*70th Annual*
*Soma Weiss*
*Medical and Dental*

# Student Research Day

January 14, 2010


# Book of Abstracts

HARVARD MEDICAL SCHOOL
Office of Enrichment Programs

# The Soma Weiss Student Research Day

This day honors the memory of Soma Weiss, MD (1899-1942), an inspiring teacher and physician at HMS and an ardent supporter of student research. Soma Weiss was born January 27, 1899 in Bestercze, then a part of Hungary. He immigrated to New York in 1920 and graduated from Cornell Medical College in 1923.

Soma Weiss came to Harvard Medical School in 1925 when he was appointed assistant at the Thorndike Memorial Laboratory and Research Fellow in the Department of Medicine. He rose rapidly, demonstrating his great ability as an investigator, teacher, administrator, and clinician. Within four years, Dr. Weiss was appointed Assistant Professor of Medicine. His medical capabilities, his diplomatic handling of difficult situations, and his amicable personality led to his appointment as Director of the Second and Fourth Medical Services at Boston City Hospital in 1932. In this position, he took charge of the fourth year medical students, winning their admiration and affection. One of the important contributions he made to teaching was in his development of the Clinico-Pathological Conference at the City Hospital. His own bi-weekly Pharmacological-Therapeutic Conference gave the students unusual insight into the use of drugs.

Soma Weiss possessed all the qualifications necessary for the great clinician. He was a master of observation. His ward rounds were excellent; while conducting them, he never neglected the patients, the students, or the visiting physicians. He kept them all in proper balance while he dominated the whole. He wisely insisted that clinical work must be the basis for the study of disease.

Soma Weiss became the second Physician-in-chief of the Peter Bent Brigham Hospital in 1939. He died January 31, 1942 from the rupture of a congenital intracranial aneurysm. In the intervening years, his generous spirit, his eager and able services for the Hospital, his great abilities as a physician, investigator, and teacher, left an indelible imprint on the many students he mentored.

*Harvard Medical School wishes to thank the Weiss family for their generous donation in support of the Annual Soma Weiss Student Research Day.*

*Soma Weiss*
1899 -1942

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# HARVARD MEDICAL SCHOOL

## *70TH ANNUAL*
## Soma Weiss Student Research Day

## January 14, 2010

**Poster Sessions**
1:30 - 3:30 PM
Atrium of the Tosteson Medical Education Center
260 Longwood Avenue, Boston, Massachusetts

**Student Presentations and Poster Awards**
3:30 – 5:00 PM
Room 209, Tosteson Medical Education Center

| | |
|---|---|
| **Welcome** | **Jeffrey Flier, MD**, *Caroline Shields Walker Professor of Medicine*<br>*Dean of the Faculty of Medicine, Harvard Medical School* |
| **Introductions** | **Eleftheria Maratos-Flier, MD**, *Associate Professor of Medicine, Beth Israel Deaconess Medical Center*<br>**Patricia D'Amore, PhD,** *Professor of Ophthalmology (Pathology), Schepens Eye Research Institute*<br>*Chair, Faculty Committee for Student Research* |
| **Student Speakers** | **Charles Carspecken** (Holmes)<br>Characterization of the Role of Angiopoietin-Like Proteins in Blood Stem Cell Development |
| | **Carolyn Olson Walsh** (Peabody)<br>Postprandial Energy Availability with Three Popular Diets During Weight Maintenance after Weight Loss |
| | **Ethan Sanford** (London)<br>Genetic Mechanism Underlying Lung Hypoplasia in Congenital Diaphragmatic Hernia |
| | **Matthew Vanneman** (Holmes)<br>Using NKG2D/Fc Fusion Protein to Modulate Immune-mediated Tumor Destruction Against a Broad Spectrum of Cancer Types |

**Awarding of Poster Prizes**

Elizabeth D. Hay Prize for Basic Science Research
Judah Folkman Prize for Clinical / Translational Science Research
Charles Janeway Prize for International Research or Service
Robert Ebert Prize for Health Delivery or Service

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**Welcome to the 70th Annual Soma Weiss Student Research Day Assembly. The first of these assemblies, held in 1940, was organized by a group of medical students to:**

- **Highlight the investigative work of their colleagues in the field of medical science.**
- **Provide those students engaged in investigative work with the opportunity to present their research before an interested assembly.**
- **Demonstrate the breadth of possibilities of medical student investigative work.**

**The assembly honors the memory of Soma Weiss, a Harvard Medical School teacher and physician who was noted for his inspirational support and dedication to the advancement of medical science.**

**Funding for student research and investigative work is provided, in part, through endowment accounts and fellowships that are managed by the Office of Enrichment Programs and Harvard Medical School. The following Endowments and Fellowships are representative of those used to support our students:**

**Rishon M. Bialer Fund**
**Marshall A. Barber, PhD Memorial Fund**
**William B. Christensen Fund for Student Research**
**Class of 1955 Research Fund**
**Doris Duke Charitable Foundation**
**Myer Dana and Etta Dana Fund**
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**Arthur T. Hertig Fellowship Fund**
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**PASTEUR**
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**John Ware Memorial**
**Paul Dudley White Traveling Fellowship Fund**
**George Bernays Wislocki Scholarship**
**Laurence Ellis Lecture Fund**
**Serena & Eugene Schnitzer, MD Fund**

**The abstract included in this volume are reflective of the diversity and quality of research experiences available to all Harvard medical and dental students. The presentations at today's assembly are a tribute to the memory of Soma Weiss, and a testimony to the tradition of excellence in medical and investigative research at Harvard Medical School and Harvard School of Dental Medicine.**

***We congratulate all of our student researchers, investigators, and their sponsors.***

**Phase I Trial of Autologous Tumor Vaccine and a GM-CSF-expressing Bystander Cell Line in Patients with Recurrent Glioma**

**Pankaj K. Agarwalla**
**Harvard Medical School, Walter Bradford Cannon Society, Class of 2010**

**William T. Curry, Jr., MD**
**Department of Neurosurgery, Massachusetts General Hospital**

Malignant glioma is the most common malignant primary central nervous system tumor and is associated with significant morbidity and mortality. Cancer immunotherapy such as vaccination with tumor cells and GM-CSF (granulocyte-macrophage colony-stimulating factor) is a promising new approach for malignant glioma.

Our objective was to determine the feasibility, safety, and biologic activity of vaccination with a combination of irradiated autologous glioma cells and bystander cells engineered by gene transfer to produce GM-CSF (GM-K562 cells) in patients with recurrent glioma.

IRB approval was obtained for a Phase I dose-escalation study of GM-CSF-based vaccination with autologous tumor cells. Patients with recurrent glioma undergoing repeat craniotomy and tumor resection were eligible and enrolled prior to re-resection. At time of re-resection, patient tumor cells were harvested, processed, and cryopreserved. Patients underwent the vaccination (between $1x10^5$ to $5x10^7$ tumor cells per vaccination) on day 0, 7, 14, 28, 42, and 56 as tolerated. GM-CSF was produced locally at the site of vaccination by simultaneous injection of dose-level appropriate numbers of GM-K562 cells, which are poorly immunogenic cells derived from a leukemia line in blast crisis and engineered to secrete a consistent amount of GM-CSF after lethal irradiation. Patients have been enrolled into two GM-K562 dose levels: $5x10^6$ and $1x10^7$ GM-K562 cells per dose. Primary outcomes include measurement of safety, as by documentation of serious adverse events (NCI-CTCAE grade III or higher) and feasibility, defined as the ability of patients to receive at least one vaccination. Secondary outcomes include biological activity and antitumor efficacy. Biological activity is being measured by clinical and histologic evaluation of delayed-type hypersensitivity reactions and by punch biopsies of vaccination sites. Further biological activity is being measured by flow cytometric analysis of the phenotypes of white blood cell subsets. Antitumor efficacy will be measured by survival and radiographic evidence of freedom from tumor progression.

To date, 11 patients have enrolled in the trial. There have been no serious adverse events and all enrolled patients have been able to receive and tolerate at least one vaccination. Biological activity has been demonstrated by the presence of immune infiltration at vaccination sites and marked delayed type hypersensitivity (DTH) reactions after 4 vaccinations. One patient had a striking radiographic and clinical response to treatment. Overall survival is still being measured. Flow cytometry of whole blood reveals several trends for immune cell subset response to vaccination.

Vaccination with irradiated autologous glioma cells and a GM-CSF-producing bystander cell line is a safe and feasible against malignant glioma in the recurrent setting. Lymphocytic infiltration at vaccination sites, marked DTH reactions, and radiographic and clinical responses signify some development of anti-tumor immunity and justify future research with our immunotherapeutic approach.

**Improving Access to Health Services: The Implications of Eliminating User Charges for Caesarians in Mali**

**Katherine H. Albutt**
**Harvard Medical School, William Bosworth Castle Society, Class of 2012**

**Kim A. Wilson, MD**
**Assistant Professor of Pediatrics**
**Children's Hospital Boston, Harvard Medical School**

Mali is plagued by elevated and stagnant morbidity and mortality rates and poor indicators of maternal health. The adjusted maternal mortality remains exceptionally high and its burden is disproportionately clustered among the poor. The lifetime risk of dying in pregnancy in Mali is 1/15 and caesarian rates are under 1% for 80% of the population, indicating extremely low access to critical obstetric care. Increasing skilled birth attendance and improving access to life-saving obstetric procedures is critical to ensure better maternal health. To address the problem of financial barriers to care, Mali abolished user fees for caesarians in public sector facilities in 2005 and has since provided facilities with the supplies and equipment to perform a basic caesarean section. It remains unclear, however, how effective the free caesarian policy is in increasing access to adequate obstetric care, especially for the poorest segments of the population. Program evaluation of the free caesarian initiative, in collaboration with ATN Plus and the Ministry of Health, is in progress to reduce obstacles to care and increase access to high impact maternal health services in Mali.

This program evaluation seeks to evaluate the impact of eliminating user charges for caesarians on maternal health outcomes and access to care in Mali. The specific objectives of the evaluation are: (1) to evaluate the impact of eliminating user charges for caesarians on maternal health outcomes and access to care; (2) to identify the remaining barriers and challenges to implementation of the free caesarian initiative; and (3) to evaluate the clinical appropriateness of caesarians in the public sector.

The program evaluation employed diverse research methods. An exhaustive literature review was conducted to identify key factors affecting access to institutional deliveries and caesarean sections in Mali. Analysis of pre- and post-policy data was conducted to assess trends in the caesarean section rate. A proxy SES questionnaire was developed, piloted, and adapted for prospective data collection. Instruments for community-level qualitative research and for facility-based research on policy implementation were created and piloted.

Analysis of trends in existing data indicate that there has been a clear increase in clinically indicated caesarean sections in Mali and in all regions over time on the order of 200% nationwide. The caesarean section rate has increased from 0.94% to 2.17% nationally and post-caesarean maternal and neonatal deaths and post-operative complications have declined. The evaluation remains in progress and as such final results and conclusions are pending.

**Arthroscopic-Assisted Biceps Tenodesis using a Suture Anchor**

**D. Edmund Anstey**
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**Thomas J. Gill, MD**
**Department of Orthopedic Surgery, Division of Sports Medicine**
**Massachusetts General Hospital**

**Background**: The anatomic location of the long head of the biceps (LHB) makes the tendon particularly susceptible to injury as it passes over the glenohumeral joint. This is a common source of shoulder pain and must often be treated surgically. The two primary surgical approaches practiced are tenotomy and tenodesis. Tenotomy, which is a surgical release of the tendon, effectively can relieve pain but often results in distal retraction of the LHB resulting in a cosmetic deformity known as a "popeye sign." For this reason, some physicians and patients prefer tenodesis, which involves releasing the tendon from its origin and inserting it into the bicipital groove. While avoiding distal retraction and the potential for a cosmetic deformity, tenodesis has the additional benefit of maintaining function of the LHB. The purpose of this study is to evaluate the clinical outcome of a novel method of tenodesis that involves arthroscopic ligation of the biceps tendon and subsequent open, suture anchor fixation at the inferior portion of the bicipital groove.

**Methods**: Clinical results of arthroscopic assisted biceps tenodesis were evaluated using subjective shoulder value, ASES score, Constant score, and visual analog pain scale. Comparisons were made to the contralateral limb. Patients were asked about the acceptability of the scar and whether they would have the surgery again.

**Results**: Forty eight patients who underwent arthroscopic-assisted biceps tenodesis were evaluated at a mean of 4.1 years postoperatively. Visual analog pain scores were 0.84 ± 1.59 for the operative and 1.05 ± 1.86 for the non-operative limb. ASES scores were 91.22 ± 15.15 for the operative and 89.35 ± 17.96 for the non-operative limb. Constant scores averaged 83.79 ± 14.18 for the operative and 82.90 ± 15.06 for the non-operative limb. There were no reoperations for biceps tendon tenderness. Forty six of the patients found scar appearance acceptable, and 40 replied they would have the surgery again (with 7 not responding).

**Conclusions**: Arthroscopic-assisted biceps tenodesis produces motion, pain and function that is similar the patient's non-operative limb. Reoperation does not appear to be necessary. Overall patient satisfaction is high and the surgery produces acceptable cosmetic results.

**Impact of a three-year quality improvement initiative on departmental performance on communication of critical test results**

**Shawn G. Anthony**
**Harvard Medical School, Francis Weld Peabody Society, Class of 2010**

**Ramin Khorasani, MD**
**Center for Evidence-Based Imaging, Department of Radiology**
**Brigham and Women's Hospital**

Optimal communication of critical test results (CCTR) is a National Patient Safety Goal. We investigated the impact of a three-year quality improvement initiative on communication of critical and discrepant imaging results.

This HIPAA-compliant quality improvement study, exempt from IRB approval, was performed in a 752-bed adult urban tertiary teaching hospital with over 600,000 radiology procedures annually. We developed a departmental CCTR policy in February 2006 based on recommendations from the Joint Commission, the American College of Radiology, and the Massachusetts Coalition for the Prevention of Medical Errors. The policy defined types of findings, urgency level, timelines for notification, acceptable modes of communication, escalation process, and documentation requirements. The primary outcome measure was compliance with CCTR policy, measured by periodic review of radiology reports between February 2006 and May 2009. We used chi-square tests to assess trends in compliance.

12,193 radiology reports were reviewed during 17 quality assurance audits. 9.2% (1,126/12,193) of all reports reviewed met CCTR policy criteria for critical results. The proportion of critical results fully compliant with CCTR policy increased from 28.6% in February 2006 to 94.6% by May 2009 ($p < 0.001$). The proportion of critical results with documented communication but not meeting the policy timeline decreased from 19.0% to 3.1% ($p < 0.05$), and the proportion of critical results without documentation of communication decreased from 52.4% to 0.2% ($p < 0.001$).

Development, implementation, monitoring, and reinforcement of CCTR policy resulted in substantial improvement of departmental performance for CCTR. Further studies will be useful to determine whether automation and integration with workflow will further improve individual physician and departmental performance.

**Significance of Overdiagnosis in Lung Cancer Screening**

**Ryan Arakaki**
**Harvard Medical School, Francis Weld Peabody Society, Class of 2012**

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**Institute for Technology Assessment, Department of Radiology**
**Massachusetts General Hospital, Harvard Medical School**

Lung cancer is the leading cause of cancer death in the United States. Lung cancer accounts for approximately 6% of all deaths each year in the U.S. There were approximately 215,000 new cases of lung cancer identified in 2008 with 80% of these cases caused by smoking. The high rate of lung cancer in people that smoke makes smokers a readily identifiable population that is at high risk for this disease. This makes them a candidate population for cancer screening. It is important to identify those at high risk of lung cancer because in approximately 75% of individuals that develop lung cancer, symptoms only occur once the tumor has already spread to distant sites. The proposed method of screening is the use computed tomographic (CT) screening due to its ability to detect small tumors when they are still in the early stages of growth before they have metastasized.

The sensitivity of CT that allows it to detect early stage lung cancer also creates a risk of overdiagnosis. The definition of overdiagnosis in cancer screening is the detection of cancer in a patient that would not have become clinically evident in that patient's lifetime. Several studies have attempted to estimate lung cancer overdiagnosis rates when CT screening is used, however, one of the difficulties in trying to compare these studies is the use of different methods that have been used to calculate overdiagnosis.

In our work, we are examining the different methods that have been used to estimate lung cancer overdiagnosis in an effort to clarify the results that have been reported by various studies. We are also investigating how the rate of overdiagnosis changes over time when CT screening is implemented in a specific population and what types outcomes are observed when overdiagnosed lung cancer cases occur. The results of this study will improve our understanding of the consequences of using CT to detect lung cancer by further describing one of the potential drawbacks to implementing lung cancer screening protocols.

**Micronutrient Supplementation Practices in HIV Care and Treatment**

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**Harvard Medical School, Francis Weld Peabody Society, Class of 2012**

**Kevin Sztam, MD, MPH**
**Children's Hospital Boston, Department of Gastroenterology/Nutrition**

The World Health Organization (WHO) estimates there are over 33 million people living with HIV worldwide, with 20 million infected in Africa alone, most in the area of the sub-Sahara. Almost 2 million children are infected [1,2]. In addition there is a geographic overlap of HIV and malnutrition in sub-Saharan Africa, where approximately 30% of adults are food insecure [3,4]. Those with limited or monotonous food supplies may face specific micronutrient deficiencies. These deficiencies have individual pathologies causing deficiencies in innate, humoral, and cell-mediated immunity. Micronutrient deficiencies of vitamins A, B, E and others, commonly observed in HIV-infected patients with advanced disease, are associated with HIV disease progression and mortality [5-9]. Many micronutrient deficiencies are associated with immune effects, [8,10] thus prevention of deficiency is critical to optimize outcomes in a disease such as HIV, where both viral infection and nutritional deficiencies may synergistically affect immune status.

Baseline intake is still a prime determinant of micronutrient status. In clinic sites in our HIV treatment programs in Kenya and Tanzania, HIV-infected patients are often admitted with malnutrition and signs and symptoms of micronutrient deficiencies: low body mass index, presence and severity of infections, and dermatologic findings. The provision of MMS (multiple micronutrient supplementation) through routine HIV care is therefore important adjunctive therapy.

To better understand access to MMS we piloted an assessment of MMS use in the care of HIV/AIDS patients in 9 comprehensive care and treatment centers in Central Province in Kenya. A survey was administered to pharmacists and nutritionists at each site with questions pertaining to content, availability, source, and funding of MMS. Preliminary results showed noticeable differences. For example, one site may have MMS available for pregnant and lactating mothers, children, and adults, whereas another may only have MMS for adults or substitute an MMS meant to target one population for another. This was most highlighted by differences between district hospitals, which usually had a larger supply of commodities compared to their satellite sites. This may be a result of a delay in dispersal or what some pharmacists referred to as "hoarding", where district sites hold on to supplies meant for satellite sites to serve as backup. Nonetheless, on average all sites had at least one MMS available at the time of the assessment, but many did not have a reliable supply or source. Further analysis and studies are required to better understand procurement and distribution of MMS in resource-limited areas.

**Utilizing subgenome capture strategies to interrogate sequences and dosage of known, candidate, and newly discovered Congenital Heart Disease (CHD) genes**

**Omar Badri**
**Harvard Medical School, Walter Bradford Cannon Society, Class of 2012**
**PASTEUR Fellowship**

**Christine Seidman, MD**
**Department of Genetics and Department of Cardiology**
**Brigham and Women's Hospital and Harvard Medical School**

Congenital heart malformations are among the most common birth defects with approximately 35,000 U.S. infants born annually with Congenital Heart Disease (CHD). Ony a few decades ago, 20% of children born with CHD survived to adulthood. By the 1980s this figure stood at 85% and continues to increase. This patient population is now so large (~1 million in the US) that it is known by an acronym, GUCH ("Grown-Up Congenital Heart" disease), and the management of the special health needs of this group has become a subspecialty of cardiology. The aim of my study is to define the genetic causes of CHD and to use this information to understand the clinical course of affected patients and to define molecular pathways required for heart development. Ultimately we hope these insights may provide new therapeutic opportunities.

