . Compared to rest, attention decreased the connectivity between the primary somatosensory cortex (S1) and the cingulate cortex. Compared to rest, distraction increased the connectivity between the right prefrontal cortex (rPFC) and insula, S1, thalamus, and periaqueductal gray (PAG). Distraction decreased the connectivity between the secondary somatosensory cortex (S2) and insula as well as between S1 and S2. Compared to distraction, attention decreased the connectivity between the rPFC and PAG and S1 and thalamus and increased the connectivity between S2 and the thalamus.

The results of this pilot study show that distraction compared to either attention or rest result in significant changes in functional connectivity between brain regions implicated in pain processing. In particular, the rPFC altered its connectivity with multiple regions when distraction was compared to rest, suggesting that this area may be an interesting target for future real-time feedback in studies with chronic neuropathic pain patients.

## **RUSSIAN ORTHODOX END-OF-LIFE BIOETHICS**

**Mengkai Shieh**, David J Holloway, Samuel W Lebaron, Department of History, Department of Political Science, Department of Medicine - Family & Community Medicine

Because the Russian Orthodox Church is an influential voice in Russian society today, the purpose of this project has been to understand how the Russian Orthodox Church deals with bioethical issues related to end-of-life healthcare. This understanding should help in cultural competence and in promoting more effective collaborations with Russians in healthcare and research efforts. There is increasing discussion in our medical community on the need to increase focus on non-physical patient needs. The knowledge from this study should help contribute to these efforts.

The approach to this study has involved consultations and interviews with experts, study of relevant scholarly and religious texts, and participant-observation in Russian healthcare environments and society. Russian Orthodox bioethics is a system of bioethics which is located within a larger structure of Orthodox spirituality. The aim of Orthodox spirituality is union with the personal God, Who is described as being knowable through His uncreated energies. The epistemology of this system is founded upon spiritual knowledge derived from this union. Union with God is considered the purpose and goal of human existence, and this goal is the primal orienting principle for all elements and activities within this system. In this system, there exist no bioethical principles or values which possess inherent value in and of themselves, as they are only considered to possess a significance in accordance with their relation to this primal orienting principle.

This research sheds light on how Russian Orthodox patients and healthcare providers view and utilize modern healthcare. Some of the concepts and themes of the study have been found to be relevant in understanding other Eastern philosophical systems, such as Confucianism, as well as for elucidating aspects of our own historical-philosophical heritage. The student plans to return to Russia to continue this research through further participant-observation in Russian healthcare and society.

## LIVER TUMOR MOTION USING DIFFERENT FIDUCIAL LANDMARKS

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Organ motion remains a significant problem when treating liver tumors with stereotactic body radiotherapy (SBRT). This study was performed to determine how implanted fiducials correlated with the diaphragm as a potential surrogate for tumor position.

Twenty-seven patients with primary and metastatic liver tumors who had implanted gold fiducials in the liver in preparation for SBRT at Stanford University were included in this study. Each patient underwent a 4-dimensional CT scan as part of treatment planning after fiducial placement, and image analysis was performed using commercial software (MIMContouring; MIMVista Corp., Cleveland, OH). The positions of the fiducials, GTV center, and diaphragmatic apex were recorded on both the end-expiration phase (50%) and the end-inspiration phase (0%) scans. The relationships between the seeds and the diaphragm apex to the GTV center at both phases were evaluated. Wilcoxon rank-sum tests and Pearson correlation coefficients were performed with commercial software (SPSS Statistics 18; SPSS Inc., Chicago, IL).