Human mutations that have been previously identified to cause familial CHD alter transcription factors and regulatory genes that orchestrate cardiac development, a highly conserved process among vertebrates. The consequence of virtually all of these CHD mutations is altered gene dosage: either haploinsufficiency (null allele mutation or mutations that functionally inactivate the translated protein) or over-expression (chromosomal duplication or gain of function mutations), information that indicates precise levels of these proteins us critical for normal cardiac morphogenesis. From this observation, the Seidman laboratory hypothesized that structural variations in the human genome, or copy number variations (CNVs) that alter the dosage of cardiac development genes, also cause sporadic cases of CHD. Using genomic platforms they identified regions on chromosomes 1 and 7 that are altered by CNVs in children with tetralogy of Fallot.

My goal is to find the disease genes within each genomic interval. Because there are no obvious candidate genes (e.g., genes known to be involved in cardiac embryogenesis) encoded in these intervals I will sequence the entire intervals using subgenome capture technology. To date, I have created filters (nitrocellulose membranes with amplified genomic regions containing target genes encoded on chromosome 1 (~62.9kb, 8 gene and 44 conserved regions) and chromosome 7 (~44.3kb, 20 conserved regions). I am currently validating these filters for efficient capture of these sequences using control samples. Once validated, I will use these filters to capture each genomic region from DNA samples obtained from children with severe CHD (tetralogy of Fallot, double outlet right ventricle, hypoplastic left heat syndrome) and their unaffected parents. I have processed approximately 100 patient samples into genomic libraries, for filter capture and sequencing. I will compare sequences in CHD samples with the reference human genome. If point mutations that encode nonsynonymous variants, insertions, or deletions are identified, I will determine if these are present or absent from parental samples. Nonsynonymous variants found in the same gene from multiple affected

children that are absent from controls and parental samples will provide evidence for their causality. The work is ongoing.

**Informing Families of Their Child's Disability: Evaluation of a Training Program for Health Care Professionals**

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**Director of Psychiatry, University Center for Excellence in Developmental Disabilities, Division of Developmental Medicine, Children's Hospital Boston**

Each year, thousands of families around the world are informed by a health care professional about their child's disability. When families are told this news in an insensitive or inappropriate manner, the disclosure can cause hardship for the family, their child, and their health care provider. Using research regarding the needs of families and their care providers, a set of guidelines for best informing families of their child's disability were developed by the National Federation of Voluntary Bodies in Galway, Ireland.

A training program based on these guidelines was developed for health care professionals and piloted at a university in Ireland. The training course included information on the guidelines, the screening of a video about two families who had vastly different experiences learning of their child's disability, and a presentation by the mother of two children with disabilities. Training was given over several sessions to groups of medical students, nursing and midwifery students, and junior physicians. Participation in the training was voluntary and participants were asked, but not required, to fill out pre- and post-training questionnaires. These were matched using unique identifiers. The questionnaires measured the participants' self-reported levels of comfort and confidence on a 5-point scale. Knowledge was measured using multiple choice and fill-in-the-blank questions, which were scored by matching answers to the guidelines.

Data collected from 254 participants was analyzed using SPSS version 17.0. Significant improvements in comfort, confidence and knowledge were found in the medical and nursing students. Junior physicians improved in their comfort and confidence. Further data analysis is ongoing.

These data indicate that the training program was effective for participants in increasing their comfort, confidence, and knowledge when faced with the role of informing a family. As such, it would be beneficial to increase training opportunities for health care professionals using this program based on best practice guidelines. The training may be especially useful for those early in their careers who can benefit the most from increased knowledge regarding the best methods of informing families of their child's disability.

**Developing a Culturally Appropriate Intervention for Breast Cancer Screening among Underserved Women in Mulukukú, Nicaragua**

**Devika Bhushan**
**Harvard Medical School, Oliver Wendell Holmes Society, Class of 2012**
**Class of 1984 Scholarly Project Enrichment Fund**

**Rosemary B. Duda, MD, MPH**
**Associate Professor of Surgery, Harvard Medical School**

Breast cancer accounts for the greatest number of cancer-related deaths among women worldwide, and in Nicaragua, it is reported to be the second most frequent type of cancer among women. In Mulukukú, a rural setting where clinical breast-examinations (CBE) are not routine and there is no access to mammographies, we aimed to promote awareness and early detection of breast disease by training women on a rudimentary breast health module, covering symptoms, risk factors, and the correct performance of a breast self-examination (BSE).

The project relied on a network of local community leaders to disseminate the breast health messages to procure legitimacy and maximize uptake of the teaching. Five community leaders, three nurses from the Ministry of Health (MINSA) outpost in Mulukukú, and five community health providers from the Cooperativa Maria Luisa Ortiz, were trained to conduct this intervention. 198 women were reached with the breast health module, with pre- and post-intervention surveys administered to assess the intervention's impact on their breast health knowledge, attitudes, and behaviors (KAP). Suspected abnormalities were referred to the regional MINSA hospital for follow-up.

The mean age of the participants was 30.0 years (range: 18 to 63 years). Sixty-one (31.1%) had no formal education, while 65.7% could both read and write. Over half (53.5%) had never been married and 79.2% had at least one child.

74.1% of the women were previously aware of breast cancer, but only 5.1% had performed a BSE and 8.1% ever had a CBE performed (and only for specific patient-directed problems).

Women with a CBE history were significantly more likely to know how often to perform a BSE (OR = 8.18 [2.49, 26.88], p = 0.001) and to understand family history-related risk (OR = 7.62 [2.10, 27.70], p = 0.002). Women who had previously heard of breast cancer were more likely to indicate that women's breasts can vary significantly in shape and size (OR = 2.96, [1.43, 6.13], p = 0.004) and to affirm that both breasts should be examined with the BSE (OR = 4.34 [1.62, 11.63], p = 0.003).

97.4% of the women stated being comfortable with the technique and 99.4% intend to perform it monthly. When judged against eight key criteria, BSE technique was found to be correct 94.2% of the time (range: 81.6% - 99%). Participants showed significant improvement on several key questions, and BSE and CBE performance are first steps towards preventing breast cancer mortality.

**Total Energy Expenditure Declines Despite Stable Physical Activity After Significant Weight Loss**

**Christine E. Bookhout**
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**David S. Ludwig, MD, PhD**
**Director Optimal Weight for Life (OWL) Program, Division of Endocrinology, CHB**
**Associate Professor of Pediatrics, Harvard Medical School**

The US epidemic of overweight and obesity is a major public health concern because excess weight increases risk for morbidities such as cardiovascular disease and type II diabetes. Although dieting is often recommended to treat obesity, maintaining body weight after significant weight loss can be difficult. The concept of a "body weight set point" suggests that weight loss induces physiological adaptations that promote weight regain, including hunger and reduced energy expenditure. We hypothesized that a decrease in total energy expenditure (TEE), the overall number of calories expended per day, would occur after significant weight loss. Since TEE reflects the calories one can consume without weight fluctuation, quantifying its change with weight loss is relevant to weight stabilization and prevention of weight regain.

We conducted an analysis as part of a larger, ongoing feeding study. During the run-in phase of weight loss, an energy-restricted diet provided 60% of energy needs until the participant lost 10-15% of baseline body weight. After stabilizing the participant's weight, three energy-balanced test diets were provided in a randomized crossover design. TEE was measured using doubly-labeled water methodology before weight loss and during each of the three test diets. Duration of moderate-to-vigorous activity was assessed using an MTI Actigraph accelerometer worn for one week in each phase.

Results for 11 completed participants showed an average baseline TEE of 2816±670 kcal compared with 2114±496 kcal as the average of three post-weight loss measurements ($p=0.01$, T-test). The within-subject decrease in TEE from baseline to post-weight loss averaged 701±368 kcal ($p<0.0001$, 95% CI 454-948 kcal), representing a 24.1±10.6% decline in TEE ($p<0.0001$, 95% CI 17-31%). In contrast, minutes of moderate to vigorous physical activity did not change significantly (47.2±16.7 min/day baseline vs. 45.1±24.2 min/day as the average of 3 measures post-weight loss, $p=0.82$).

We speculate that weight loss could reduce TEE through a decline in basal metabolic rate (resting energy expenditure) and/or through a reduction in calories burned during exercise. Our data indicate that physical activity duration did not significantly decline following weight loss, although fewer calories could have been expended per unit time due to lower body weight. A decline in resting energy expenditure may explain much of the decrease in TEE, providing a physiological explanation for the difficulty of maintaining a reduced weight over time.

**Memory and Executive Function in Children with a History of Institutional Care**

**Karen Bos**
**Harvard Medical School, Oliver Wendell Holmes Society, Class of 2010**

**Charles A. Nelson III, PhD**
**DMC Laboratories of Cognitive Neuroscience**
**Children's Hospital Boston**

Throughout the world orphaned and abandoned children are frequently raised in institutions, which elevates their risk for later developmental delays and disorders. Though previous research has identified a pattern of general intellectual impairment among children exposed to early institutional deprivation, the contributions of specific underlying processes (such as memory, attention, perception, and problem solving) that may influence cognitive performance in this group are not as well understood. This study examined memory and executive function in children with a history of early institutional care.

The data for this study were collected through the Bucharest Early Intervention Project (BEIP). The BEIP is the first randomized clinical trial to evaluate foster care as an alternative to institutional care for abandoned children. In this study, institutionalized children in Romania were randomly assigned to continued institutional care or foster care, with an average age at foster care placement of 22 months. A third group of never-institutionalized children was also enrolled.

At eight years of age participants enrolled in the BEIP completed a touch screen-based, automated neuropsychological battery (Cambridge Neuropsychological Test and Automated Battery; CANTAB), which has been extensively validated for children in this age group. The CANTAB focuses primarily on measuring functions of the temporal and frontal lobes, with tests falling into three domains: visual memory, visual attention, and planning/working memory.

At this time, preliminary results are available from 72 subjects (46%) enrolled in the BEIP. There are statistically significant differences on two tests of visual memory (Delayed Matching to Sample and Paired Associates Learning) and on one test of executive function (Spatial Working Memory). For the percent correct for the Delayed Matching to Sample, the mean for the EIG was 56% correct, while the mean for the NIG was 69% correct ($p=0.003$). For the Paired Associates Learning, for total errors the mean was for the EIG was 28 and for the NIG was 13 ($p=0.002$). For the Spatial Working Memory, for total errors the mean was 68 for the EIG and 61 for the NIG ($p=0.035$). The preliminary data does not, however, suggest a significant difference in memory and executive function among children in the institutional care group ($n=22$) compared to the children assigned to the foster care intervention ($n=34$) on either the tests of visual memory or of executive function.

These analyses of memory and executive function will contribute to our understanding of several key aspects of institutional experience on later neuropsychological function.

**Hepatitis C at the MGH HealthCare Centers**

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The Hepatitis C Virus is estimated to be one of the most prevalent blood-borne pathogens in the United States. An estimated 1.6% of the U.S. population carries anti-HCV antibodies and 1.3% have an active HCV infection. Chronic hepatitis puts a strain on healthcare resources, and as the population of infected patients age and the disease progresses, more and more patients are predicted to burden the healthcare system due to HCV related complications. Identifying who is at risk for infection and the characteristics of who is and isn't likely to be treated is an essential first step to determining how the health care system and providers can respond to barriers to preventing and treating HCV.

The aim of this study was to determine the characteristics, treatment rates, and barriers to treatment of patients infected with the HCV under the care of primary care physicians in three community clinics serving underserved populations: Massachusetts General Hospital (MGH) Healthcare Centers of Charlestown, Revere, and Chelsea. The first step to critical analysis was to identify characteristics and health maintenance details sure to provide the most useful information for comparing sub-populations of positive-HCV patients between and within each health center. A broader goal included creating a database that would follow and identify targets for future interventions that will increase the effectiveness of prevention and antiviral treatment through the primary care delivery system.

Patient characteristics were chosen after reviewing current literature on a.) treatment and epidemiology of HCV and b.) trends in health quality research. Patient data collection began after the demographic and health information to be investigated was carefully chosen. A list of current MGH HealthCare Center patients with HCV was generated by query of all patients from 2000 to 2008 who had a history of a positive HCV antibody test. Then, data collection on medical and psychiatric history, social history, substance abuse history, HCV treatment status, and contact with providers at each clinic began.

Data collection is still in process. The developing database will be used for scientific analysis as well as a tool for physicians at the three health centers to track the progress of interventions and treatment of HCV in their specific patient population. Finally, development of the database will continue to adapt to new collaborations between a growing number of physicians with the desire to help alleviate the burden of HCV on their patients and their communities.

**SNF8 binds TRPC6 and enhances channel activity**

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Canonical transient receptor potential 6 (TRPC6) is one of seven members of the transient receptor potential (TRP) family of calcium-permeable cation channels that allow for an increase in intracellular calcium following activation of G-protein coupled receptors and receptor tyrosine kinases. Mutations in TRPC6 are a cause of an autosomal dominant form of inherited kidney disease known as focal segmental glomerulosclerosis (FSGS). All of the known FSGS mutations are located within the cytoplasmic segments of the cation channel, suggesting that these domains play a critical role in the proper regulation of channel activity.

To discover potential binding partners of TRPC6, we performed a yeast two-hybrid screen using the intracellular amino-terminal domain of TRPC6 as bait and isolated SNF8 as a TRPC6 binding partner. SNF8 is the mammalian counterpart to yeast VPS22, a component of the ESCRT-II endosomal trafficking complex required for the endocytosis and lysosomal degradation of transmembrane proteins. The interaction was confirmed by co-immunoprecipitation from eukaryotic cell extracts. The amino-terminal 107 amino acids, which do not contain any reported FSGS-associated mutations, are necessary and sufficient for the interaction. Overexpression of SNF8 enhances both wild-type and mutant TRPC6-mediated whole-cell currents in HEK293T cells. Furthermore, activation of NFAT-mediated transcription by FSGS-associated TRPC6 mutants is enhanced by overexpression of SNF8, and partially inhibited by RNAi-mediated knockdown of SNF8. Although the ESCRT-II complex functions in the endocytosis and lysosomal degradation of transmembrane proteins, SNF8 overexpression does not alter the amount of TRPC6 present on the cell surface.

Taken together, these results identify SNF8 as a novel regulator of TRPC6 and suggest that modulating the SNF8-TRPC6 interaction may influence the ability of TRPC6 mutations to cause FSGS. Experiments are currently underway to address whether SNF8 binds to TRPC6 alone, or as part of the ESCRT-II complex. Additional electrophysiology experiments will be needed to determine whether SNF8 acts to alter the open probability or unit conductance of TRPC6.

**Characterization of the Role of Angiopoietin-Like Proteins in Blood Stem Cell Development**

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Understanding the molecular and genetic basis for hematopoietic stem cell (HSC) differentiation and expansion carries profound clinical implications for patients with cancer and other hematologic pathologies. By definition, HSCs can self-renew and become any blood cell in the body; however, the signals that control their fates are not completely understood, and HSCs are difficult to expand outside the organism in culture. In this study, zebrafish were used to examine the role of Angiopoietin-like proteins (angptls) in the expansion of HSCs during embryogenesis. Recent studies suggest that exogenous angptls can expand adult HSCs *ex vivo* and that lack of angptls have antiapoptotic activities. Currently, the receptor and signaling pathways involved with the angptl function have not been identified.

Four transgenic zebrafish lines for each of the four isoforms of Angptls (1, 2a, 2b, 3) were established to better understand how these proteins confer phenotypes *in vivo*. We first made a heat shock-inducible angptl transgenic fish, Tg(hsp70:angptl2a) and observed an increase *cmyb*+ HSCs in the aortic gonado mesonephros (AGM), the site of definitive hematopoiesis in the embryos. To determine whether angptls are required for definitive HSC formation, we performed antisense morpholino knockdown of the angptl 1 and 2a or 3 alone. We found loss of AGM HSCs as well as defective vascular development including decreased angiogenic sprouting and defective arterial-venous specification. Both hematopoietic and vascular defects in these double morphants could be rescued by a constitutive active form of Akt, implicating Akt as an important downstream mediator of these two processes.

Because the angptl receptor is still unidentified, we took a candidate approach in identifying potential interacting pathways with angptl signaling. Activation of notch, wnt or prostaglandin (PGE2) signaling pathways has been shown to be important in HSC formation during hematopoiesis. We performed double morpholino injection into the notch and wnt reporter fish and observed decreased notch and wnt signaling in both, signifying that angptl may act upstream of notch and/or wnt pathways during hematopoiesis. In addition, crossing Tg(hsp70:angptl2a) into a notch mutant knockout, *mindbomb*, results in rescue of mutant HSC phenotype, further strengthening the interaction between angptl and notch signaling pathways. In contrast, PGE2 could not rescue the HSC phenotype in the angptl double morphants, excluding its interaction with angptl signaling. These studies demonstrate the necessary role of angptls in developmental hematopoiesis and have translational potential in improving embryonic cord blood transplants that reconstitute a patient's blood cells following chemotherapy.

**CD133 regulates apoptosis in melanoma cells by modulating the calcineurin/NFAT-signaling pathway**

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Malignant melanoma, considered the most aggressive and lethal form of skin cancer, possesses high metastatic potential and is notoriously resistant to existing therapies. In its disseminated metastatic form, malignant melanoma carries with it an extremely poor prognosis for patients, with an abysmal median survival rate of 6 months and a five-year survival rate of 5%. It is believed that the lethality of malignant melanoma can be largely attributed to its enhanced survival properties, inherently low rate of spontaneous apoptosis, and resistance to drug-induced apoptosis. Recent studies have offered some preliminary clues into its anti-apoptotic capacity, particularly the expression of the cancer stem cell (CSC) marker, CD133, which supports the existence of a subpopulation of melanoma cancer stem cells with tumor-initiating and self-renewal capabilities that can promote melanoma cell survival.

The aim of this study was to analyze the role of CD133 in malignant melanoma and to elucidate the downstream signaling events promoting cell survival. Previous work in the laboratory had demonstrated that lentiviral-mediated silencing of CD133 in human B16-F10 metastatic melanoma cells caused a four-fold reduction in the size of primary tumors derived from these cells when transplanted into mice. Additionally, annexin and tunel stains of CD133 silenced melanoma cells revealed a two-fold increase in the rate of apoptosis, and immunohistochemistry showed a preferential expression of the anti-apoptotic survival factor, Bcl-2. Even more intriguingly, diminished calcium levels were consistently detected in these cells.

Taken together, these results suggest that CD133 plays a key role in melanoma cell survival and exerts its anti-apoptotic effects through a $Ca^{2+}$-dependent mechanism. To further investigate this possibility, we examined the calcineurin (Cn)/NFAT-signaling pathway, in which cytoplasmic $Ca^{2+}$ influx activates the Cn-phosphatase to dephosphorylate NFAT, and thus facilitates NFAT's entry into the nucleus, where it can activate gene transcription. We identified the expression of one NFAT family member, NFATc1, in melanoma cells by Western blot. Immunostaining and confocal microscopy of NFATc1 in CD133-silenced melanoma cells showed reduced expression and nuclear localization of NFATc1. Moreover, inhibition of NFATc1 in CD133-expressing melanoma cells with the calcineurin inhibitor, FK506, phenocopied the effect of silencing CD133, thus further supporting the idea that CD133 promotes cell survival through the Cn/NFAT-signaling pathway.

This study is the first to demonstrate a requirement for CD133 in the regulation of apoptosis and a role for the Cn/NFAT-signaling pathway in mediating this process in melanoma cells. These findings not only enhance our understanding of the mechanisms underlying melanoma, but also represent a promising new avenue for developing novel therapies to eradicate melanoma tumor cells in a more highly specific manner.

**Fructose Induced Insulin Resistance via Modulation of AMP-activated Kinase (AMPK) Levels**

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Diabetes and obesity are two of the most widespread conditions in the American population. With increased consumption of processed foods, the incidence of diabetes and obesity among younger individuals has increased greatly. Previous studies have shown that diets rich in fructose can cause insulin resistance. However, not much is known about the pathway through which insulin resistance develops. One protein of interest is AMP-activated kinase (AMPK) which has been shown to act as an energy sensor in the cell. During periods when ATP levels are low and AMP levels are high, AMPK activates a variety of mechanisms which allow for increased cellular uptake and metabolism of glucose, thereby reducing serum glucose levels. Alternatively, AMPK lies downstream of the insulin signaling pathway, and can be activated when insulin is present. We present the hypothesis that fructose metabolism may modulate insulin action by altering AMPK levels.

In this study, we examined fructose induced alterations in the activity of AMP-activated protein kinase. Hepatic HepG2 cells were cultured for 72 hours in media with physiological levels of glucose (5mM) and fructose (0.5mM) and diabetic range glucose (11mM) levels. Cells were then treated overnight with 2 mM metformin and/or 1mM insulin. Subsequently, cells were harvested, and western blot analysis was performed to examine expression of phopshorylated AMPK and phosphorylated acetyl-coA carboxylase (ACC), which is directly phosphorylated by AMPK.

Our results demonstrated that both fructose and metformin induced increased AMPK activation, which at first seems paradoxical in that metformin is a widely used anti-diabetic drug, while fructose intake has been linked to insulin resistance. However, fructose metabolism includes addition of two phosphate groups from ATP molecules, leading to local depletion of ATP and a corresponding increase of AMP leading to AMPK activation. Metformin on the other hand, indirectly and independent of ATP levels activates AMPK, although the exact mechanism is not known. We hypothesize that the depletion of ATP during fructose metabolism may activate other pathways which lead to an impaired ability to respond to insulin. In addition, fructose induces AMPK independently of the insulin signaling pathway. Finally, at physiologic glucose levels, insulin treatment increased AMPK activity, whereas at high glucose levels, basal AMPK activity decreased, and addition of insulin further decreased AMPK activity. Cells treated with fructose showed the same pattern as high levels of glucose, with high fructose treated cells showing a decrease in AMPK activity upon stimulation by insulin.

**Vioptix Monitoring System Decreases Flap Loss in Microsurgical Breast Reconstruction**

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Deep inferior epigastric artery perforator (DIEP), Superficial inferior epigastric artery perforator (SIEA), and Superior gluteal artery perforator (SGAP) flaps are the latest innovations in microsurgical breast reconstruction after mastectomy. Vascular compromise in these free flaps can be difficult to detect in the immediate post-operative period, potentially leading to flap loss and subsequent patient distress. Early detection and intervention increases the salvage rate. Traditionally, clinical evaluation includes assessment of the capillary refill, color, temperature and turgor; however, these criteria are highly subjective and dependent on the assesor's clinical experience in free flap monitoring. Tissue oximeter (ViOptix) monitoring potentially offers a more objective method for detecting vascular compromise. It is a non-invasive monitor of real-time flap perfusion through the emission of near-infrared light and measurement of local tissue oxygen saturation.

A retrospective database review of patients undergoing DIEP, SIEA, SGAP breast reconstructions from 2004 to 2008 at an academic teaching hospital was preformed. Traditional clinical evaluation was used to monitor flaps prior to May 2008 and the ViOptix monitor was utilized after June 2008. The ViOptix monitor was used immediately after the completion of the surgery to provide continuous monitoring of the flap. Operative re-exploration was indicated when the oximeter showed a 20 point decrease within 1 hour or an absolute reading below 30. Additionally, a retrospective chart review was conducted for patient demographics, operative details, and flap complications including return to OR and flap loss.

A total of 448 flaps (from 356 patients) were identified and divide into two groups: 372 flaps were monitored without ViOptix and 76 flaps monitored with the ViOptix system. Flaps that were monitored clinically experienced a 5.9% rate of re-exploration, while flaps monitored by ViOptix had an re-exploration rate of 9.2%. However, the control group experienced a 3.0% flap loss while the Vioptix group experienced 0% flap loss.

Since utilizing the ViOptix monitor, we have seen a higher re-exploration rate, but more importantly, no flap losses. The primary reason for this improvement in patient care is the earlier detection of vascular problems and intervention before significant flap ischemia or necrosis occurs. We feel that the increased rate of re-exploration reflects our initial learning curve as the prevention of flap loss represents a major improvement in patient care. Continuing education of nursing staff and residents will be necessary for accurate interpretation of the monitor.

**Assessment of a non-invasive diagnostic method for salivary gland carcinoma using Fourier Transform Infra-Red Spectroscopy**

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In the United States, there are approximately 5000 new cases of salivary gland neoplasms each year. These neoplasms can be malignant or benign and they can arise from the parotid, submandibular, sublingual or minor salivary glands. The malignancy of the neoplasm is determined pre-operatively using fine needle aspiration cytology (FNAC) or ultra-sound guided core biopsy (USCB). Based upon these results, a conservative, superficial or total resection of the salivary gland will be considered. The inherent problem of using FNAC is its inability to distinguish between in situ and invasive carcinoma and difficulties in diagnosing common salivary gland neoplasms such as pleomorphic adenoma. USCB has a greater diagnostic advantage compared to FNAC but it is more invasive and requires local anaesthesia.

The aim of this study is to assess the use of the WQF-660 FTIR spectrometer in conjunction with or to replace FNAC or USCB to improve pre-operative diagnosis of salivary gland carcinoma. This spectrometer uses mid-infrared radiation, which has no known harm to the human body, to generate spectroscopic data that can be used to differentiate between malignant and benign neoplasms at the molecular level.

To test the sensitivity and specificity of the spectrometer, 56 patients with salivary gland neoplasms of unknown malignancy were screened pre-operatively. Biopsies of the neoplasms were examined post-operatively by a pathologist, who was blinded to the results of the spectral data. The diagnoses made from the biopsies were considered to be the gold standard by which the spectral data were compared to after being analyzed using stepwise logistic regression.

To find the highest classification accuracy, logistic regression was performed using a model with 14 independent variables which shows a perfect fit with Cox & Snell $R^2$ of 0.65 and a Nagelkelke $R^2$ of 1.00. This model classifies the cases with 100% accuracy in comparison to the diagnosis made from the biopsies with 13 malignant and 43 benign neoplasms.

The high sensitivity and specificity of the WQF-660 FTIR spectrometer indicates its clinical potential to be used as a non-invasive, simple and rapid diagnostic tool for determining the malignancy of salivary gland neoplasms. Due to the nature of the mid-infrared radiation, anaplasia at the molecular level can be detected using the WQF-660 FTIR spectrometer.This information obtained could be used clinically to reduce occult metastases in patients with a clinically negative neck and to determine the margin of resection to allow complete removal of the neoplasm.

**Combination-Based Photodynamic Therapy to Disrupt Compensatory VEGF Signaling in Pancreatic Cancer**

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Pancreatic cancer is the fourth-leading cause of cancer death in the United States. Photodynamic therapy (PDT) is an emerging modality in the treatment of this disease as it induces localized tissue death using relatively nontoxic reagents and non-ionizing light. One concern, however, is that PDT insult can induce upregulated expression of the cytokine vascular endothelial growth factor (VEGF) in cancer cells. VEGF signaling is known to promote tumor angiogenesis and survival, proliferation, and migration in both cancer cells and epithelial cells. As a result, disruption of this VEGF signaling may improve the efficacy of cytotoxic therapies such as PDT.

To achieve this, our group has developed a combination strategy that involves the simultaneous delivery of the clinically-approved, anti-VEGF antibody bevacizumab (Avastin) and the photosensitizer benzoporphyrin derivative (BPD) directly into cancer cells. This represents a novel use of Avastin, as it is normally administered extracellularly to target secreted VEGF protein. Central to this strategy is the use of a liposomal construct known as a nanocell to encapsulate both drugs, enabling intracellular delivery. Our group demonstrated that this strategy significantly increases treatment cytotoxicity in the AsPC-1 pancreatic cancer line in cell monolayer as compared to strategies that do not involve intracellular delivery of Avastin.

The hypothesis for this project is that the enhanced killing seen with this combination therapy results from effective elimination of the intracellular stores of VEGF that would normally be secreted following PDT insult, thus abrogating a vital survival signal that allows cancer cells to resist PDT's phototoxicity. This study supports this hypothesis through *in vitro* data demonstrating the therapy's remarkable efficacy in neutralizing both intracellular and extracellular pools of VEGF protein compared to standard extracellular application of Avastin. Furthermore, the temporal characteristics of this VEGF neutralization are characterized, and are shown to persist for a minimum of 72 hours. Finally, cell viability studies performed under a variety of conditions demonstrate that the enhanced killing is achieved only with the intracellular delivery of Avastin, thereby illustrating the significant improvement to cell killing that can be achieved with the targeting of intracellular VEGF stores.

**N and C terminal mutations of PsbO, the manganese stabilizing protein of Photosystem II**

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Man-made pollution has come to have an ever increasingly negative impact on population health. One strategy to solving this dilemma is to create new technologies that can make clean burning fuels with fewer detrimental byproducts. A quickly developing candidate, biofuel, utilizes the sugars naturally made by plants and shows great promise in this area. However, biofuels currently do not seem usable on a large scale basis due to the lack of abundance of usable plants relative to fuel demands. In order to create more efficient synthetic technological innovations that can improve upon the natural photosynthetic yield, it is first essential to understand the chemistry behind photosynthesis.

The active site of photosystem II (PSII) uses manganese, calcium, chloride, and a protein called PsbO to catalyze photosynthesis. Due to current limitations in x-ray crystallography, it is not possible to directly see the relationship between each factor in the active site, making it challenging to infer the exact chemistry behind the oxidation reaction. Our research utilizes mutant PsbO proteins to indirectly determine the relationship between chloride and manganese so that the chemistry driving photosynthesis can be better understood. Here, we report on the role of three PsbO mutants, ΔL6M, W241F, and ΔL6MW241F in retaining inorganic cofactors in the PSII active site, and the function of these mutants with regards to stability of the manganese cluster. The results indicate that the mutants with the highest affinity for chloride maintain manganese stability at wildtype levels for the longest duration, while the mutants with the lowest affinity for chloride exhibit markedly decreased manganese stability regardless of whether the mutation is N or C-terminal in origin. These findings suggest that the chloride ions retained by PsbO provide stability to the four manganese atoms in the active site of PSII. Specific N or C-terminal residues do not appear to play a direct role in the stability of the manganese cluster.

**Contamination of common household surfaces with influenza virus: A prospective case-control study in urban Thai households**

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**Background**: Rationale infection control and community pandemic mitigation guidance require an improved understanding how of influenza is transmitted. Indirect contact via inanimate objectsfomites may be an important mode of transmission that frequent hand washing could mitigate. We studied households with an influenza-infected child to measure the prevalence of influenza virus contamination, determine the effect of an intensive hand washing intervention, and explore potential associations with humidity and temperature.

**Methodology**: In the context of a larger study of influenza transmission, we identified febrile pediatric outpatients with laboratory-confirmed influenza and randomly assigned their households to hand washing intervention and control arms. Six surfaces common to all households and the finger pads of the index case and symptomatic family members were swabbed. Specimens were tested by real-time reverse transcription-polymerase chain reaction (RTPCR) and positive specimens were placed on tissue cell culture. A handheld, calibrated psychrometer was used to measure absolute humidity and temperature.

**Results**: 64 of 90 (71.1%) study households had pediatric index cases infected with the 2009 pandemic influenza A (H1N1) virus. Sixteen of 90 (17.8%) households had at least one influenza positive surface by RT-PCR and only one pandemic virus was successfully cultured. The fingertips of 15 (16.6%) index patients were influenza positive. Control arm households had a higher prevalence of surface contamination (11/45; 24.4%) than households in the hand washing arm (5/45; 11%), though this was not significant:; Prevalence Risk Difference (PRD) 13.3% (95%CI: -2.2-28.9%; p=0.09). Households where the index case was infected with a seasonal influenza virus had a significantly higher prevalence of surface contamination (PRD: 18.3%: (95% CI: -1.2, 37.8%; p=.04). Households where the age of the index case was $\leq$ 8 years had a significantly higher prevalence of surface contamination (PRD 19.1% :95% CI 5.3, 32.9% p=0.02). Among households with low compared to high absolute humidity, the PRD was 37.9% where the index case was infected with seasonal influenza and 38.5% when secondary infections were present (p=.07).

**Conclusions:** We documented influenza virus contamination on household surfaces and on the fingertips of ill children. Households with young influenza infected children and those infected with seasonal influenza viruses had increased rates of surface contamination. Increased hand washing can reduce the prevalence of influenza virus surface contamination in the home.

**Therapy-Related Oral and Maxillofacial Complications in Hodgkin's Lymphoma Survivors: A Sib-Pair Analysis**

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Late and long term effects of Hodgkin Lymphoma (HL) treatment is an important issue given the early age at diagnosis and relatively high cure rate of this type of cancer. Late medical effects of cancer treatment have been explored for HL, but there is a lack of knowledge of how these therapies affect the oral health of the individual. Most oral complications resolve when cancer treatment ends and the patient's overall condition improves. However, some complications, such as xerostomia, may persist.

The objective of this study was to assess the oral health and evaluate the incidence of oral complications in HL survivors as compared to their unaffected siblings. We hypothesize that the higher prevalence of oral and maxillofacial related complications in HL survivors correlates with HL treatment.

We analyzed data collected in a cross-sectional study of patients treated for HL between 1969 and 1996 at Brigham and Women's Hospital, as well as their siblings, who served as the control population. The patients were derived from a preexisting HL database that captures all HL patients seen at the department of radiation oncology at four Harvard-affiliated hospitals. 512 HL survivors and a comparison group of 224 siblings completed the 500-item questionnaire that included questions regarding oral health.

Based on the date collected, HL survivors were: 12 times more likely experience an abnormal sense of taste than their unaffected siblings (OR 11.9, 95% CI=3.7, 38.2, p=0.0001), 9 times more likely to experience loss of sense of taste or smell lasting 3 of more months than their unaffected siblings (OR 9.4, 95% CI=2.9, 30.5, p=0.0001), and almost 5 times more likely to experience problems with chewing/swallowing than their unaffected siblings (OR 4.7, 95% CI=2.1, 10.3, p=0.0001). There was no significant difference found in the number of recent visits to the dentist or the number of permanent teeth lost.

The data indicates that HL survivors are significantly more likely to experience oral complications such as abnormal sense of taste, and problems chewing/swallowing compared to their unaffected siblings. This study demonstrates that HL survivors are at a higher risk for oral complications than a control population which may be attributed to their previous cancer therapy. There is still much to learn about the late and long term oral and maxillofacial complications in this population; thus further studies to explore the severity and impact on quality of life are needed.

**The Metabolic and Hemodynamic Effects of Coronary Artery Bypass: A Prospective Study**

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Coronary artery disease (CAD) is the leading cause of death in the United States. An estimated 16 million Americans have CAD and its prevalence is expected to rise with an increase in diabetes and obesity. Coronary artery bypass grafting (CABG) is indicated for patients with severe blockages and/or CAD that cannot be treated by other methods. CABG is a procedure where a healthy blood vessel from another part of the body is used to bypass the blocked coronary artery to provide oxygenated blood to the distal myocardium.

CABG procedures are typically performed with the assistance of a cardiopulmonary bypass (CPB) machine, which temporarily takes over the function of the heart and lungs during surgery to maintain circulation and oxygenation. However, CPB is associated with adverse effects, including systemic inflammation and reperfusion injury. Off-pump coronary artery bypass grafting (OPCABG) avoids the use of CPB. However, no study to date has been able to determine if OPCABG is superior to CABG. It is important to clarify differences in postoperative metabolic effects, nutritional markers and glycemic control, and hemodynamic effects, need for pressors and inotropes, between CABG and OPCABG.

Based on adverse effects linked to time on CPB, we hypothesized that patients undergoing OPCABG would require less metabolic and hemodynamic assistance in the intensive care unit (ICU). Additionally, we hypothesized a "dose effect" of time on CPB: the longer on-pump procedures would be correlated with increased metabolic and hemodyamic effects. To test these hypotheses, all patients undergoing CABG (n=29), OPCABG (n=26), and CABG + other intervention (n=29) in a nine-week period were enrolled in the study for prospective data collection and pre-operative/intra-operative chart review. All standard hemodynamic, metabolic, endocrine, and nutritional markers and interventions already part of mandatory dynamic nurse-driven ICU protocols were collected, as well as all medications administered during the inpatient stay.

Preliminary regression analysis of variables with an absolute value correlation coefficient $\geq 0.5$ yielded several statistically significant ($p<0.05$) linkages to certain outcomes. For all patients in the study, administration of tube feeds and total parenteral nutrition was correlated with median time to extubation, time on CPB and hours on pressors were correlated with time to ICU discharge, and lowest albumin level was correlated with days to hospital discharge. Since a few of the patients in the study remain in the ICU or step-down unit; a more comprehensive statistical analysis will be conducted once the data set is complete.

**Screening for major depression in post-myocardial infarction patients: operating characteristics of the Beck Depression Inventory-II**

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Major depression (MDD) affects 15-30% of patients who have suffered a myocardial infarction (MI), and is associated with cardiac mortality, recurrent cardiac events, and impaired health-related quality of life in the year following the MI. Despite its prevalence and association with poor outcomes, depression in post-MI patients usually goes unrecognized and untreated. Although both the American Heart Association and the American Academy of Family Physicians have recently recommended routine depression screening for all post-MI patients, there is little consensus about which screening tool to use. In this exploratory study, we assessed the operating characteristics of the Beck-Depression Inventory-II (BDI-II) and the BDI-II cognitive subscale (BDI-II-cog) in screening for major depression (MDD) in post-myocardial infarction (MI) patients.

Between October 2003 and July 2005, 131 post-MI patients admitted to an urban academic medical center completed the BDI-II and a semi-structured interview for depression within 72 hours of symptom onset. Sensitivity, specificity, positive and negative predictive values, overall correct classification, and likelihood ratios for various cutoff values on both scales were evaluated by comparing BDI-II scores to interview diagnosis of MDD. Receiver-operator curves (ROC) were also calculated and area under the curve (AUC) measured.

The AUC was 0.96 for the BDI-II and 0.89 for the cognitive subscale (BDI-II-cog). The optimal cutoff value for the BDI-II was ≥16, with a sensitivity of 88.2% and a specificity of 92.1%. Cutoff values of ≥3 (sensitivity=88.2%, specificity=81.6%) or ≥4 (sensitivity=82.4%, specificity=88.6%) were both acceptable for the BDI-II-cog.

These preliminary results suggest that the BDI-II and its cognitive subscale are very effective tools for screening for MDD in post-MI patients. Future studies should evaluate the BDI-II and the BDI-II-cog in a larger cohort of MI patients, and investigate the feasibility of using these scales in a screening program linking patients to treatment in the clinical setting.

**Rethinking War and HIV Transmission: Urbanization and Aid Dependency in Post-Conflict Northern Uganda**

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At approximately 11 to 12 percent, prevalence of HIV in northern Uganda is double the national average. Since the late 1980s, the region has been affected by a war involving the Lord's Resistance Army/Movement and the national government, driving most from villages into towns or internally displaced persons' camps. Many studies have suggested that the elevated prevalence of HIV is linked to the war, in the form of rape, transactional sex, the social intimacy of camps, and limited healthcare, among other forms of physical and structural violence. Other scholars speculate that the war has in fact protected against transmission, limiting movement and privacy while increasing knowledge of HIV/AIDS. What are the structural factors contributing to HIV transmission, and how and why does prevalence remain high in the north compared to other parts of the country?

In order to answer these questions and produce knowledge useful in developing prevention strategies, I conducted a qualitative study consisting of 30 life history interviews with HIV outpatients at St. Mary's Hospital Lacor, Gulu District; focus group interviews with persons living with HIV at Comboni Samaritan and Pabo camp; interviews with officers of World Vision, UN World Food Programme, and Gulu Youth Centre/Straight Talk; and participant observation at discos, bars, and churches.

While many elements of war – including camps, physical violence, and mobility of sexually active soldiers – have contributed to the transmission of HIV, other consequences of the war, especially in the recent post-conflict years, have been generally overlooked. In order to reduce transmission, more efforts must be made to recognize the intersection of humanitarian dependency and urbanization. Expectations of food and other aid have disrupted the generational development of productive lives, as children born and raised in camps are unaccustomed to farming and are inclined to enter into transactional sexual relationships that attempt to reproduce the structure of now-discontinued humanitarian handouts. Compounding this problem is difficulty in addressing urban-rural transitions. As a result of the war, a largely rural north consisting of clan-based villages was urbanized into camps and towns, expanding sexual networks and offering different lifestyles, including better health facilities and schools as well as the development of new consumer desires. Wealth and material inequalities that have accompanied this uncertain transition are contributing to HIV transmission. Current efforts aimed at reducing transmission must acknowledge the limitations of humanitarian and NGO efforts while addressing inequality, urban-rural transitions, and poverty, rather than focusing on conventional consequences of war.