A total of 37 tumors and 109 seeds were evaluated. Absolute GTV motion was as follows: anterior/posterior, median=3.33mm, range: 0.24 – 12.73mm, 95<sup>th</sup> percentile=6.53mm,  $p<0.000$ ; superior/inferior, median=7.36mm, range: 0.00 – 20.70mm, 95<sup>th</sup> percentile=16.92mm,  $p<0.000$ ; right/left, median=2.28mm, range: 0.02 – 16.80mm, 95<sup>th</sup> percentile=6.97mm,  $p=0.780$ . The diaphragm apex to the GTV center was noted to change by a median difference of 5.96mm (range: 0.07mm – 25.23mm, 95<sup>th</sup> percentile=21.31mm,  $p=0.982$ ) between the 50% and 0% phases. This difference did not seem to be correlated with the superior/inferior distance of the GTV isocenter from the diaphragm (Pearson correlation,  $r=-0.110$ ,  $p=0.518$ ), indicating that this change cannot be explained by the proximity of the tumor to the diaphragm. In the superior-inferior direction, the median difference was 5.00mm (range: 0.00mm – 17.5mm, 95<sup>th</sup> percentile=16.25mm,  $p=0.819$ ) between the 50% and 0% scans. The median difference between the 50% and 0% scans in the distance from GTV center to the fiducials was 2.92mm (range: 0.028mm – 37.03mm, 95<sup>th</sup> percentile=15.66mm,  $p=0.092$ ). This difference appeared to be correlated with the distance of the fiducial from the GTV ( $r=-0.180$ ,  $p=0.026$ ). In the superior-inferior direction, the median difference was 3.75mm (range: 0.00 – 37.50mm, 95<sup>th</sup> percentile=17.50mm,  $p=0.155$ ) between the 50% and 0% scans, however this difference in distance did not seem to be correlated with superior/inferior GTV distance from the fiducial ( $r=-0.080$ ,  $p=0.33$ ).

Liver tumor motion due to respiration is substantial. Using the diaphragm alone as a surrogate for tumor position when treating liver tumors may not be adequate, leading to an error of >5 mm. Implanted fiducials correlate better with tumor position.

## **ASSESSING THE IMPACT OF DISEASE-SPECIFIC INTERVENTIONS ON POPULATION HEALTH: AN EVALUATION OF *PEPFAR* OUTCOMES.**

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Vertical (disease-specific) programs and horizontal (broader-based primary care) programs have long been debated by experts and practitioners with minimal quantitative evidence provided in support of the comparison. This study seeks to provide quantitative evidence of the impact of a major vertical program, the President's Emergency Plan for AIDS Relief (PEPFAR), on the population health of its focus countries.

This study focuses on the countries in Africa that receive PEPFAR funding. Countries without epidemiological data or without a generalized HIV epidemic have been excluded. Sub-Saharan countries with a generalized HIV epidemic but without PEPFAR funding are designated as control countries. Five indicators are being used to evaluate population health: life expectancy at birth, infant mortality rate, under-five mortality rate, DTP3 immunization coverage rate, and MCV immunization coverage rate. Two time periods are considered for analysis: 1997-2002 for the time period prior to initiation of PEPFAR funding and 2004-2007 for the time period following initiation of PEPFAR funding in 2003. Control variables accounted for in this study are population, HIV prevalence in 1997, gross domestic product, funding disbursed by The Global Fund from 2002-2006, and four measures of governance.

Keeping in mind the limitations of this study, there are three possible outcomes: improvement in population health, worsening of population health, and no change. Outcomes must be examined alongside evaluations of the disease-specific aims of PEPFAR. The results of this study will be a limited but important step to moving the debate between vertical and horizontal programming towards an evidence-based approach to better inform the decision making of donors and policymakers.

## OPTIMIZING ENROLLMENT IN RESEARCH STUDIES USING ONLINE SURVEYS

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Conducting population-based research using a survey method for data collection can be challenging in achieving adequate response rates necessary to meet enrollment targets and minimize subject bias. Web-based surveys represent a recent technological development that provides an alternative method for data collection and analysis.

We used an online survey program Surveyor developed by the Information Resources and Technology department at Stanford School of Medicine. Surveyor is an online, free software tool that creates HIPAA-complaint surveys linked to a web domain and hosted on a server. We created an online survey to assess risk factors for development of stress fractures in high school runners. We visited 28 local high schools and collected email addresses from each athlete. Athletes were contacted separately and provided the link to the web-based survey with instructions and a unique study code. Athletes who did not complete the survey initially were provided multiple reminders to increase participation. Female athletes were initially contacted in Fall 2008 and Spring 2009 (time one,  $t_1$ ). We opened enrollment for additional female and male athletes in Fall 2009 ( $t_2$ ). Recruitment was closed March 2010.