**Promoting the Adoption of Health Information Technology: An Experience in the Office of the National Coordinator for HIT**

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Despite a widespread consensus that electronic health records (EHRs) can improve the quality, safety and efficiency of healthcare, few U.S. hospitals and providers have adopted EHRs or integrated them into clinical practice. Perhaps as few as 17% of physicians and 10% of hospitals have even basic EHR systems and substantial technical and logistical challenges hamper adoption.

The American Recovery and Reinvestment Act of 2009 (ARRA), makes a significant commitment to encouraging the adoption of EHRs nationwide. The health information technology (IT) portion of the law—the Health Information Technology for Economic and Clinical Health Act (HITECH Act)—allocates $19 billion for the promotion of health IT and EHRs. Significantly, ARRA does not envision EHR use for it's own sake: the $17 billion set aside for Medicare and Medicaid incentive payments to providers and hospitals can only by paid to those who demonstrate "meaningful use" of health IT (i.e. those who use health IT to improve the care of patients). ARRA assigns the task of defining meaningful use to the Office of the National Coordinator for Health IT (ONC) and the Centers for Medicare and Medicaid Services (CMS) in the U.S. Department of Health and Human Services. ONC also works to set privacy and security measures for EHRs; to create regional extension centers that will provide technical expertise; to develop, recognize and implement standards and certification criteria for EHR products and to create grant programs for state governments to promote EHR adoption.

I worked on a diverse set of issues at ONC from June through August 2009. My principle projects focused on meaningful use of EHRs and privacy and security of personal health information. Towards creating meaningful use definitions, I participated in ONC discussions about meaningful use and helped ONC to engage experts from CMS, the Agency for Healthcare Research and Quality and the Institutes of Medicine. I also helped read, catalogue and analyze more than 700 public comments on proposed meaningful use definitions. This data was presented to the Federal Advisory Committee that recommends meaningful use definitions to ONC. Regarding privacy and security, I drafted guidelines for compliance with the *Nationwide Privacy and Security Framework for Electronic Exchange of Individually Identifiable Health Information*. This document establishes principles for the ethical collection, use and exchange of individually identifiable health information. Compliance with the guidelines will be required for entities receiving Medicare or Medicaid incentive payments for EHR use.

**Needs Assessment and Asset Map of Women in Zwedru, Liberia: A Photovoice Project**

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*Background*: Women in Liberia represent a particularly vulnerable social group. Sexual and gender based violence is prevalent and has significantly impacted the lives of women. By the end of the civil war, 77% of women had been victims of rape and other forms of sexual abuse. With a total fertility rate of 6.2 and low access to maternal care, Liberian women experience among the highest rates of maternal mortality in the world. Discrimination against girls is common, leading to limited access to education. About one-third of women are literate compared to sixty percent of men living outside of the capital, Monrovia.

*Aims:* 1) To empower women through a community-based participatory Photovoice project. 2) To increase awareness of needs and assets of women in Zwedru, Liberia.

*Methods:* We trained four female Tiyatien Health community health workers (CHW) to co-facilitate the project. The CHWs then recruited eighteen women from communities all over Zwedru to participate in the Photovoice project. The eighteen women consisted of single mothers, people living with HIV/AIDS and included women ages 19-65. We then held an empowerment workshop in which the women discussed the strengths and challenges that exist in their communities. Each woman received a digital camera to document her life and her community. We conducted a home visit with each woman in which we collected the photographs and conducted in-depth audio-recorded interviews. We held a second workshop for critical reflection on the photographs and audio-recordings.

*Results:* The participants took several thousand photographs, and we collected hours of audio recordings during the home visits. The project gave participants the opportunity to document their communities through their own eyes. Many women reflected that they felt proud to take photographs and participate in the project. In addition, through these photographs and interviews, we were able to identify many of the most significant challenges facing women in the community, including poor living conditions, food insecurity, and lack of access to educationand clean water. Using the photographs and audio, we created a fifteen-minute film entitled "A Life in Zwedru." The community women who participated in the project decided to form a women's group, Zwedru Women United for Change.

*Conclusions:* The project gave Tiyatien Health an opportunity to launch its first program focused on women's issues and empowerment. In addition, it allowed the participants to form a group that will serve as a source of empowerment, moral support, and economic improvement.

**Family Burden/Impact In Caregivers To People With Intellectual Disability, Schizophrenia, or Dual Diagnosis**

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Intellectual disability (ID) refers to a particular state of intellectual and adaptive functioning, beginning in infancy, in which limitations in intelligence coexist with attenuated cognitive, social, and practical skills. ID has a prevalence of 1.5% in western countries with the potential to increase to 4% in less developed nations as a result of factors including the presence of neurotoxic agents or nutritional deficiencies during pregnancy. The complexity of care and treatment of individuals with ID may be compounded by the presence of underlying mental illness such as schizophrenia, the combination of which is referred to as Dual Diagnosis (DD). Studies have shown higher rates of mental health disorders in people with Intellectual Disability (ID), compared to those without ID, suggesting increased biopsychosocial vulnerability.

'Family burden' was initially proposed as a term to describe the emotional and economic strain that family members experience while taking care of a relative with some kind of disability; 'impact' has recently been advocated as an alternative to 'burden' to suggest that providing care may include positive consequences in addition to adverse costs. The family impact on people experiencing severe mental disorders has long been acknowledged, and studies focusing on individuals with ID are being introduced due to the progressive recognition of rights in this population and recent de-institutionalization and changes in family structure.

The ECFOS-II / SOFBI-II scale (*Entrevista de Carga Familiar Objetiva y Subjetiva* / *Objective and Subjective Family Burden Interview*) was used to assess the family impact to cargivers in 3 different populations: ID (179), schizophrenia (356), and DD (150). We hypothesized that the presence of psychiatric symptoms in combination with ID would be associated with a greater family impact, and that the distinct modules assessed by this scale might demonstrate differential importance amongst comparison groups to potentially inform design of interventions.

Statistical analysis revealed that the family impact on caregivers to individuals with DD as compared to individuals with ID or schizophrenia was significantly different in all modules assessed, including *Assistance with Activities of Daily Life*, *Supervision of Behavioral Problems*, *Dedication and Replacement by Other Carers*, and *Emotional Concern about the Relative*. These differences highlight the importance of timely and accurate diagnosis of psychiatric symptoms in the ID population. Proper classification of an individual's disorder(s) will be essential in providing appropriate preventative and educational services to the caregivers as a means to relieve specific areas of burden and reduce associated comorbidities.

**Are Prolonged QTc Intervals Predictive of Ventricular Dysrhythmia in Drug Overdose Emergencies?**

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Drug overdoses account for over two million emergency department visits each year in the United States. Overdose can be defined as intentional or unintentional overexposure to illicit or prescription drugs. Ventricular dysrhythmia, including ventricular tachycardia and fibrillation, is a potentially fatal outcome that can result from drug overdose. Electrocardiogram (ECG) analysis of QTc interval duration may provide prognostic information regarding the risk of ventricular dysrhythmia in overdose patients. While some drugs are known to produce prolonged QTc intervals, specific exposure information cannot always be ascertained in emergency settings. Furthermore, there are currently no prognostic tools to predict the occurrence of ventricular dysrhythmia in overdose emergencies, and standard cardiac care guidelines may not be optimal in these situations.

We hypothesized that prolonged QTc intervals will predict ventricular dysrhythmia in drug overdose emergencies. Our first aim was to describe the incidence of prolonged QTc intervals and to characterize initial patient presentations and course of care in overdose emergencies. Our second and ongoing aim is to investigate the relationship between QTc interval and the occurrence of ventricular dysrhythmia in the study population. Data was collected from 108 overdose patients presenting to Mt. Sinai and Elmhurst Emergency Departments in New York City from January to August 2009. Data includes ECGs and past medical history such as demographics, specific toxin exposure, pre-hospital treatment, and medical conditions and medications that may increase risk of ventricular dysrhythmia.

A diverse patient population (51% female, 35% Hispanic, 29% Caucasian, 32% African American, 2% Asian, and 2% other) was represented. The average number of drug exposures was 1.9, and 50% of patients presented with multiple toxicities. The most frequently encountered toxin exposures included sympathomimetics (28.7%), opioids (25.0%), cardiac medications (14.8%), benzodiazepines (12.0%), antidepressants (11.1%), antipsychotics (9.3%), acetaminophen (9.3%), and anticonvulsants (6.5%). Of these exposures, 83% were intentional exposures and 36% were suicide attempts. Over half (53%) of the patients were brought in by ambulance. Upon admission to the emergency department, 82% of patients received a serum toxicity screen and 52% received a urine toxicity screen. Significantly, ECGs in 19% of patients demonstrated prolonged QTc intervals (Female > 470ms, Male > 450ms).

These data indicate that a significant number of overdose patients display prolonged QTc intervals upon admission, which may be correlated to an increased risk of ventricular dysrhythmia. Ventricular dysrhythmia outcome data will be assessed following further patient collection.

**Implementation of Education Tools to Enhance Uptake of Congenital CMV Screening in Infants at Risk for Sensorineural Hearing Loss**

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Primary maternal CMV infection during pregnancy can cause congenital CMV disease at a rate of 30-40%. Congenital CMV infection occurs in 1-2% of live births and is the most common congenital infection that causes long-term sensorineural hearing loss and neurological impairment. Specifically, congenital CMV is estimated to be responsible for 10-30% of childhood hearing loss. While no vaccine for prevention of CMV infection and disease is approved for use, there is evidence that early treatment of infants at less than 1 month of life with the nucleoside analog ganciclovir may halt or delay the progression of hearing loss. However, because there is currently no universal diagnostic testing for congenital CMV carried out or mandated by the state, infants with congenital CMV are being diagnosed late (>30 days) or not at all, which limits therapeutic options. This indicates a need both to increase congenital CMV testing uptake and to screen infants at an earlier age.

We hypothesized that implementation of educational tools about congenital CMV will increase the knowledge-base of providers and ultimately increase congenital CMV screening in infants who fail the newborn hearing screen and are therefore more likely to have CMV.

We designed a prospective cohort study in which education tools will be implemented in and prospective data on knowledge/participation will be collected from the following provider populations at Brigham and Women's Hospital: hearing screeners, postpartum nurses, and pediatricians. Six months post-implementation, prospective data regarding frequency of CMV testing will be obtained to assess the influence of provider education on the rate of congenital CMV screening.

Educational tools developed for hearing screeners and nurses are 30 minute in-services consisting of a 15 minute Powerpoint presentation about congenital CMV and hearing loss, a question/answer session, and pre- and post-tests assessing basic knowledge about the given topic. Tools for physicians consist of an email survey assessing basic knowledge about the given topic and CMV screening routines. Educational tools are being implemented in September 2009.

Results will be presented and it is hoped that this study will lead to enhanced information about the role of provider education in increasing congenital CMV screening. It is also hoped that this study will increase knowledge and awareness about CMV and congenital hearing loss and ultimately, increase the frequency of CMV testing in infants who fail their newborn hearing screen.

**Proteomic Analysis of the New Modality for the Mutated WW Domain of YAP: Phosphotyrosine Recognition**

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The WW domain is a protein-protein interaction module that recognizes ligands containing PPxY motifs. Phosphorylation of the Y within the PPxY core abrogates complex formation with WW domains. Based on data obtained from screens of large repertoires of YAP WW domain mutants, we converted the wild type WW domain of YAP (a Notch-type transcriptional co-activator) into a module that recognizes PPx(phospho-Y). Three amino acids (L, H, and Q) located within the binding pocket of the wild type YAP WW domain were mutated to R, R, and K, respectively. The LHQ-RRK YAP-WW domain mutant was expressed in bacteria, purified, and used to probe SPOT arrays composed of 1,895 twelve-mer peptides that represent the full complement of the PPxY-containing proteins of the human proteome. Two versions of the arrays were used. One version contained peptides with non-phosphorylated Y, while the other contained peptides with phosphorylated Y. This analysis confirmed the switch of binding specificity for the WW domain mutant and identified numerous sites that could serve as WW or SH2 binary switches in signaling. The majority of phospho-peptides were recognized only by the RRK-WW-YAP mutant and not the by the wild-type WW domain. Conversely, the majority of non-phosphorylated peptides were recognized only by the wild-type WW-YAP, while not by the RRK mutant.

In summary, we have generated a pair of WW domains that could be used to quantify the level of tyrosine phosphorylation of specific sites in various signaling proteins. Since it was possible to engineer a pair of WW domains that recognize ligands exclusively in either phosphorylated or non-phosphorylated states, it should also be possible to use a similar approach for other modules that recognize modified residues, including SH2, PTB, Chromo, Bormo, and other domains. These pairs of domains may represent valuable probes for various proteomic platforms used in basic and applied research.

**Drug-eluting bead hepatic arterial chemoembolization (DEB-HACE) in the treatment of carcinoid metastases to the liver**

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Carcinoid tumors are indolent neoplasms of neuroendocrine orgin that frequently only manifest in the presence of metastatic disease. Despite the heterogeneity in location, size, and other characteristics among primary carcinoid tumors, 75% of patients develop hepatic metastases. Currently, surgical resection is the primary intervention employed in patients with limited hepatic disease and has proved to significantly increase long-term survival. However, only a fraction of patients are eligible for resection, leaving most with transarterial therapy as the primary treatment option.

Drug-eluting bead hepatic arterial chemoembolization (DEB-HACE) has emerged as a frontline transarterial therapy for patients with unresectable livers. However, its performance in patients with primary carcinoid tumors has yet to be described. The present study assesses the short and long-term tumor response and time to progression of patients treated with DEB-HACE and compares these results to transarterial chemoembolization (TACE), an older but still widely employed therapy.

The angiography and interventional radiology HI-IQ database and electronic medical record (EMR) were queried for all patients treated with DEB-HACE between October 2005 and June 2009. Inclusion criteria included: 1) history of metastatic carcinoid tumor to the liver, 2) pre-procedure contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) available for review, 3) <3 month post-procedure contrast-enhanced CT or MRI available for review, 4) patient had given prior consent for use of their medical record for research purposes. All enhanced CT and MRI were evaluated from the study period. The EMR was searched to determine which patient had undergone prior HACE without DEB and to record complications. Tumor response was recorded as complete response (CR), partial response (PR), stable disease (S), or progressive disease (P), based on both RECIST and EASL criteria. Objective response (OR) was defined as either a CR or PR by RECIST or EASL.

18 patients underwent 40 DEB-HACE procedures on 38 hepatic lobes. At short-term follow-up (< 3 months; range: 8-134 days; mean: 59 days) 22 lobes (58%) had an objective response and the remainder had stable disease. 26 lobes were evaluated at long-term follow-up (> 3 months; range: 163-1247 days; mean: 445 days). 17 (65%) had an objective response and the remainder had either stable or progressive disease. Probability of progression-free survival was approximately 93% at 3 months, 87 at 6 months, and 60% at 1 year.

These results in comparison to previously published data on TACE suggest that DEB-HACE performs comparably to TACE in the short-term. Accurate long-term assessment requires a head-to-head prospective, randomized trial that definitely compares DEB-HACE to TACE.

**Inflammation in periodontal ligament cells of a mouse model of Marfan syndrome**

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Marfan Syndrome (MFS) is a multi-system disorder caused by mutations in fibrillin-1, the structural component of extracellular microfibrils and a regulator of latent TGFβ activation. In accordance with the latter function of fibrillin-1, TGFβ antagonism through the action of losartan ameliorates aneurysm progression in mouse models of MFS and pediatric patients. MFS1 manifestations occasionally involve severe periodontitis: an inflammatory disease of the periodontal ligament (PDL). PDL is located between the cementum that covers the root of the tooth and the alveolar bone socket. The fibers that comprise PDL all contain large amounts of fibrillin-1. Previous studies have correlated inflammatory markers like interleukin-1 beta (IL-1B), tumor necrosis factor (TNF), interleukin-6 (IL-6), and IFN-stimulated gene-15 (ISG-15) to periodontitis. Hence it has been argued that expression of these inflammatory markers in MFS periodontitis may be affected as a result of heightened TGFβ signaling.

To test this hypothesis, we examined periodontitis in wild type and Fbn1 mutant mice treated with placebo or Losartan. Specifically, mRNA was extracted from the whole tooth and PDL and reverse transcribed into cDNA. Real time (RT)-PCR was then performed using forward and reverse primers for the above-mentioned inflammatory markers as well as β-actin (which is present in all tissues) to serve as a control. To confirm the findings presented by RT-PCR, mandibles from these four groups of mice were embedded in MMA and then sectioned and stained with H&E for histological comparison.

Preliminary RT-PCR data were not statistically significant excluding a possible linkage between losartan blunting of TGFβ signaling and decreased inflammation in MFS periodontits. Ongoing analyses are focusing on histological comparisons in order to rigorously evaluate whether or not Losartan is a beneficial treatment for periodontitis in MFS.

**Study of Extended Duration Counseling to Quit Smoking: Preliminary Outcomes**

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Cigarette smoking is the single largest preventable cause of premature death in the United States. Tobacco control efforts require more effective cessation treatments for active smokers who want to quit smoking. Recent research has indicated that extended duration counseling, in conjunction with nicotine replacement therapy, produces high patient engagement and significantly higher quit rates.

The overall objective of this 5-year randomized controlled trial is to compare smoking abstinence rates after 3, 6, and 12 months of counseling. All participants will be followed for 2 years beyond their quit-dates to assess both cessation status and the durability of the counseling intervention. This preliminary outcome analysis used data available after 10.5 months of enrollment, in order to determine whether abstinence rates for those with most extended counseling duration were significantly higher than those receiving shorter durations of counseling.

Participants were 60 males and 40 females, aged 22-72 years (M=45.0, SD =11.2). The majority were employed, 67.0% were white, and 27.0% were married or living with an intimate partner. Their dependence on cigarette smoking varied widely (measured by Fagerstrom Test of Nicotine Dependence; M=5.1, SD=2.2).

Quit-rates at 9 months post quit-date were compared for the eligible participants who had been enrolled long enough. As expected, the 12-month counseling group had the highest abstinence proportion (0.44) compared to the 6 and 3 month groups (0.10, 0.22). These results were not significant (p=0.3782), but they may achieve significance as sample sizes increase. Quit rates did not differ significantly by age group or gender.

This knowledge may lead to improved treatment options for cigarette smokers. Also, since environmental tobacco exposure contributes to significant morbidity and premature mortality, this study will benefit those who share air spaces with current smokers. In conclusion, the final results of this study will improve information available for individual patient care decisions, system-wide health policy decisions, and the direction of future research.

**Efficacy of a Prototype Endoscope with Two Deflecting Working Channels for Endoscopic Submucosal Dissection (ESD)**

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**Background**: Optimizing the visualization of the cutting line of the submucosal layer is essential to performing an effective and safe endoscopic submucosal dissection (ESD).

**Objective**: To evaluate a prototype endoscope with two deflecting working channels compared to a conventional double-channel endoscope, in time required for ESD of submucosal lesions in distinct anatomical locations of the stomach.

**Design**: A prospective, comparative ex-vivo study.

**Methods**: An ex-vivo endoscopy simulator utilizing fresh porcine stomachs was used for the resections. Forty lesions located in distinct locations (greater curvature, lesser curvature, anterior and posterior wall) were randomized to undergo ESD with either the conventional endoscope (n = 20) or the prototype endoscope (n = 20). The main outcomes measured were: procedure time (primary endpoint), specimen size, submucosal injection frequency, en bloc resection rate and perforation rate (secondary endpoints).

**Results**: In the subgroup of resections in the greater and lesser curvature, the mean procedure time was significantly less in the prototype endoscope group compared to the conventional group (8.4 ± 2.1 min vs 11.3 ± 2.1 min, respectively; P = 0.006) and the mean submucosal injection frequency was significantly less in the prototype endoscope group compared to the conventional group (1.9 ± 0.6 vs 2.5 ± 0.5, respectively; P = 0.025).

There were no significant differences in procedure time, specimen size, submucosal injection requirements, en bloc resection rate and perforation rate between the two endoscopic groups in the anterior and posterior group or in all locations pooled.

**Conclusions**: ESD utilizing the prototype endoscope with two deflecting working channels may provide an improved platform for quicker ESD with equivalent safety, especially in greater and lesser curvature anatomical lesions of the stomach.

**Development of a Pancreatic Tumor Model and Evaluation of the Safety of NOTES® Tumor Enucleation**

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**Background**: Laparoscopic distal pancreatectomy is associated with a high morbidity and mortality. Natural Orifice Translumenal Endoscopic Surgery (NOTES) tumor enucleation may provide an alternative to distal pancreatectomy.

**Objective**: The goal of this study is to determine the feasibility of NOTES tumor creation and enucleation as a multidisciplinary approach.

**Methods**: Seventeen swine underwent general anesthesia. Continuous measurement of cardiac output (CO), peripheral vascular resistance (PVR), and extravascular lung water (EVLW) was conducted using a thermodilution technique via central venous and arterial access. A linear-array EUS endoscope was used to inject a thermosensitive ABA tri-block polymer mixed with methylene blue through the stomach wall and into the distal pancreas using a 22-gauge EUS needle. Due to its thermosensitive character, the polymer solidifies in response to body temperature, creating an artificial tumor. A double-channel gastroscope was used to create a gastrotomy. Endoscopic pancreatic tumor enucleation was performed using an electrocautery knife, snare, and forceps. The closure of the gastrotomy was performed with t-tags in addition to investigational closure clips using an over-the-scope technique. Nine non-survival animals were sacrificed immediately after the procedure, with subsequent necropsy. Eight survival animals were observed for 14 days, with sacrifice and necropsy thereafter.

**Results**: The procedure was performed successfully in all seventeen pigs studied, 9/9 non-survival (100%) and 8/8 survival (100%) animals, using a pure NOTES approach. All animals successfully underwent continuous intraoperative monitoring of CO, PVR, and EVLW. Complications included two esophageal dissections (1 in non-survival group, 1 in survival group), from the introduction of the endoscopic overtube (2/17, 12%), unrelated to the actual surgical procedure. In the survival animals, there were two small splenic lacerations from retraction with the endoscopic forceps, for which hemostasis was achieved prior to closure of the gastrotomy (2/7, 29%). At necropsy of the survival animals, there was sufficient closure of 6/7 gastrotomy sites (86%). There was evidence of insufficient closure of one gastrotomy site. Around that site, there was evidence of infection.

**Conclusions**: The creation of artificial pancreatic tumors by EUS-guidance is feasible. Pancreatic tumor enucleation using a transgastric NOTES approach is technically feasible. NOTES tumor enucleation was performed successfully in all animals and could be an alternative to laparoscopic distal pancreatectomy. Further adoption and adaptation of this technique will require the development of more appropriate specialized tools, to improve the safety profile of the procedure. The continuous impact of a complicated NOTES procedure on hemodynamics can be successfully monitored.

**A novel approach to pretargeted radioimmunotherapy**

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One of the largest challenges in designing and administering radionuclide-based cancer therapeutics is delivering a lethal dose to the tumor without damaging surrounding tissues. One way to accomplish this is to directly chelate radionuclides to tumor-specific antibodies in a process known as radioimmunotherapy (RIT). However, because of the long half-life of antibodies in blood, RIT is dose-limited by significant bone marrow toxicity and can prevent delivery of a lethal radiation dose to the tumor. A solution to this problem was discovered by uncoupling the tumor targeting step from the drug delivery step. This method is known as pretargeted radioimmunotherapy (PRIT) and uses a bispecific targeting molecule that binds to both the tumor and to a radionuclide-chelating molecule. Because the targeting molecule is harmless, it can be administered at doses high enough to saturate the tumor. Once the tumor is saturated, the radionuclide chelator can be administered at a much lower dose and will be selectively retained in the tumor.

There are currently two main PRIT strategies. The first leverages the high-affinity streptavidin-biotin interaction to deliver a radionuclide to tumor-bound antibodies. This method has limited clinical value, however, because streptavidin is retained at high levels in the kidney, resulting in a toxic renal dose. The second involves conjugating antibody Fv domains, one specific for the tumor and the other specific for a peptide-based delivery vehicle. This approach avoids the high renal dose, but is complicated by the steps required to produce both the bispecific antibody and the delivery vehicle. We have developed a new PRIT approach that will address the shortcomings of existing strategies. Our delivery vehicle is DOTA, a small molecule radionuclide chelator with established safety in humans. We have designed a bispecific antibody by recombinantly fusing a high-affinity DOTA-binding scFv to an IgG specific for a tumor antigen of choice. This bispecific antibody is modular in design and is easy to express and purify with standard antibody purification protocols. I designed and characterized this bispecific antibody *in vitro* against CD20, a B-cell antigen expressed on many types of lymphomas. The antibody's binding constants for CD20 (600pM) and for DOTA (20pM) indicate that this bispecific antibody will be able to achieve tumor-to-normal tissue ratios equal to or greater than those of existing strategies without the disadvantages of those approaches.

**Immunohistochemical characterization of cellular events involved in porcine mandibular distraction wound repair**

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Distraction osteogenesis (DO) is a minimally invasive procedure used to gradually increase bone length through fixation of an expandable mechanical device across a surgical osteotomy. In this study the serial cellular events that take place during DO were investigated by immunohistochemical analysis of PCNA and type II collagen (LSAB-HRP method).

Sixteen female Yucutan minipigs with mixed dentition (age 4-6 months) underwent unilateral mandibular DO with protocol of zero day latency, expansion rate of 1mm/day for 12 days followed by 24 day fixation. Two animals underwent acute (12mm) lengthening and one animal was used as a sham control. The animals were sacrificed and mandibles were harvested at different stages throughout treatment.

Through observational analysis, cell proliferation appears to be highest during mid-distraction with the periosteum and endosteum containing the highest concentration of PCNA positive cells. A progressive decrease in PCNA positive cells is observed throughout the course of treatment with end-fixation having the least concentrated number PCNA positive cells. Small islands of type II collagen were identified during distraction.

Cell proliferation occurs in a predictable manner as the need for increased cell numbers and the biosynthesis of tissue are greatest during the expansion of callous in early distraction. Intramembranous ossification is the primary mechanism for mandibular bone formation during DO and is supplemented by the endochondral process at a lesser degree as represented by only small islands of cartilage.

**DISC1 and Its Interaction with APP in a Neuronal Precursor Cell Migration Pathway**

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Proper neuronal precursor cell migration is important for normal neurodevelopment. APP (amyloid precursor protein), a factor implicated in Alzheimer's Disease in the Aβ hypothesis, has been found to be necessary for proper cortical cell migration through knock-down experiments. Recently, knock-down experiments of DISC1 (Disrupted in Schizophrenia-1), a gene which when mutated is implicated in schizophrenia, have shown a quite similar phenotype to APP knock-down experiments. In fact, DISC1 over-expression rescues APP-deficient cells, indicating that both proteins are functioning within the same cell migration pathway and that DISC1 is downstream of APP.

APP and DISC1 were hypothesized to bind together in this pathway, so experiments were performed to help verify the existence of this binding and better characterize it. Different DISC1 truncation mutants were constructed in order to establish the binding region of DISC1 on APP's C-terminal cytosolic domain using transient transfection in COS tissue culture cells. Once the cells were harvested 48 hours following co-transfection of DISC1 and APP, immunoprecipitation of DISC1 followed by Western blots for APP and DISC1 were used to establish whether the region of DISC1 included in the over-expressed truncation mutant was necessary and sufficient for APP binding. It was determined that DISC1's binding region appears to be between amino acids 110 and 220 of the protein. Without this region, strong binding between APP and DISC1 was not evident.

In order to establish whether phosphorylation of threonine at position 668 of APP alters the DISC1-APP binding interaction, mutations at position 668 were developed in order to investigate this hypothesis. These constructs included a mutation of threonine to glutamate, designed as a "phosphomimetic" form of the protein, as well as a mutation of threonine to alanine, designed as a "phospho-dead" form of APP. Studies are ongoing using immunoprecipitation and Western blot to characterize the possible alterations of the DISC1-APP binding interaction as a result of threonine 668 phosphorylation.

Studies of the normal functions of both DISC1 and APP, and their possible neurodevelopmental interaction in the same cortical cell migration pathway, is very important for better elucidation of the mechanism of disease in schizophrenia. Additionally, this study has potential therapeutic design impact for schizophrenia since DISC1 defects may become a target for both testing and treatment of schizophrenia.

**Proteomic Profiling of the Alzheimer's disease β-site APP Cleaving Enzyme (BACE)**

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Alzheimer's disease is the most common neurodegenerative disorder, affecting more than 5 million Americans and over 20 million people worldwide. Accumulation and deposition of the amyloid-beta (Aβ) protein is thought to be a precipitating factor driving the progression of Alzheimer's disease. One of the proteins essential for the production of Aβ is the beta-site APP Cleaving Enzyme (BACE). Though much is understood about BACE's role in Aβ production, very little is known about the other biological processes that BACE is involved in, and how BACE achieves its specificity for these pathways. To better understand the normal cellular functions of BACE, we used an unbiased proteomic approach to survey all of the proteins released from cultured cells by BACE processing. Through quantitatively comparing the abundance of proteins shed from cells that overexpress BACE to control cells, we were able to identify 68 BACE substrates from the analysis of two cultured cell lines. Many of these proteins are known to play pivotal roles in neurodevelopment and immunology, which implicates BACE as a key player in these systems. An analysis of the topologies of these proteins reveals that BACE only cleaves Type I, Type II and GPI-linked proteins, indicating a preference for proteins that have only one domain extending from the lipid bilayer. Of these putative substrates, we selected several of the most biologically interesting candidates and attempted to validate them by cDNA cloning, addition of an epitope tag, and expression in a cell culture system. Of the six proteins analyzed in this way to date, each has been validated as a BACE substrate. These results implicate BACE as a regulator of several key cellular signaling pathways through proteolytic processing, and highlight the potential complications of BACE inhibition as a therapeutic approach to Alzheimer's disease. Future work will probe for sequence motifs within the identified substrates that dictate BACE cleavage, and further studies will analyze the functional consequences of BACE inhibition *in vivo*.

**Proteasome Inhibition as a Promising Target for Subtype-Specific Glioblastoma Therapy**

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Glioblastoma (GBM) is the most common and malignant primary brain tumor in adults. Current standard of care for newly diagnosed glioblastoma is surgical resection followed by adjuvant radiotherapy and temozolomide. Despite this trimodality treatment, only a minority of patients survive beyond two years. We therefore sought to identify molecular targets that may suggest more directed or effective therapies. Oncogenic mutations are highly prevalent in brain cancer, altering the regulation of signaling pathways and allowing unchecked cellular proliferation and survival. However, recent evidence has demonstrated the emergence of a new class of proteins, non-oncogenes, which are rate-limiting in their tumor-promoting pathways. These proteins are critical for cancer cell viability and represent potential cancer therapeutics.

A genome-wide RNAi screen was performed in GBM cell lines to identify in an unbiased fashion the genes most critical for tumor cell proliferation and survival. The screen yielded a diverse set of genes whose depletion impaired the viability of GBM cells. Among these, we identified the proteasome subunit PSMA1. We examined the prognostic significance of PSMA1 expression by querying a database of 531 patients in published studies with clinically annotated tumors that have undergone gene expression profiling. High (greater than mean) PSMA1 expression correlated with decreased patient survival, with a median overall survival of 12.2 months versus 14.8 months (lower than mean). The two-year survival rates were 29.1% versus 17.5%, respectively (log rank P = 0.017).

Further, we classified the 531 tumor specimens into four transcriptome subtypes based on the work of Verhaak and Hoadley et al. In the subset of GBM patients with a proneural gene expression profile, high PSMA1 expression correlated more strongly with decreased patient survival, with a median OS of 9.4 months versus 16.5 months for those with low PSMA1 expression. Taken together, these data suggest proteasome inhibition as a strategy for treating patients with glioblastoma, most specifically those with the proneural subtype.

**Nutrition Knowledge and Food Security Assessment in HIV/AIDS Patients of Port Elizabeth, South Africa**

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Nutrition undeniably plays a large role in positive health and well-being especially among HIV/AIDS patients. The pervasive weight loss and malnutrition among HIV inflicted patients speeds the disease development and increases death rates due to nourishment's influences on immune function. Specifically, malnutrition in HIV infected patients is attributed to an increased need for energy and protein, inadequate nutritional intake, and poor absorption. HIV and nutrition are further linked through food insecurity, which is defined as a lack of sustainable food access due to economic or physical availability factors. The addition of an inability to obtain foodstuffs further intensifies the problem of adequate nutritional health.

It was hypothesized that there is a disparity of nutrition knowledge between HIV/AIDS patients and their healthcare providers. It was further hypothesized that there is a food insecurity presence among the HIV/AIDS patient population. These hypotheses were based on two objectives. First, this project investigated the system of nutritional education within the clinical setting by testing differences in nutritional knowledge between clinicians and HIV/AIDS patients. Second, this project reviewed the capabilities of food obtainment among the same HIV positive individuals. The combination of these two goals allowed for the investigation of a disparity between the medical advice and provisions offered to patients and also of the capability for patients to adhere to nutritional guidelines.

The objectives of the study were achieved through a cross-sectional observational design conducted at the Port Elizabeth Health Complex (PEHC) hospitals. A total of 96 HIV/AIDS adult patients (24 males/72 females) and 19 healthcare providers were recruited for brief, in-person surveys. Nutrition knowledge was assessed with a shortened and culturally tailored version of the "General Nutrition Knowledge Questionnaire;" the food security questionnaire was adopted from the USDA Current Population Survey (CPS) Food Security Supplement.

Observationally the data demonstrates a difference in nutrition knowledge between patients and their clinicians with averages of 62.1% and 85.3% correct, respectively. The patient population also averaged with a food security level greater than "very low food insecure." Stratification based on age, gender, education, and socioeconomic status and a student t-test analysis are pending.

With the addition of recent findings on nutrition's roles in HIV-related symptoms onset delay, nutritional modifications via education and supplementation may pose to be a cheap and beneficial treatment. This study has demonstrated the need of an effective educational system on nutrition and resourceful food interventions that address food security for the PEHC patients.

**Mental Health Needs Assessment in Niger**

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Niger is a land-locked country in sub-Saharan West Africa with one of the lowest average incomes and highest infant mortality rates in the world. The majority of public health initiatives are aimed at malnutrition and prevention and treatment of infectious diseases such as malaria, tuberculosis and HIV/AIDS. Unfortunately, addictive and mental disorders, which make up the leading causes of worldwide disability, are frequently overlooked in health policy planning. Affordable and feasible treatments exist, with the potential to profoundly improve the lives of people who suffer from psychiatric disorders. The National Mental Health Program in Niger now hopes to scale up mental health services in order to address this underserved population.

As part of the plans to enhance psychiatric care in the country, we conducted 40 semi-structured interviews with policy makers, clinicians and service users to assess barriers to accessing mental health care. Our interviews included information on needs, attitudes and existing resources. Through this process we compiled a list of recommendations for the National Mental Health Program. These suggestions encompass training for health workers, increased availability to medication, resources for traveling between health care facilities and villages and partnership building with traditional healers.

**Quantitative Study of Mammalian Transcriptional Regulation from High-throughput Sequencing Data**

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In eukaryotic organisms, cellular functions and behaviors are largely determined by context-dependent gene expression. Therefore, studying transcriptional regulation of gene expression is important for understanding the mechanisms of cellular responses to pathophysiological changes. While the key players of transcriptional regulation (RNA Polymerase II, transcription factors (TFs), histone marks) have been identified through classical molecular studies, how these factors quantitatively influence genome-wide expression patterns in mammalian cells is largely unknown. High-throughput sequencing technologies allow genome-wide analysis of *in vivo* protein binding sites, in addition to permitting a more precise measurement of the transcriptome. Thus, this project aims to provide quantitative insights in transcriptional regulation through analysis of the binding information of its key players and gene expression data obtained from high-throughput sequencing experiments. We have developed a computational model to predict gene expression patterns in mouse neurons based on the binding data in the promoter proximal region.

The four TFs studied in the project, CBP, CREB, Npas4 and SRF, have previously been shown to be important in neural development. To quantify the impact of TF binding on gene expression, an association study was conducted. In this study, if one of these TFs was able to bind to a gene in a designated window proximal to the promoter, the gene had a probability of expression that was 14-64 times higher than those of genes without the TF binding. The enrichment of these TF binding sites was considered as an indicator for TF binding strength. One finding in this study was that a statistically-significant correlation between TF binding enrichment and gene expression level usually existed for genes that were highly expressed in the cells. For genes that had two different TF binding sites, the correlation was often better for the TF pairs that were closely-situated as opposed to the TF pairs situated further apart.

After the quantitative analysis for TFs, a multiple linear regression model incorporating the binding information of TFs, RNA polymerase II and histone marks was used to predict gene expression patterns in mouse neurons. When the model was fitted to the changes in gene expression level and factor binding status between two time points, the goodness-of-fit, $R^2$ was ~0.12. In other words, this model could explain 12% of the data variation in the gene expression patterns. Despite its relative low value, the $R^2$ was highly significant and consistent with the prediction rate for mammalian cells reported in the literature.

**Fate-mapping the Primary Mouth Region**

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Craniofacial abnormalities are among the most common birth defects, appearing in 1 out of every 700 live births. However, the etiology of most craniofacial birth defects is unknown. This stems from our limited understanding of early craniofacial development and the genes regulating it. Specifically, the opening and subsequent development of the primary mouth, which is the first conduit between the foregut and the outside world, has received little attention, despite being crucial for eating, breathing, and speech. The primary mouth is the central location around which the neural crest migrates and ultimately forms both the face and the secondary mouth made up of the teeth, jaws, and tongue. Though primary mouth development is an early, central phase of development, there are few images available and the morphological and molecular underpinnings are largely unknown. To better understand the process of primary mouth development, fate-mapping studies were conducted in *Xenopus laevis* embryos to determine whether primary mouth tissue is retained and if so, what craniofacial structures arise from primary mouth tissue. Stage 20 *Xenopus laevis* embryos were injected with a fluorescent, lipophylic dye in the region that forms the primary mouth. The dye, known as DiI, permanently labels the cell membrane. The labeled embryos were photographed over the next week to follow the primary mouth tissue as the mouth opened and developed. After the embryos began to feed, they were fixed, sectioned, and viewed to determine whether the labeled tissue remained and what internal structures were derived from the labeled tissue. The sections were also labeled with tissue specific antibodies. In all older embryos, the primary mouth tissue was retained, at least in part. The tissue appears to give rise to structures directly surrounding the mouth orifice and oral cavity. The types of tissues derived from the primary mouth are still being studied by labeling the sections with tissue specific antibodies. Using these techniques, we will be able to define the tissues derived from the primary mouth and better understand early craniofacial development.

**Development of a Pandemic Influenza Business Continuity Plan for International Organization for Migration Thailand Mission**

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This project was to prepare and test an influenza pandemic business continuity plan (BCP) for the Thailand Mission with Regional Functions of the International Organization for Migration (IOM) located in Bangkok. IOM is an intergovernmental organization that has a mandate to serve refugee and migrant populations, and it has over 300 staff in Thailand. In Bangkok, the IOM office oversees operations in Thailand and also serves as a regional resource office for Southeast Asia (Cambodia, Lao PDR, Malaysia, Myanmar, Indonesia, Thailand and Viet Nam).

Over a seven-week period, I reviewed previous pandemic preparedness documents prepared by the regional and national migrant health program units at IOM Bangkok. The drafting of the full BCP followed guidelines issued by IOM headquarters in Geneva and the pandemic BCP developed by the United Nations Country Team in Thailand (including institutions such as WHO, UNHCR, and UNDP) of which IOM is a partner agency.

I met with and interviewed various program coordinators that managed IOM programs to identify how IOM activities would be affected in a pandemic emergency. These programs include refugee resettlement, medical assessment and tuberculosis treatment, counter-trafficking, labor migration, and migrant health. I conducted three site visits in Thailand to become familiar with IOM operations in refugee camps and migrant health program activities, and to observe a functional pandemic simulation conducted with a provincial ministry of health.

The structure of the BCP includes a crisis decision-making and implementation structure, policies to ensure staff safety and communications, specific operation plans for program units, guidelines for IOM participation in a country-wide pandemic response, and next steps to advance pandemic preparedness.

After the draft of the BCP was completed, my on-site mentor and I asked for feedback for the policies and procedures that we created. We planned and conducted a full-day functional pandemic simulation exercise at IOM Bangkok with program coordinators and Head of Offices from IOM offices in Thailand, Malaysia, and Lao PDR. From the experiences and feedback from this simulation, we drafted a summary report and made significant revisions to BCP, particularly addressing ambiguities and redundancy in the crisis decision-making structure. It is currently being released to IOM Bangkok staff as well as other IOM missions in the Southeast Asia region to serve as a resource and template for BCP development.