Of female athletes contacted  $t_1$ , 71.5% (211 of 295) completed the survey. At  $t_2$ , 62.8% of females (235 of 374) and 59% of males (311 of 527) completed the survey. Response rate for  $t_1$  was higher than  $t_2$  when compared to both female and male subjects ( $P < 0.05$ ). No difference was seen in response rates between genders at  $t_2$ . Mean response time and range of response times were greatest for female subjects recruited during  $t_1$  ( $28.16 \pm 72.86$  days,  $P < 0.05$ ; range 1-422) than at  $t_2$  for males ( $8.43 \pm 11.13$  days; range 1-59) or females ( $18.85 \pm 29.17$  days; range 1-199). In all populations, recruitment was improved by sending multiple reminder email messages. We conclude that online surveys can be used to enroll subjects for research studies and can meet the standard of published surveys of 60%. Target enrollment may be reached using a shorter recruitment period and is optimized with multiple reminder messages.

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## NEUROPSYCHOLOGICAL CHARACTERISTICS OF CHILDREN AND ADOLESCENTS AT RISK FOR BIPOLAR DISORDER

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Bipolar disorder (BD) is a chronic, recurrent disease associated with significant morbidity and mortality, especially when it begins in childhood. BD is known to be an inherited disorder that has a prodromal phase in most cases. Children and adolescents with a parent with BD, who had mood dysregulation but not full BD (putative prodromal BD), were compared to healthy peers across several neuropsychological domains. Social reciprocity was measured by the Social Responsiveness Scale (SRS), theory of mind was measured by use of the NEPSY, and affect recognition was measured by the Diagnostic Test of Nonverbal Accuracy (DANVA) and the NEPSY. Deficits in neuropsychological functioning may precede the first episode of mania in children and adolescents at risk for BD. Careful characterization of the prodromal BD profile may help to define an endophenotype and provide opportunities for early detection and intervention.

Eighteen subjects with prodromal BD were compared to 9 subjects with no psychopathology. Subjects with prodromal BD demonstrated impairment in social skills. There were significant group differences in social reciprocity ( $F=11.144$ ,  $p=.003$ ) across all domains, including Social Awareness ( $F=4.75$ ,  $p=.041$ ), Social Cognition ( $F=14.3$ ,  $p=.001$ ), Social Communication ( $F=11.9$ ,  $p=.003$ ), Social Motivation ( $F=11.49$ ,  $p=.003$ ), and Autistic Mannerisms ( $F=11.84$ ,  $p=.003$ ). There were no significant group differences in performance on Theory of Mind or affect recognition tasks.

Subjects with prodromal BD experienced a relatively early onset of mood symptoms, which may alone have negatively affected their social development. It could also be the case that innate differences in the neural circuitry of prodromal subjects contribute to mood dysregulation and social deficits. The lack of impairment in Theory of Mind and affect recognition suggests that social skills are disproportionately affected in prodromal BD. However, it is possible that there were differences that we failed to detect. In general, all subjects made few errors in these tasks, so perhaps the measures themselves are not sufficiently difficult *i.e.* sensitive enough to detect group differences. Further exploration is warranted.

## **<sup>18</sup>F-5-FLUOROURACIL DYNAMIC PET/CT REVEALS DECREASED PERFUSION AFTER BEVACIZUMAB IN COLORECTAL METASTASIS**

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In patients with advanced colorectal cancer, recent clinical trials have shown that the addition of anti-angiogenic agents such as bevacizumab to standard chemotherapy increases treatment response rate and prolongs overall survival. However, long-term or high-dose anti-angiogenic therapy may lead to a vasculature that is inefficient for drug delivery and resistant to current treatment standards. Recent evidence supporting the 'Jain hypothesis' suggests that treatment with bevacizumab improves chemotherapy efficacy by normalizing tumor vasculature leading to more efficient oxygen and drug delivery. However, no studies have yet been conducted in human subjects to determine the most effective time to administer bevacizumab to maximize its effects. This study is designed to determine whether using a radiolabelled analog of 5-fluorouracil, [<sup>18</sup>F]-5-fluorouracil, for PET/CT imaging can directly demonstrate differential chemotherapy delivery to known tumor sites before and after administration of bevacizumab.