**Boston Public Health Commission Sexual Health Report of Boston Teenagers**

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According to the Center for Disease Control, the rate of unprotected sex, teen pregnancy and sexually transmitted infections (STIs) among adolescents have been on the rise in Boston, MA since 2005. The 2007 CDC Boston Youth Risk Behavioral Survey (YRBS) shows that more than half of high schools students in Boston (62%) have had sexual intercourse previously. Concurrently, only 59% of Boston high school students were taught how to use the condom in 2007. The Massachusetts Department of Health (MA DPH) showed that the age bracket of 15-19 year olds have the largest proportion of Chlamydia and Gonorrhea cases among all age groups in Massachusetts. Given this evidence, it is only appropriate that the Boston Public Health Commission (BPHC) research methods to reduce the high rates of teen pregnancy and STIs in Boston.

The BPHC is formulating a "Sexual Health of Boston Adolescents Report" that will address the rise in STI and teen pregnancy rates among adolescents (age 13-19) in Boston. Upon completion in January 2009, the report will be distributed to the Boston public, health professionals, and high school administrators with the most up-to-date, comprehensive information about the sexual health of Boston adolescents. The report intends to recommend policy change and intervention for the city of Boston, including increasing access to reproductive health services and improving the sexual education curriculum of Boston Public Schools.

Quantitative data is based on the analysis of recent data collected from various Boston specific sources, including the 2009 Boston YRBS and MA DPH, and the Boston Youth Survey. This quantitative data includes information regarding the rate of teen pregnancy, STIs, condom/contraceptive use, and racial disparities among Boston teens. To best capture qualitative data, we will administer surveys and focus groups of teenagers and their parents, evaluating the knowledge base, concerns, and sexual practices of teens. A third section of the report highlights nationally recognized best practice models of sexual health delivery and education. In the concluding section, the BPHC will make recommendations based on the analysis of quantitative, qualitative, and best practices data on methods to best counter the growing rates of STIs and pregnancy rates among teenagers in Boston.

**Adolescent Perspectives on Family Planning and Pregnancy for Quality Improvement of Teen Family Planning Initiative**

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Despite a 14-year decline, adolescent pregnancy rates in Boston rose 12.2% between 2005 and 2007. Especially affected are those neighborhoods with historically lower educational attainment and a higher poverty rate. Not only a marker of social deprivation, adolescent pregnancy confers medical and developmental consequences for teenagers and their children. Socioeconomics and access to health services play a role, but factors mediating adolescent pregnancy are complex and locally specific.

The Dimock Teen Initiative, part of the Obstetrics and Gynecology Department of the Dimock Center in Roxbury, seeks to address the burden of adolescent pregnancy by providing outreach, education and confidential contraceptive services to local teens. To adequately meet the needs of its target population, the Teen Initiative aimed to utilize teen perspectives to guide program planning and evaluation. Eliciting teen perspectives on family planning and pregnancy, thus, became a major goal of this quality improvement project.

To this end, we developed a semi-structured interview script to ascertain current contraceptive use, past experiences with family planning, influences relevant to use of services, and emotional response and support after discovery of pregnancy. We conducted 20 interviews with women ages 15 to 20 who utilized services at the Dimock Center. Interviews were coded into thematic categories, including communication, barriers to family planning care, and health education.

The qualitative data analysis yielded several observations that can be used to inform the further development of the Teen Initiative. First, respondents desired open, non-judgmental conversation with both doctors and parents, but fear of judgment from both groups (55%, 40%, respectively) was a significant barrier to discussions about sex. Second, respondents forgot to use a birth control method (BCM) (50%), considered BCM a general hassle (45%), or were concerned about side effects (60%). Compounded with limited health literacy about medical terms in general, and specifically the term "family planning" (90%), one can conclude that teenagers harbor misconceptions and fears about BCMs not addressed by parents or the medical community.

Lack of communication between teens and their parents created barriers to family planning care that could be addressed with confidential services, but counseling must supplement these services to empower teenagers to assert control over their reproductive lives. Since many teens expressed interest in hearing first-hand accounts from "real teen moms," one recommendation for the Dimock Teen Initiative involves connecting non-pregnant teens with teen parents who can ground teenagers in the realities of being young and pregnant.

**Providers' perceptions of a clinician-based intervention to reduce racial disparities in diabetes outcomes**

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This study explored the impact of an intervention aimed at reducing disparities in the intermediate outcomes of black and white patients with diabetes. The intervention provided primary care providersin a multispecialty medical group with individualized performance reports on disparities in diabetes outcomes among their own patients over a 12-month period. The intervention also provided cultural competency training about the causes of disparities and suggestions for strategies to improve communication and diabetes care for black patients.

This qualitative study explored clinicians' experience of the intervention as well as their perceptions of the causes and potential solutions to those disparities. It was conducted throughsemi-structured interviews with 17 providers, followed by thematic analysis by the research team.

Most of the clinicians recognized the presence of disparities in diabetes control among their patients. They described a complex set of causal factors driving those disparities only some of which they perceived to be within their power to change. All the clinicians were able to identify additional services or programs that they felt would help them tackle disparities. A minority of clinicians did challenge the premise of the intervention, namely that a patients' race or ethnic group was the primary factorunderlying the disparity, and instead drew attention to the importance of socioeconomicfactors. The performance reports and training were generally well received but some clinicians did not feel empowered to act on the information presented.

Evaluation of the intervention demonstrated no differences in the control of blood pressure, hemoglobin A1c, or LDL cholesterol for black patients whose clinicians were in the intervention compared with those in the control group, but awareness of disparities was more common among intervention clinicians. Several possibilities for the apparent lack of connection between performance feedback and outcomes were identified through this qualitative study. The outcomes may not be a function of clinician performance given the complex structural determinants of health, or lack of resources or services that are required for clinicians to make progress in reducing disparities. Limited and inaccurate physician recall of the intervention suggests suboptimal implementation. While a straightforward target for addressing the apparent ineffectiveness, improved implementation cannot address the barrier that some clinicians did not accept the underlying premise of the intervention: that racial and ethnic disparities exist in diabetes care. In this study, improving clinicians' awarenesswas not sufficient to reduce racial disparities in measures of disease control for patients with diabetes.

**The Effect of Vascular Endothelial Growth Factor on Pulmonary Hypoplasia in a Rat Model of Congenital Diaphragmatic Hernia**

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Background: Congenital diaphragmatic hernia (CDH) is a developmental condition characterized by incomplete diaphragm formation in utero. Abdominal viscera, including intestines, stomach, spleen, and liver, may herniate through the diaphragm defect, compressing the lungs and resulting in pulmonary underdevelopment. One-year survival has been cited as 50%, with mortality over 90% in infants with severe anomalies. Survival is largely dependent on the severity of pulmonary underdevelopment. Vascular Endothelial Growth Factor (VEGF) has been implicated in numerous studies as a primary mediator of lung regeneration.

Nitrofen (2,4-dichlorophenyl-P-nitrophenyl) is a herbicide and a known teratogen. Previous studies have demonstrated that nitrofen exposure to pregnant rats causes a fetal phenotype very similar to that of CDH (including the development of pulmonary hypoplasia).

Objective: We hypothesize that exogenous vascular endothelial growth factor will ameliorate pulmonary hypoplasia in fetal rats with nitrofen-induced congenital diaphragmatic hernia.

Methods: Time-dated pregnant rats underwent gavage feeding of 100 mg of nitrofen dissolved in 1mL of olive oil on embryonic day 8. On embryonic day 17, 1ml of of saline (control) or 0.8mg VEGF in 1ml saline (experimental) was injected into the peritoneum of the pregnant rats. Fetal rats were harvested by cesarean section on embryonic day 21. The lungs of the fetal rats were fixed at 15mmHg via intracheal instillation of 10% formalin. Total lung volume, air space volume, alveolar tissue volume, and alveolar surface density will be measured by stereologic methods. Vascular density, cellular proliferation, and apoptotic indices will be measured. Statistical analysis for the lung morphometry data will be performed using the Students two-tailed, unpaired t-test for comparisons between groups ($\alpha$=0.05). Raw counts from the immunohistochemistry data will be subjected to Mann-Whitney rank sum test using Sigma Stat (SPSS, Chicago, IL).

Results: 73.9% of all rat fetuses in the control group had diaphragmatic hernias: 47.1% had hemidiaphragm defects, 47.1% had postero-lateral defects, and 5.9% had retrosternal defects. 72.7% of all rat fetuses in the experimental group had diaphragmatic hernias: 59.1% had hemidiaphragm defects, 31.8% had postero-lateral defects, and 9.1% had retrosternal defects. The morphometric analysis of lung tissue samples is ongoing; no data on lung volumes is currently available.

Conclusions: The administration of nitrofen was highly effective in creating a rat model of congenital diaphragmatic hernia. Analysis of tissue samples is currently in progress, and conclusions regarding the effectiveness of vascular endothelial growth factor in ameliorating pulmonary hypoplasia are forthcoming.

**Ethical and Policy Issues in Reimbursement for Renal Transplantation: Proposal for a Sustainable Model**

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Kidney transplantation is the best treatment for patients with end-stage renal disease. Despite the science and success of transplantation, the demand for organs far exceeds the supply. In the United States, there are currently over 80,000 candidates on the renal transplant waiting list while only a little over 16,000 kidney transplants are performed each year. Organ shortage is a serious issue and reimbursement of live kidney donors has been proposed as one solution. However, reimbursement can quickly become an inducement, exploiting the poor undereducated; it may also open the gateway to the crime of organ trafficking. Reimbursement is a significant moral issue as donation is viewed as a truly altruistic sacrifice that cannot be given a value. At this time, the United States does not reimburse living donors.

Iran is currently the only country that reimburses its kidney donors; their renal transplant waiting list was abolished in 1999. However, studies suggest that many who donate in Iran do so for the compensatory gain. Organ trading has become a worldwide problem; cases have been documented in Brazil, South Africa, Thailand, and India. In 2008, a summit was held in Istanbul to combat organ trafficking and the Declaration of Istanbul was drafted with established guidelines for ethical organ transplantation.

Singapore is a very modern, Western civilization with the 5$^{th}$ highest incidence of kidney failure in the world. The government has addressed its organ shortage issue since establishing the Human Organ Transplant Act in 1987. The most recent and controversial transplant bill approved in March 2009 now allows for the reimbursement of living kidney donor expenses.

There are serious ethical, legal, and social issues surrounding the kidney shortage and kidney donor reimbursement policy. We propose a sustainable and fair approach to reimbursement in Singapore, incorporating both fixed and case-by-case reimbursement. Donors will receive a subsistence allowance, an insurance premium for 30 days post-donation, and reimbursement for hospital costs , travel from a foreign country, and loss-of-income. A recipient with the means will provide the funds for the donor reimbursement; if not, the state, a VWO, or a third party donor fund will provide the money.

Singapore's decision to reimburse donors will remove any financial disincentive for donation; our proposal accomplishes this goal adequately and ethically for both local and foreign donors and can serve as a model for other countries.

**Comparison of Clinical Decision Support content developed by six members of the Clinical Decision Support Consortium**

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**Background:** Clinical decision support (CDS) systems are manual or computer-based information systems that assist in clinical decision-making. Although CDS systems have been shown to improve the quality of care and increase workflow efficiency, CDS has not been widely adopted because such systems are difficult and expensive to create and are not easily shareable.

**Methods:** In this study, we compared clinical decision support systems in six collaborating sites of the Clinical Decision Support Consortium (CDSC). We gathered clinical decision support content within the areas of diabetes mellitus (DM), hypertension screening, and coronary artery disease management and surveyed institutions on characteristics of their electronic medical record (EMR) system and general characteristics of their site.

**Results:** The approach to CDS varied among sites. Three of the sites purchased their EMR system while the other three developed their own. The characteristics of the decision support content varied among sites. Some commonalities included the use of clinical data for CDS systems; providing some customizability by role or user; making CDS use automatic; allowing CDS messages to be actionable; and use of CDS at the time of decision-making. Most messages were recommendations, although forcefulness of the message varied as did provision of an explanation or evidence for the message. Each site also identified a unique purpose of their CDS, such as quality measurement and pay for performance. In DM care, almost every site had monitoring rules (e.g. to assess HgA1c), but fewer had rules that suggested specific treatments and only one of the sites had a diagnosis rule. Many sites had sophisticated exclusion criteria, such as exclusion of patients at the end of life or those with contraindications to treatments. A breadth and depth of prevention rules were seen, and all sites had rules for lipid screening, eye exam, influenza vaccine, pneumococcal vaccine, and nephropathy screening reminders.

**Discussion and Conclusion:** The details of CDS systems vary widely. However, institutions looking to start their own CDS system can begin by implementing an automatic CDS system based on clinical data, used at time of decision-making, providing messages that are recommendations for action, and making messages actionable. Implementation of CDS in a new site should focus on preventative care first, since many sites have found prevention rules most feasible to implement.

**Role of plasma fatty acid analysis and advanced MR imaging in diagnosis of chronic pancreatitis**

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Chronic pancreatitis can be a difficult disease to diagnose. Imaging and lab tests are frequently normal. ERCP and secretin-pancreatic function tests are routinely performed to diagnose this disease, but are both invasive. The purpose of this study is to determine whether fatty acid abnormalities, a reflection of Cystic Fibrosis gene (CFTR) dysfunction, and advanced MR imaging techniques may provide accurate and less invasive methods of diagnosing chronic pancreatitis.

Using a case control study design, subjects with chronic pancreatic and healthy controls will be enrolled. Subjects with chronic pancreatitis will be divided into those with imaging positive disease and those with positive secretin tests in the setting of negative imaging. Each of the three arms of the study will enroll ten patients. Blood samples will be obtained for fatty acid analysis and MRI/MRCP utilizing arterial spin labeling (ASL) technology will be performed. We hypothesis that subjects with chronic pancreatitis will demonstrate decreased plasma levels of docosahexaenoic acid (DHA) and will have decreased pancreatic perfusion on ASL MRI following cholecystokinin stimulation in comparison to healthy controls.

This study is of significance because there is currently no minimally invasive diagnostic test for cases of chronic pancreatitis in which conventional imaging is negative. If our hypothesis is correct, ASL MRI and plasma fatty acid analysis could provide viable options for minimally invasive early diagnosis of chronic pancreatitis, as well as new potential therapeutic targets.

**Associations of known early childhood obesity predictors with serum adipokines at 3 years of age**

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Blood levels of leptin and adiponectin may be better markers of adiposity in young children than are anthropometric measures. No prior studies have examined associations of pre- and peri-natal predictors of obesity with childhood leptin and adiponectin. We examined associations of gestational diabetes, maternal smoking, gestational weight gain, infant sleep duration, breastfeeding duration and infant weight gain with these adipokines at age 3 years.

We studied 730 mother-child pairs in Project Viva, a prospective pre-birth cohort study. Main outcomes were serum leptin and adiponectin at age 3 years. We performed multivariable analyses to examine each outcome separately.

Mean serum leptin concentration was 1.95 (SD 1.99) ng/ml and mean serum adiponectin concentration was 22.3 (5.6) µg/ml. After adjusting for maternal age, BMI, education and parity, paternal BMI, household income, and child's sex, race, and age we found that children exposed *in utero* to gestational diabetes or impaired glucose tolerance had higher leptin concentrations (0.84 ng/ml [95% CI: 0.15, 1.52, $P = 0.02$] and 0.88 ng/ml [95% CI: 0.14, 1.62, $P = 0.02$], respectively) Higher leptin concentrations were also present in children exposed to smoking during gestation (0.61 ng/ml [95% CI: 0.04, 1.18, $P = 0.03$]). Compared with children who had been breastfed for ≥ 12 months, those breastfed for 0-3 months had higher leptin (0.87 ng/ml [95% CI: 0.35, 1.41]), and those breastfed for 6-12 months had lower adiponectin (-2.2 µg/ml, 95% CI: -3.4, -0.9, $P = 0.001$).

At age 3 years, children exposed to maternal gestational diabetes, impaired glucose tolerance, or smoking during pregnancy, and those who had been breastfed 0-3 months, but not longer or shorter, had increased serum leptin concentrations. Associations with 3-year adiponectin were few.

**Knowledge of Oral Lesions Associated with HIV/AIDS Affecting Oral Examinations Performed in South Africa**

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Oral lesions present early and in recognizable forms in HIV/AIDS infected individuals. Lesions such as *Oral Candidosis, Hairy Leukoplakia, Kaposi's sarcoma,* and *Squamous Cell Carcinoma* are extremely common in the oral cavities of infected patients, with studies showing 75% of advanced AIDS patients having histological alterations of the tongue. *Oral Candidosis* has repeatedly been identified as one of the earliest manifestations of HIV and serves as a strong predictor of AIDS related illness or death. Furthermore, an increase in oral lesions has been shown to correspond with a decrease in CD4+ counts, suggesting that oral lesions can provide important prognostic information and can also be used as a low-cost monitoring tool for CD4+ levels. Proper identification of any of these lesions could indicate a need for diagnostic testing in a previously undiagnosed patient or the failure of anti-retroviral medications in a known infected individual.

The aforementioned lesions can be identified easily on physical examination and are most frequently located on the tongue, floor of the mouth, gingiva, and palate. It is therefore important, especially in regions of the world rife with HIV/AIDS infection (South Africa had 5.7 million documented cases in 2007), that clinicians examine the oral cavity as part of a routine physical exam. Based on this information, we hypothesized that clinicians that were more highly knowledgeable on lesions associated with HIV/AIDS would include the oral cavity more frequently in routine physical examinations. To test this hypothesis, physicians and nurses at public hospitals and primary health care clinics in Port Elizabeth, South Africa (n=23) were observed as they routinely cared for patients. The number of times they looked into the oral cavity (Oral Exam Score, OES) was recorded as a fraction (on a scale of 0-1; 0 = oral cavity never included, and 1= oral cavity included in every examination). After the observation, an oral health questionnaire was administered that tested knowledge of oral lesions associated with HIV/AIDS.

The data suggests a positive correlation between increasing knowledge of oral lesions associated with HIV/AIDS and increasing Oral Exam Scores. Clinicians in the poorly knowledgeable category had an average OES of .42, while clinicians in the satisfactory and highly knowledgeable categories had OES averages of .56 and .63 respectively. The data indicates a need for increased emphasis on the oral cavity in medical education and in performing physical examinations in areas heavily burdened with HIV/AIDS.

**Assessing the Oral-health Related Quality of Life among the HIV-infected in Port Elizabeth, South Africa**

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Port Elizabeth has been greatly challenged by healthcare issues related to the high prevalence of HIV/AIDS (34.5%). It is estimated that more than 30% of HIV infected individuals have oral conditions that arise because of their weakened immune systems. Although antiretroviral therapy has helped alleviate some of these burdens, additional oral health concerns may arise as a result of treatment. Oral pathology has been linked with HIV infection, yet the impact of oral health on these patients' lives has not been closely studied.

Based on the existing knowledge of HIV related to oral health, we hypothesized that the overall quality of life for individuals with HIV would be significantly affected as a result of poorer oral health. To test these hypotheses, we utilized the validated, short form Oral Health Impact Profile (OHIP-14) to measure oral health-related quality of life (OHRQOL). Subjects were recruited from 5 different government hospitals located throughout Port Elizabeth. HIV positive subjects (n=120) were recruited from HIV treatment clinics, while negative subjects (n=38) were recruited from out-patient clinics.

The OHIP-14 provides a scientific framework for understanding oral disease and its clinical and psychosocial consequences. The questions assess different dimensions of oral health: functional limitation; physical pain; psychological discomfort; physical, psychological, social disability; and handicap. Subjects were asked to report the frequency with which their oral health has had an impact on their lives. The possible responses (never, hardly ever, occasionally, fairly often, very often) were then assigned a numerical value from 0 to 4. Responses for each subject were summed to formulate an individual OHIP-14 score. Greater OHIP-14 scores suggest a poorer OHRQOL.

The mean additive OHIP-14 scores for the HIV positive and negative groups were 10.80 (SD=10.98) and 6.19 (SD=6.48), respectively. Based on preliminary analysis, HIV infected individuals appear to have a poorer OHRQOL than uninfected individuals. However, possible confounding factors such as socioeconomic status, general health, and antiretroviral therapy have not yet been controlled for and will be taken into consideration for final analysis.

Upon completion, results will be shared with the hospital sites at which data was collected. Any significant findings showing a disparity in OHRQOL for HIV infected individuals can be used to further incorporate oral healthcare in a more comprehensive treatment plan for HIV/AIDS patients. Shedding light on existing disparities could potentially benefit many patients, since several oral conditions linked with HIV can be treated with over-the-counter creams, mouthwashes, and prescription drugs.

**A comparison of the accuracy of impression techniques for implants with varying angulations**

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In order to restore a large amount of damaged teeth from fractures and caries are with artificial materials it is necessary to make a copy of the patient's dentition and fabricate prostheses indirectly on a model outside the patient's mouth. An impression refers to such an inverted copy of the patient's mouth. When finished prostheses are taken back to the patient's mouth, their final fit is a measure of their success. Unlike natural teeth, implants cannot move to compensate for even a small misfit once they have integrated into the bone. Complications of a misfit include screw loosening or fracture, ceramic or veneer fracture, implant fracture, peri-implantitits and soft tissue complications, and bone loss. The fit of prostheses, and therefore the success of dental implants, greatly depend on the accuracy of impression taking.

The aim of this study was to compare the accuracy of three different impression techniques for bone level implants, with a special focus on implant angulations. In general, simple impression technique requires less time but possibly less accuracy. It was hypothesized that the relative accuracy of the three impression techniques would change, depending on the implant angulations. With the results of the study, a guideline will be set on which impression techniques to use in different cases with various implant angulations. Such a guideline will help dentists and patients to save time, obtain optimal implant-prosthesis fit and minimize complications of a dental implant treatment.

Seven different models were created, mimicking two-implant-unit cases for three missing teeth in the mandible. The anterior implant was placed parallel to its adjacent natural tooth, whereas the posterior implant was mesially angled. Seven models differed only in the mesial angulation of the posterior implant: $0^o$, $5^o$, $10^o$, $15^o$, $20^o$, $25^o$, or $30^o$. We are currently in the process of impression taking and accuracy measurements. Impressions will be taken from each of the seven master models, using the closed-tray method, the open-tray non-splint method, and the open-tray splint method. A verification jig will be made to fit perfectly on a master model, and its fit on experimental models will be quantitatively measured and compared for statistical significance.

An expansion of the study will include patient trials. An IRB application was recently approved, and we are in the process of recruiting patients. The open-tray and the closed-tray impression accuracy will be compared using a verification jig as in the *in vitro* study.

## *In vitro* study of interactions between key enamel matrix proteins and their calcium-binding properties

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**Background:** The formation of tooth enamel, the hardest tissue in the body, is intricately regulated by essential extracellular enamel matrix proteins, such as amelogenin and enamelin. While studies have indicated the functional importance of amelogenin and enamelin during enamel development, key aspects of the enamel formation process, *amelogenesis*, remain unknown. In particular, the molecular interactions between such enamel matrix proteins and interactions between protein and developing mineral, guiding the overall formation of bundles of highly organized hydroxyapatite (HA) crystals, are not clearly understood. Recent studies have shown that certain amelogenins have the capacity to stabilize amorphous calcium phosphate and regulate the rate of its transformation to HA to varying degrees. The ability of enamel matrix proteins to bind calcium could similarly affect the rate of HA formation and may also influence protein-protein interactions. Therefore, the calcium-binding properties of selected enamel matrix proteins were initially studied.

**Hypothesis:** We hypothesize that calcium binding by enamel matrix proteins is dependent on the nature of the protein and its degree of phosphorylation.

**Methods:** Recombinant (non-phosphorylated) and native (one phosphorylated site) porcine proteins were used. Full-length recombinant amelogenin (rP172), full-length native amelogenin (P173), native (P148) and recombinant (rP147) proteolytic amelogenin cleavage products (lacking the hydrophilic C-terminus) were investigated under various calcium concentrations at pH 7.4 (TRIS/HCl), 37ºC, with an ionic strength of 163 mM and a protein concentration of 2 mg/mL. The 32kDa cleavage product of native enamelin was similarly investigated. Calcium-binding properties of each protein were determined using a calcium ion-selective electrode.

**Results:** While P173 and P148 exhibited minimal calcium binding (*e.g.,* 4±1% and ~0% bound, respectively, at 2.5 mM $Ca^{2+}$), both non-phosphorylated rP172 and rP147 proteins showed relatively greater binding (*e.g*., 13±2% and 9±2% bound, respectively, at 2.5 mM $Ca^{2+}$). The presence of the hydrophilic C-terminus had a smaller influence on calcium-binding properties. In contrast, phosphorylated enamelin exhibited greater calcium binding (*e.g*., 9±2% bound at 1 mM $Ca^{2+}$; 20±5% bound at 2.5 mM $Ca^{2+}$).

**Conclusions:** The surprising difference in calcium-binding capacities between the non-phosphorylated and phosphorylated amelogenin homologs (*i.e.,* r172 vs. P173; rP147 vs. P148) suggests that phosphorylation may affect protein conformation or self-assembly in a manner that influences calcium-protein interactions. Greater calcium binding by 32kDa enamelin appears to be consistent with the presence of multiple phosphate groups that would be expected to enhance its affinity for calcium. Transmission Electron Microscopy analyses currently in process should aid in the interpretation of these findings.

**Treatment Decisions in the Management of Asymptomatic Third Molars**

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Third molar (M3) removal is one of the most common surgical procedures carried out in the United States. Managing symptomatic third molars (M3s) requires a straightforward decision-making process, i.e. therapeutic extraction vs. symptomatic management with analgesics and antibiotics. The management of asymptomatic, disease-free M3s, however, is one of the more challenging daily decisions an oral and maxillofacial surgeon makes.

A current Cohrane systematic review concluded that there was no clear evidence supporting or refuting the removal of asymptomatic disease-free M3s to prevent problems in the future. Other systematic reviews suggest that active surveillance of asymptomatic M3s is the recommended management strategies. Other data suggest that removal of M3s before age 25 is associated with a decreased risk for complications and better postoperative recovery. As such, both clinicians and patients must weigh and measure unknown benefits and quantifiable risks of operative intervention versus active surveillance of M3 overtime and also incorporate the patient's assessment of the risks and benefits.

The purpose of this study is to address the following clinical question: Among patients presenting for evaluation of their third molars, do those who choose extraction, when compared to those who choose active surveillance, differ in important demographic, clinical, anatomic, or radiographic variables?

The study hypothesis is: among patients presenting for evaluation of third molars, the investigators hypothesize that there exists a set of ≥ one variable that predict patient treatment choice, i.e. extraction vs. monitoring

The study's specific aims are to design a retrospective cohort study and enroll a sample composed of patients presenting for third molar evaluation in order to: 1) estimate the frequency of asymptomatic M3s, 2) estimate the frequency of treatment recommendations (active surveillance vs. extraction), 3) estimate the frequency of patient treatment choice (monitoring vs. extraction) and 4) Identify factors associated with patient treatment choice, and 4) develop a statistical model to identify variables that may be associated with patient choice.

**Mitochondrial Energy Metabolism in Genetically Defined Subsets of Diffuse Large B Cell Lymphoma**

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Tumors often rewire their metabolism to ensure steady supply of ATP, reducing equivalents and intermediary metabolites for growth. Certain tumors prefer glycolysis over oxidative phosphorylation (OxPhos) even when sufficient oxygen is present (aerobic glycolysis). This metabolic switch leads to marked increase in the contribution of glycolytic ATP to total cellular ATP, a phenomenon known as the Warburg effect. Although the Warburg effect has been reported in many cancers, recent re-evaluation of tumor energy metabolism indicates that it is not a universal feature of all tumors. Indeed, evidence for both an oxidative class of tumors relying primarily on OxPhos for energy and tumors with simultaneous enhancement of glycolytic and oxidative flux exists. This variability in the tumor metabolic profile likely mirrors heterogeneity in their genetic makeup and tumor micro-environment, and importantly, underscores the tremendous flexibility of the tumor metabolome.

Recent analysis of gene expression signatures in a large number of newly diagnosed Diffuse Large B Cell Lymphomas (DLBCLs), provided evidence for three subtypes: an "Oxidative Phosphorylation" (OxPhos) cluster showing up-regulation of multiple mitochondrial genes; a "B-Cell Receptor/Proliferation" (BCR) cluster demonstrating increased expression of B-cell receptor signaling genes; and a "Host Response" (HR) cluster boasting up-regulation of inflammatory response genes. In an effort to validate these genomics data and to gain insight into the contribution of energy metabolism to the development, maintenance or clinical outcome of DLBCL, we have initiated a detailed analysis of the OxPhos tumor metabolome. Two dimensional proteomic analysis comparing the OxPhos and BCR mitochondrial proteomes revealed significant differences in expression of enzymes involved in the TCA cycle, fatty acid oxidation, ketone body and amino acid metabolism. In addition, assessment of mitochondrial function in DLBCL cell lines with OxPhos versus BCR genetic signatures indicates significant differences in fuel metabolism, oxygen consumption rates, mitochondrial membrane potential and reactive oxygen species. Studies are underway to examine whether and how changes in these metabolic programs may impart OxPhos DLBCLs with a bioenergetic advantage for tumor growth and survival. Our findings may uncover novel insights into targeting energy metabolism in DLBCL.

**Pharmacological Resuscitation Attenuates Cellular Injury Response in Lung Following Hemorrhagic Shock**.

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Hemorrhagic shock can lead to systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and death. These effects may be due to cellular stress signals, inflammation, and host immune responses. We have previously shown that pharmacological resuscitation using histone deacetylase inhibitors (HDACIs) significantly improves survival in a lethal model of hemorrhagic shock (60% blood loss) with no fluid resuscitation. The aim of the current study was to examine the effects of HDACIs on mitogen activated protein kinase (MAPK) signaling pathways, which are known to promote inflammation and apoptosis.

To eliminate *survival bias*, we created a sublethal model of hemorrhagic shock. Wistar-Kyoto rats (250-300g) were subjected to 40% blood loss then randomized to valproic acid (VPA) treatment (300 mg/kg IV dissolved in 750 µliters/kg) or saline vehicle control (750 µliters/kg 0.9% saline). Animals were sacrificed at 1, 4 and 20 hours after treatment. Lung tissue samples were analyzed by Western blotting for degree of acetylation and expression of active (phosphorylated) and inactive forms of extracellular signal-related kinase (ERK), c-Jun N terminal Kinase (JNK), and p38 MAPK. Serum was screened for inflammatory mediators using Meso Scale Discovery (MSD) multi-cytokine assay and results confirmed by ELISA.

Hemorrhaged animals demonstrated significant increases in phosphorylated ERK compared to sham at 1 hour and 4 hours, phosphorylated p38 at 1 hour, and phosphorylated JNK at 4 hours ($p<0.05$) post hemorrhage. VPA treatment significantly attenuated these changes ($p <0.05$). No differences were seen in MAPK protein activation at the 20 hour time point. MSD screening identified elevated levels of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in the serum of vehicle animals at 4 hours compared to sham and VPA treated groups. Serum ELISA verified that this was a significant finding ($p <0.05$).

Hemorrhagic shock led to acute activation pro-inflammatory MAPK signaling pathways and neutrophil-recruiting chemokine CINC-1 whereas VPA modulated these effects. VPA mediated attenuation of MAPK pathway activation and neutrophil chemoattractants may denote an early, immunomodulatory mechanism underlying VPA treatment's survival advantage in hemorrhagic shock and may suggest a role for VPA in preventing secondary inflammatory sequelae.

**Interference of *Streptococcus sobrinus* Biofilm Formation By Anti-Peptide Antibody To Functional Domains Of Glucosyltransferase**

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Dental caries is the destruction of tooth tissue by the action of bacteria. *Streptococcus mutans* and *Streptococcus sobrinus* are the two species most closely associated with this disease in humans. Their cariogenicity is caused by secretion of lactic acid, a metabolic end product. For lactic acid to be present in quantities large enough to cause a detrimental effect, a significant number of cariogenic bacteria must accumulate on the teeth.

Immunization using bacterial antigens involved in attachment or accumulation, e.g., glucosyltransferease, may be effective in caries control. The purpose of this study is to determine whether rat IgG antibody, directed to CAT/GLU functional domains of *S. sobrinus* glucosyltransferase, can interfere with biofilm formation *in vitro*.

This was accomplished by purifying IgG antibody, using protein G technology, from the sera of sham-immunized, diepitopic (CAT/GLU) peptide-immunized, or Gtf enzyme-immunized rats. Gtf-binding was then measured in ELISA. Anti-peptide IgG antibody significantly bound Gtf, albeit at a 32-fold lower dilution than anti-Gtf enzyme IgG antibody.

The ability of antibody to inhibit biofilm formation was measured in an *in vitro* 96-well assay, which consisted of polypropylene round-bottomed plates fitted with tops containing plastic pins that extend into each well providing a surface on which biofilms can form. The wells contained 124ul *S. sobrinus* culture, 62ul anti-peptide IgG antibody, and 62ul sucrose. Control wells contained polyclonal anti-Gtf or sham IgG antibody with or without sucrose. After incubating the plates, the pin-containing tops were stained with 0.2% crystal violet dye. The stain was eluted and read spectrophotometrically at $A_{575}$. The absorbance is positively correlated with the number of cells in the biofilm formed on pins.

The polyclonal anti-Gtf IgG antibody preparation (positive-control) showed significant inhibition (89%) at a concentration of 0.08 mg/ml, similar to inhibition seen with the six 1/400 diluted sera. Despite being assayed at IgG concentrations ranging from 0.3-0.7 mg/ml, none of the six anti-CAT/GLU IgG antibody preparations showed significant biofilm inhibition compared to biofilms formed in the presence of the six negative control IgG.

Therefore, Gtf binding activity with the anti-peptide antibody in ELISA did not directly correlate with biofilm inhibition. This may be attributed to the necessity for higher antibody concentrations required for biofilm inhibition, or because the synthetic peptide epitopes did not induce antibody of sufficiently high affinity.

**Accessing child mental health services:**
**An ethnographic study on mainland migrants in Hong Kong**

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From 1985 to 2004, 807,431 mainland Chinese migrated to Hong Kong, accounting for 54.9% of population growth in that period. It is likely that migrants from mainland China will remain the most important source of population inflow into Hong Kong in the coming years. The process of migration is often associated with a variety of factors that impact mental health, such as acculturative stress, discrimination, social marginalization, or difficulties navigating unfamiliar institutions in order to attain education, employment, etc. Migration is also linked to multiple obstacles to both identifying and accessing mental health services, including lingual, educational, socioeconomic, and institutional barriers.

The goal of this study was to use qualitative interviews to examine the experiences of migrant families from mainland China in the course of accessing child mental health services in Hong Kong. The study focused on the impact of migrant status on becoming aware of the children's mental health conditions, factors that impeded or facilitated their access to treatment, and general experiences with mental health services in the context of migrant status.

35 qualitative interviews were conducted with the family members of child psychiatry outpatients at two sites in Hong Kong: Kwai Chung Child and Adolescent Psychiatric Centre at Princess Margaret Hospital and Yaumatei Child Psychiatric Centre. 5 additional interviews were conducted with non-migrant family members, and 6 interviews were conducted with staff members at the centers and workers at a local NGO. Audio recordings of the interviews were transcribed and annotated for identification of prevalent themes and analytical categories.

The interviews revealed that parents often missed or misinterpreted the children's psychiatric symptoms, with some parents attributing symptoms to superstitious causes or misbehavior. Many parents had not heard of their child's current condition prior to diagnosis, or did not realize that their child's symptoms were suggestive of the condition. The most common routes of entering the mental health care system were via the intervention of school social workers and teachers, or through a hospital referral. Other common themes included fear of others knowing about their children's psychiatric disorders, generally not knowing where to seek mental health services, and frustration with the long waiting time needed to secure an appointment with the public mental health services.

Both practical and cultural barriers affect migrant families when accessing child mental health care, and factors such as communication barriers and low socioeconomic status may also exacerbate obstacles otherwise common to all patient families.

**Autologous cell-based high precision, closed-loop, optically-controlled insulin delivery**

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There is great interest in developing cell-based therapies to replenish beta cell population in patients with diabetes. The pancreatic beta cell has excitable cell membrane which depolarizes in the presence of high glucose mediated by an ATP-sensitive potassium channel. Depolarization triggers opening of voltage-gated calcium channel and allows for extracellular calcium entry into the cell. Calcium then signals degranulation of pre-packaged insulin granules.

In this study, we explored the possibility of coupling a light device to signal insulin release from skin derived endothelial cells. Conversion of light signal to membrane depolarization was achieved by expressing Channelrhodopsin-2 (ChR2), a light activated cation channel. Our lab has previously shown that ChR2 and other light activated ion channels and pumps can be powerful tools in controlling neuronal membrane excitability with light.

Thus, we hypothesized co-expressing insulin, L-type voltage-gated calcium channel, and ChR2 in human dermal microvascular endothelial cells (HEMECs) would be sufficient to create a new source of insulin secreting cells under the control of a light device.

To test this hypothesis, five genes including the three subunits of L-type voltage-gated calcium channel, insulin, and ChR2 were PCR-amplified and cloned into a lenti virus backbone with ubiquitin promoter for constitutive high-level expression.

As first step, lenti viruses containing ChR2 gene were produced, purified, and used to infect a mouse insuloma cell line (MIN-6) and HDMECs. Whole-cell patching was done to determine magnitude of light-activaed current (a.k.a. “photocurrent”).

Photocurrent in MIN-6 cells were strong enough to depolarize the cells above the threshold voltage of L-type calcium channels. However, photocurrent in HDMEC were barely detectable making us believe HDMEC does not have the right membrane electrical properties for our experimental design.

This study is still ongoing and we plan to test other cell types.

**Bridging the Gap: Medical and Dental Students Advocating for Boston-area Refugee and Immigrant Families**

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*Bridging the Gap* (BTG), a 10-year-long ongoing collaboration between Harvard Medical School and MGH Chelsea, provides volunteer medical and dental students opportunities to work as advocates for refugee and immigrant families in the Boston area. Between 2000 and 2004, over 172,000 immigrants came to Massachusetts. A disproportionate number of Boston-area immigrants and refugees have settled in Chelsea, an inner urban suburb with a population of over 35,000. The Immigrant and Refugee Health Program (IRHP) at MGH Chelsea was created to help this population navigate the many complex challenges associated with settling in a new country. IRHP provides families with assistance regarding mental and physical health issues, in addition to housing and personal adjustment concerns. The program, which helps more than 900 individuals per year, has consistently been saturated with families in need, and recent increases in the Chelsea immigrant and refugee population have exceeded IRHP's capacity. The goal of BTG is to support IRHP's efforts, and, since BTG's inception, student volunteers have served as advocates for and friends to more than 180 families.

At the beginning of the 2008-2009 year, each of the 15 participating students was paired with one family. BTG families hailed from countries such as Somalia, Iraq, Afghanistan, Burundi, Cameroon, Guatemala, and Nepal. Students were required to contact their families at least twice per month and submit contact reports after each interaction. In addition, students were required to attend monthly reflection sessions, during which any concerns were addressed. The data reported were collected via student contact reports.

Over the course of the year, 92 home visits were made. On average, 4 people were helped per visit. In addition, 67 phone contacts were documented. Seventy-seven contacts involved tutoring, and 39 contacts involved help with English and/or translation. Volunteers documented 32 contacts that involved mutual cultural education. Students provided a variety of other services as well, including assistance with immigration issues, employment, health care, housing and maintenance, taxes, and summer camp arrangements. Unfortunately, because not all contacts were documented, these data are an underestimation of the actual work completed by BTG volunteers. The year culminated in a 57-attendee trip to the Boston Museum of Science for all volunteers and families in May, 2009.

The work done by BTG volunteers during 2008-2009 complemented services rendered by IRHP and allowed an additional 16 families to be assisted. BTG facilitated mutual cultural and practical learning between students and families. The experience and training that student volunteers gained through BTG will help prepare them for the challenges to be encountered as they work with underserved patients in their future careers.

**$CD11b^{+}Ly6C^{+}$ monocyte response following transient focal cerebral ischemia**

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Stroke is a leading cause of mortality and disability worldwide. Upon ischemic injury, local activation of transcription factors within the brain tissue leads to expression of pro-inflammatory factors such as cytokines and chemokines within hours. This results in increased expression of adhesion molecules on vascular endothelial cells, allowing massive influx of peripheral neutrophils, monocytes, macrophages, and T cells to the ischemic brain. There is increasing evidence suggesting that post-ischemic immune responses play a crucial role in determining clinical outcome of stroke.