In this pilot study of five patients with newly diagnosed and untreated metastatic colorectal adenocarcinoma, patients underwent baseline as well as 24-hour post-bevacizumab <sup>18</sup>F-5FU PET imaging and non-contrast CT scan. On day 0, patients received 7.5 mg/kg bevacizumab infusion over 90 minutes. The degree of <sup>18</sup>F-5FU uptake at the metastatic sites was assessed using visual interpretation and semi-quantitative SUV analyses. Our preliminary results indicate that <sup>18</sup>F-5FU PET/CT is a valuable technique in visually monitoring chemotherapy delivery to colorectal metastasis. At the 24-hour post-bevacizumab time-point, we observed a 20% decline in the amount of 5FU reaching the tumor, measured by the AUC<sub>tum</sub>/AUC<sub>aor</sub> ratio. At baseline, the mean AUC<sub>tum</sub>/AUC<sub>aor</sub> over 5-minutes was 1.24 ± 0.30 (range, 0.424 to 2.14) and was lower in patients 24-hours after the administration of bevacizumab, 1.06 ± 0.32 (range, 0.23 to 2.13), *p* = 0.04.

The <sup>18</sup>F-5FU radiotracer was adequately penetrated the organs of interest and the specific site of metastasis did not appear to alter the SUV measurements. Although, we were unable to determine the most effective dose or timing of bevacizumab to maximize tumor drug perfusion in this study, future studies will examine a 6-hour post-bevacizumab cohort as well as a 5mg/kg dosed cohort. This information will allow us to determine the optimal timing of bevacizumab infusion with respect to chemotherapy for maximum uptake of 5-FU, which will allow for improved treatment regimen design and increased survival.

## **LESBIAN, GAY, BISEXUAL, AND TRANSGENDER CURRICULAR CONTENT: MEDICAL STUDENT EXPERIENCES AND PERSPECTIVES**

### **Lesbian, Gay, Bisexual, & Transgender Medical Education Research Group**

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Lesbian, gay, bisexual, and transgender (LGBT) individuals face significant barriers to accessing safe, appropriate, and comprehensive medical care. Educating future physicians to meet the needs of LGBT persons may reduce disparities these individuals confront. Students' exposure to and experience of LGBT-related curricular content is currently unknown.

We designed a web-based survey to assess three domains: student exposure to LGBT content, perspective on LGBT content, and comfort and preparedness in caring for LGBT patients. Allopathic and osteopathic students were recruited from all medical schools in Canada and the United States via Facebook.com as well as electronic mailing distributed by professional organizations and medical school leadership.

There were 3,743 respondents, representing 170 of 176 schools. The median age was 25. First- or second-year students comprised 54.9%, and nearly 20% self-identified as sexual and/or gender minorities. LGBT topics taught varied greatly between institutions. The most common exposures to LGBT content were during required pre-clinical instruction and interactions with LGBT-identified patients. 35.9% rated their LGBT content as "fair," 34.3% as "very poor" or "poor". Most students felt more prepared as a result of their medical school training, but comfort level was most commonly reported as unchanged.

We identified extensive variation in LGBT medical curricula. Furthermore, comprehensive medical training that augments preparedness and comfort in caring for LGBT people is lacking. These results may suggest opportunities to develop standards for LGBT curricula and to create resources for institutions and their students to achieve these standards.

## **LESBIAN, GAY, BISEXUAL, AND TRANSGENDER-RELATED CONTENT IN MEDICAL CURRICULA**

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To decrease health disparities faced by lesbian, gay, bisexual, and transgender (LGBT) individuals, the Association of American Medical Colleges (AAMC) has called for LGBT content in all medical curricula. The prevalence of such content is unknown. We assessed dean-reported LGBT curricular content, perspectives on LGBT content, evaluation methods, and strategies to increase LGBT content.

Deans of Medical Education (or equivalent) at all allopathic and osteopathic medical schools in Canada and the United States were invited via telephone, e-mail, and post to complete a web-based survey.

A 65.9% response rate resulted from 116 respondents. During undergraduate medical training, LGBT content averaged 4.9 and 2.4 hours in pre-clinical and clinical years, respectively. The most common LGBT-related topics were HIV, sexual orientation, and gender identity. 49.5% of respondents rated their curriculum as “fair,” 19.3% as “poor” or “very poor.” Teaching efficacy was evaluated most frequently via written examinations and standardized patients. Additional LGBT health curricular materials and time for teaching LGBT health were the most commonly suggested strategies for increasing content. Faculty development for LGBT health was provided at 21.9% of institutions.

Substantial variation exists in the quantity and content of LGBT-related medical curricula. The majority of deans rated their curricula “fair” or below, suggesting opportunities for improvement. More teaching materials, devoted time, and LGBT content-specific faculty development could augment curricula. We offer that, in agreement with AAMC recommendations, development of LGBT-related medical education standards may reduce observed inter-institutional variation and improve self-rating while reducing health disparities.

## **INCORPORATING A CULTURE OF PATIENT SAFETY INTO STANFORD MEDICAL CURRICULUM**

Shubha L. Bhat, Natalia V. Leva, **Julia C. Pederson**, Felipe D. Perez, Stephanie M. Smith and Clarence H. Braddock. Medicine.

In recent years, the Institute of Medicine and World Health Organization (WHO) have called attention to the role of health care systems in protecting patients from medical errors and promoting high quality patient care. A new report from the Lucian Leape Institute highlights the importance of incorporating patient safety principles in all stages of medical education. Stanford University Medical Center has been active in the patient safety movement at a hospital level; however, the medical school curriculum does not currently emphasize education about patient safety principles and systems-based quality improvement.

The purpose of our project is to (1) develop core competencies around patient safety and continuous quality improvement (QI), and (2) implement changes in the medical school curriculum that emphasize these important topics. The first phase of this project is a comprehensive needs assessment in the Stanford community to identify gaps in patient safety training. We conducted one hour in-depth interviews with over 25 key stakeholders in medical education and hospital management, including clerkship directors, QI leaders, and clinical students. Common themes that emerged from these interviews include the role of the medical student as a part of the medical team and the importance of thorough communication and hand-offs.

Thus far, we have formed working relationships with each of our stakeholders, ensured two hours of space in Quarter 6 (Q6) of POM for QI and patient safety curriculum, drafted preliminary Q6 lesson plans based on input from our stakeholders, designed a pre- and post-survey to evaluate the Q6 curriculum based on established reports, and developed recommendations for core competencies to share with the curriculum committee. Looking ahead, we envision laying the groundwork for a longitudinal, integrative curriculum focused on patient safety, which will be reinforced during clinical rotations and enhanced through collaboration with the free-clinics and other schools at Stanford.

## **ELECTRONIC DECISION SUPPORT FOR DIABETES AND HYPERTENSION CARE IN TANZANIA**

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Tanzania, like most countries in Africa today is facing the “double burden of disease” that includes the continuing epidemics of infectious disease and the increasing prevalence of non-communicable disease (NCD). Yet, despite the increasing rates of NCDs such as diabetes and hypertension, these health problems receive fewer resources and less attention than infectious diseases. One reason for this is that the care of patients with chronic disease requires a more sophisticated approach by providers and a system that maintains a clinical record of patients for each visit and is available to the provider.

The challenge facing Tanzania is the shortage of staff and other resources to take on the additional challenge of chronic disease. The goal of our work is to improve the availability and quality of care available to patients with NCD's through the development of electronic algorithms, downloadable on handheld computers or cell-phones for use by nurses in public clinics treating and screening patients for diabetes and hypertension. It is our hypothesis, based on prior research in South Africa and Tanzania, that electronic algorithms can bring high quality screening and care to patients with chronic diseases who are unable to access medical specialists on a regular basis but are able to reach primary care facilities in rural and peri-urban areas of the country. In addition, the system will collect and store longitudinal data, critical to effective management of these chronic diseases, and will also be used to investigate epidemiological data, such as co-morbidities between diseases, demographic predictors or other questions about disease patterns that are at present unknown.

Through a collaboration with the Tanzanian World Health Organization, Harvard University School of Public Health, and Stanford University School of Medicine, we have developed algorithms for the management of hypertension and diabetes which incorporate evidence based guidelines and resource availability in Tanzania. We are currently in the process of iterative refinement and our finished product will soon be ready for use in the next phase of our project, which will involve validation and deployment of our algorithms in rural clinics in Tanzania.