Monocytes have been identified as a major component of innate immune response following stroke. Circulating monocyte level rises rapidly during the acute stage of ischemic brain injury. A recent study reported that $CD14^{high}CD16^{-}$ monocytes were associated with poor clinical outcome and higher mortality among stroke patients. Macrophages/monocytes can be detected in the brain as early as 12 h from the onset of focal ischemic injury in mice. While therapeutic strategies selectively targeting monocytes may prove to be effective, the exact mechanism of monocytes-mediated post-stroke brain injury remains unclear. The heterogeneity of monocytes complicates further this challenge.

$CD11b^{+}Ly6C^{+}(Gr1^{+})$ cells are a subset of murine monocytes and are functionally equivalent to human $CD14^{high}CD16^{-}$ monocyte subtype. They are readily recruited to inflamed tissues and lymph nodes *in vivo* and play an important role in acute inflammatory response in infection. The spleen was recently identified as a main reservoir of these monocytes. Using a mouse model of transient focal cerebral ischemia, we evaluated the kinetics of post-stroke $CD11b^{+}Ly6C^{+}$ response in the spleen and blood as well as its recruitment to the ischemic brain. Following 60-min right-sided occlusion of the middle cerebral artery (MCAO), the animals were reperfused for 1, 3, 7, 14, 21, and 28 d before flow cytometry analysis involving various immune cell type markers. $CD11b^{+}Ly6C^{+}$ monocytes were rapidly recruited to the ischemic brain from the periphery as early as 1d post MCAO in a biphasic manner. Gene expression study of the sorted $CD11b^{+}Ly6C^{+}$ infiltrates suggested that they differ from the $CD11b^{+}Ly6C^{+}$ splenic reservoir in functional profiles and express high levels of pro-inflammatory cytokines, including IL-1b and TNF-a. Therapeutic strategies that selectively block or immunomodulate the pro-inflammatory $CD11b^{+}Ly6C^{+}$ monocyte infiltrates may help reduce infarct size and improve clinical outcome following stroke.

**Community Health Workers and Primary Care in Rural Kenya**

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Primary health care delivery in rural sub-Saharan Africa faces difficult and mutually reinforcing constraints. At the community level, patient populations are often characterized by heavy disease burdens, high rates of poverty, difficulties in physically accessing health facilities, and low levels of health literacy. At the facility level, primary health centers often have weak infrastructure, shortages of health workers and other health resources, and a practice orientation that emphasizes acute over chronic care.

In June 2009, Ugunja Community Resource Center, an indigenous, multi-service community development organization in rural Kenya, sought to overcome these constraints by establishing a new primary care outreach program in partnership with the Kenyan Ministry of Health. The program revolves around a cadre of village-based community health workers who are each assigned twenty client households in the catchment area of a local primary health center. Each community health worker conducts regular, structured home visits to these households and carries out three principal responsibilities. First, the community health worker facilitates the adoption of preventive and health-promoting behaviors, such as insecticide-treated bed net use and cultivation of nutritious foods. Second, through structured detection of key clinical markers such as fever in a child and key socioeconomic markers such as inadequate housing and poor agricultural yields, the community health worker ensures that clients have early and smooth access to essential clinical and social services. Finally, the community health worker provides long-term home-based assistance and psychosocial support to individuals with chronic diseases such as HIV, tuberculosis, and diabetes.

Two main contributions to the new program were made through this service project. First, a training curriculum was created based on Ministry of Health guidelines, and a two-week training course was subsequently carried out during the project period. Second, an operational protocol for the program was developed, which addresses, among other things, what community health workers should do during home visits, how they should report to and liaise with the health care facility, and how they can obtain continuous mentoring and supervision through the program.

**Characterizing Sall2 Expression in Neural Development**

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Malignant gliomas are aggressive and largely incurable brain tumors with an incidence of >14,000 new cases/year in the U.S. Recently, the isolation of putative tumor stem cells from human gliomas has presented a promising avenue for better therapeutics and outcomes, as characterizing the tumor stem cells that sustain gliomas might provide therapeutic targets to effectively eliminate gliomas at their roots. Lineage restricted transcription factors are known to tightly control development of normal stem/progenitor cells, and therefore identification of new factors which control both normal and glioma stem cell populations holds significant potential to help identify, study and treat such populations.

The transcription factor Sall2 has recently been found to play a role in neural development and embryonic stem cells but its expression and function in specific CNS cell types and CNS cancers is not well understood. To address this we utilized and carefully characterized novel SALL2 antibodies and used these in immunohistochemistry and immunofluorescence approaches to characterize both the neuro-anatomical and cell-type specific locations of Sall2 expression.

IHC analysis of murine embryonic development showed that at e12.5, Sall2 was specifically expressed in all regions of the neural stem cell rich ventricular zones of the brain and spinal cord. It showed strong co-localization with the stem cell marker Sox2 and failed to co-localize with markers of more differentiated neural cells. During perinatal development (P0), Sall2 expression was still confined to the stem cell rich subventricular zones (SVZ) of the forebrain. In the adult, Sall2 expression remained high in cells of the SVZ but strong expression was also noted in populations of cells distributed throughout the gray and white matter regions. Lineage analysis by immunofluorescence defined Sall2 as a novel marker of most astrocytes and subpopulations of neurons through co-localization with GFAP and NeuN respectively. Interestingly, Sall2 did not appear to be expressed in adult oligodendroglial lineage cells as it failed to co-localize with Olig2, a pan-oligodendroglial marker.

These findings indicate a dynamic role for Sall2 in mouse neural development. At the embryonic and perinatal stages Sall2 expression is largely confined to neural stem cells. At the adult stages, Sall2 is expressed in neural stem cells as well as differentiated astrocytes and neurons. Our findings thus indicate that Sall2 plays a role in neural stem cell function and early neural development, but is not sufficient in isolation to maintain a neural stem cell phenotype given its retained expression in differentiated neural cells.

**The Use of Resolvin Family Compounds in the Generation of Tissue Engineered Cartilage**

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Microtia, the congenital malformation of the auricle, is a condition for which many pediatric patients undergo surgical reconstruction. At present, this requires a multiple stage process using a rib graft, with potential risk of intrathoracic injury. Thus, many attempts have been made to develop a technique for engineering an auricle using a patient's own donor tissue. One of the difficulties encountered thus far has been an inflammatory reaction to the scaffolding material used for delivering the donor chondrocytes.

We hypothesized that the use of the anti-inflammatory resolvin compounds, E1 or D1, as an adjuvant to the tissue engineering of cartilage in a syngeneic rat model would reduce the inflammatory reaction to the cell scaffold. The resolvins have also been shown to promote tissue repair and it was also hypothesized that they may directly promote neocartilage growth.

Costal cartilage was harvested from syngeneic rats and the chondrocytes extracted. The chondrocytes were cultured in a complete medium consisting of Ham's F12/ascorbic acid/fetal bovine serum/streptomycin at a density of 300,000 cells per T175 flask for 3 weeks. The cells were then trypsinized and seeded onto a mesh scaffold consisting of either polyglycolic acid or collagen type I. These cell-impregnated scaffolds were then cultured for another week in complete medium before implantation into syngeneic rat hosts. The rats were either injected intravenously compound or the cell scaffold was cultured with resolvin before implantation. The rats were then sacrificed at 1 week intervals up to 4 weeks. The implants were removed and analyzed for cartilage generation by gross inspection, histology, GAG/hydroxyproline assays and cartilage-specific RNA content.

Implants took on the look and texture of cartilage on gross inspection by 2 weeks implantation, but resorbed by 4 weeks. Rat groups given the highest dosage of resolvins demonstrated retardation of resorption compared to low-dose and control groups. Both GAG and HP density trended upwards over 4 weeks of implantation compared to controls. However, histology failed to demonstrate classic cartilage appearance and was negative for safranin-O staining. RNA analysis is currently in process.

Under the current dosage protocol, resolvins E1 and D1 failed to prevent implanted cell scaffolds from inducing an inflammatory reaction, which destroyed the developing neocartilage. However, higher doses appeared to retard the resorption of the implants. If resolvins do have a positive growth effect on chondrocytes, the current dosage or delivery method in this protocol may be insufficient to show an effect on cartilage development.

**A National Interactive Web-Based Physical Activity Intervention in Women: Evaluation of Choose to Move 2006-2007**

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Growing evidence underscores the role of physical activity (PA) in disease prevention. Online interventions provide a novel alternative to traditional attempts at improving PA behavior. Here we assessed participants in the web-based Choose to Move program, an American Heart Association (AHA) lifestyle intervention in women, for change in (1) PA levels, (2) quality of life measures, and (3) body mass index (BMI).

Women ≥18 years of age were recruited through national media and cross-promotion with other AHA programs. Participants gained access to 12 weekly interactive web-based modules promoting incremental changes in PA and diet. Of those who enrolled during the study period (n=15064), 3796 women completed surveys at registration ("registration cohort"), and 892 women completed surveys both at registration and at program completion ("evaluation cohort"). Self-reported information on PA, quality of life, and readiness to engage in PA was obtained at registration and completion.

Survey participants consisted primarily of white women, more than half of whom were between the ages of 35 and 54 years. The evaluation cohort did not differ significantly from the registration cohort, except with respect to PA and body image; evaluation cohort participants reported higher levels of PA, body satisfaction, and readiness to engage in PA at baseline. Participants showed significant (all $p<0.001$) and favorable changes in PA levels (baseline median 240 kcal/wk, interquartile range [IQR] 62 to 667 kcal/wk versus completion 343 [131 to 828] kcal/wk), readiness to engage in PA, and BMI (baseline median 29.3 [24.9 to 34.7] $kg/m^2$ versus completion 28.9 [24.6 to 34.2] $kg/m^2$). The proportion of participants in compliance with PA guidelines, defined as energy expenditure ≥1000 kcal/wk, improved from 15.8% at registration to 21.4% at completion, including among participants who reported no PA at registration. During the intervention period, significant improvements ($p<0.0001$) were also noted for RAND 36-Item Health Survey energy score (baseline 50 [35 to 60] versus completion 60 [45 to 70]) and well-being composite score (baseline 68 [56 to 80] versus completion 76 [60 to 84]). Factors positively and independently associated with adherence to PA guidelines at completion included weeks of program completed ($p=0.03$) in addition to measures of energy ($p=0.04$) and emotional well-being ($p=0.002$).

In this national cohort of women, a web-based intervention significantly improved PA levels and quality of life measures. With rising internet use and growing interest in online health materials, web-based PA programs may be uniquely positioned to achieve greater PA guideline compliance.

**Development of the Corticothalamic Projection in a Mouse Model for Autism**

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BACKGROUND: There is growing evidence that synapse development is disrupted in patients with autism. Yet synapses in the central nervous system exist not in isolation, but in neuronal circuits with reciprocal connectivity. This raises the possibility that focal abnormalities in synapses can propagate into broader disruptions of neuronal networks possibly explaining the puzzling developmental regression and progressively atypical neural specialization observed clinically in children with autism. To test this hypothesis, we will use the development of connectivity between the eye, visual thalamus, and cortex as a model experimental system to study reciprocal synaptic networks in autism.

Visual information encoded in the retina is transmitted from retinal ganglion cells to thalamic relay neurons in the visual thalamus, also referred to as the lateral geniculate nucleus (LGN). Feedforward connections from LGN relay cells target layers 4 and 6 of visual cortex. In turn, cells in layer 6 provide massive feedback projections back to the LGN (so called corticothalamic projections). In the mature brain, cortical feedback is known regulate moment-to-moment visual processing. However, little is known about the refinement of corticothalamic projections both in normal development and in developmental diseases such as autism.

Our laboratory has preliminary data showing that in a mouse model for Rett Syndrome (Mecp2 KO), the most common form of mental retardation and autism in girls, synapse development of the connection between the retina and the LGN is disrupted. However, little is known of whether the development of cortical feedback projections also abnormal in this mouse model.

AIMS: In this study, I characterized normal development of the corticothalamic projection to the LGN. This information will lay the groundwork for testing whether feedback connections between the cortex and thalamus are disrupted in MECP2 KO mice.

METHODS: Whole-cell voltage-clamp recordings of LGN relay neurons were obtained from thalamocortical brain slices. Single fiber strength, maximal synaptic strength, fiber fraction, and other synaptic properties were quantified at several development time points including p8-10, p15-16, p20-22, and p27-32.

RESULTS: Our preliminary results show that corticothalamic synapses remodel during normal development. The strength of AMPA and NMDA receptor currents activated by individual corticothalamic inputs as well as total amount of synaptic current evoked by cortical inputs increases over development.

CONCLUSIONS: Corticothalamic projections undergo significant remodeling during development of the visual system. By characterizing normal developmental maturation at the corticothalamic synapse, I can now examine whether development of these projections are disrupted in MECP2 KO mice.

**Changes in emergency department use as a result of health care reform in Massachusetts**

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**Background:** In 2006, Massachusetts enacted comprehensive health care reform with a goal of insuring all citizens in the state. The passage and implementation of this initiative provides an ideal natural experiment by which to characterize the effect of health insurance status on emergency department (ED) use, in particular use of the ED for lower severity visits.

**Specific Aims:** We used administrative billing data from a subset of 9 Massachusetts hospitals, accounting for approximately 400,000 ED visits per year to the emergency departments of these Massachusetts hospitals, to determine if enactment of health care reform is associated with a decrease in low severity ED visits.

**Hypothesis:** We hypothesize that the provision of health insurance will result in a decrease in ED utilization for lower severity visits.

**Methods:** This is a retrospective pre-post study using administrative billing databases collected from each participating institution. The study periods include January 1 to September 30 of 2006-2008, which includes 9 months prior to the enactment of health reform (Period 1), 9 months after the enactment but prior to the enforcement of the individual mandate (Period 2), and 9 months after the individual mandate began to be enforced (Period 3). Using a previously defined algorithm for classifying the severity of ED visits, we compared the rate of change of low severity visits between Periods 1 and 3 for our defined study group of uninsured or publicly subsidized patients (self-pay, free-care, Masshealth, Commonwealth Care) versus non-study group patients.

**Results:** Overall visits were 405,677 in Period 1 and 417,592 in Period 3. There was a 5% decrease in low severity visits in the study group (52,248 to 51,437 visits) when compared to the non-study group (56,619 to 60,864 visits) from Period 1 to Period 3 (RR 1.05, 95% CI 1.04-1.06, p = 0).

**Conclusions:** The relative decline in low severity ED visits in the group of uninsured or publicly subsidized patients may represent the positive effect of health insurance on access to a regular source of care. However, since this represents an absolute decrease in low severity visits of only 1.1% in this group, we cannot conclude that access to health insurance alone determines one's use of the ED. Further studies are warranted to investigate the longer-term effects of the reform as well as to understand the specific determinants of seeking care in the ED and in a primary care setting.

**Improving Medication Safety in Outpatient Departments Using Preprinted Formulary Prescription Forms**

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**Background.** Research from developed countries suggests that most prescription errors result from illegibility and incomplete prescribing information. One US study found a prescription medication error rate of 7.6% in the outpatient setting, with errors in frequency and dose being the most common type of error. A pre-printed order sheet was shown to reduce medication errors twofold in a randomized controlled trial in a pediatric emergency department in the United States and similar pads are increasingly popular in US clinics. Little is known about the incidence and type of medical prescribing errors in developing countries, as only a handful of descriptive studies appear in the literature. This study aims not only to document the types and rates of medication prescribing errors in a developing country but also implement preprinted prescription pads (FormularyScripts) as an intervention to reduce error.

**Introduction.** This study is being conducted in the outpatient ophthalmology clinic at Srinagarind Hospital of Khon Kaen University in Thailand. This hospital is the main referral center for a region of 20 million people in northeast Thailand. The study will take place between August 2009 and August 2010.

**Methods.** Patients are recruited from the outpatient ophthalmology department during their visit and sign informed consent after seeing a physician. All prescriptions given to these patients will be reviewed. Data will be collected for three months with handwritten scripts and three months with FormularyScripts. During both data collection periods, hospital pharmacists will document errors found in prescriptions according to the following categories: "Wrong Order", "Incomplete", "Ambiguous", "Abbreviation" and "Illegible." The prescriptions will be double checked for errors by a blinded research team member. Data will also be collected about the time used by pharmacists to correct any prescription errors. Finally, the time for a physician to write a prescription by hand and using FormularyScript will be recorded. In addition the time needed for pharmacists to process a prescription written by hand and using FormularyScript will be recorded.

**Results.** This project is still in the process of data collection and will continue until August 2010. Preliminary results are not available. Power calculations show that a comparison of 2970 handwritten prescriptions and 2970 FormularyScript prescriptions will be able to detect a 30% error reduction (from 5% to 3.5%.) The cost efficiency study is not powered for statistical significance but will serve as a pilot for larger time collection studies in the future.

**Evaluation of Mental Health Services provided by PCPs in Middle Bosnia Canton, Bosnia and Herzegovina**

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Increased knowledge of the connection between mental and physical health, as well as improved access and utilization of mental health services, justifies and encourages integration of mental health into primary care. One of the main goals of comprehensive Bosnian post-war health care reform was to transfer some of the mental health services from the highly specialized psychiatric hospitals into community primary care centers. In 2000, within the framework of the reconstruction, Harvard Program in Refugee Trauma (HPRT) trained PCPs in different aspects of mental health care. Extensive evaluation of the training and its attendees revealed a significant improvement in most aspects of management of patients with mental health problems immediately after the training and at two-year follow-up compared to pre-training. However, the improvement in PCPs' psychiatric diagnostic skills was not statistically significant. In fact, low level of diagnosis of depression and PTSD was proposed as one of the reasons for high prevalence of the two diseases in Middle Bosnia Canton, Bosnia and Herzegovina.

The goal of this study was to evaluate mental health services for patients with depression and PTSD provided by PCPs in primary care centers in this canton. Guidelines examination, interviews with 18 HPRT-trained PCPs, and review of 1341 patient charts were performed in total of five primary care centers.

No formal guidelines for managing mental health patients were found to exist in practice. We found that 33.3% of PCPs do not use any formal diagnostic instrument to diagnose depression and PTSD. After the initial diagnosis 55.4% of depression patients and 49.9% of PTSD patients are referred to a neuropsychiatrist, and then back-referred to their PCPs in 88.7% and 87.9% of cases, respectively. For both patient populations there is a 100% follow-up by PCPs. The study also found that 55.6% PCPs now refer fewer patients to mental health specialists than before the HPRT training. Database review supported all of the above findings. However, it also revealed that 42.3% and 47.4% of patients with depression and PTSD, respectively, are not diagnosed by their PCP, but by a neuropsychiatrist to whom they are referred after PCP's initial suspicion.

This nine-year post-training follow-up adds to the existing data on PCP practices in managing patients with depression and PTSD in Middle Bosnia Canton. We found that PCPs are relatively confident in management of these patients, and have relatively good communication both with the specialist services, and the patient. The data supports the effectiveness of PCP training in improving several aspects of management of depression and PTSD. However, improvement in diagnosis of these diseases was not as significant. Thus in the future mental health trainings more emphasis should be put on diagnosis of mental health disorders.

**Transcription Factor Profiling of Pathologic and Physiologic Hypertrophy**

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Heart failure affects over 5 million people in the United States, and accounts for nearly 5.4% of total health care costs. It can be caused by many different stimuli, including hypertension, myocardial infarction, and genetic mutations. In almost all cases, these insults first induce a period of compensatory cardiac hypertrophy, which, in turn, leads to fibrosis, electrical remodeling, and cardiac dysfunction. Interestingly, the heart also hypertrophies in response to physiologic stimuli, such as exercise, pregnancy, and postnatal growth. Such physiologic hypertrophy, however, is associated with relatively normal cardiac functioning and does not result in an increased risk of heart failure.

While a number of signaling pathways involved in regulating cardiac hypertrophy have been identified, the precise molecular differences between pathologic and physiologic hypertrophy remain unknown. We hypothesize that transcription factors, often thought to be master regulators of cellular processes, play an essential role in the development of the hypertrophic phenotype. To this end, we sought to obtain a better understanding of the mechanisms that govern cardiac growth by studying the differences in transcription factor expression in pathologic and physiologic hypertrophy.

Mouse models of pathologic and physiologic hypertrophy were generated using transverse aortic constriction (TAC) and swimming, respectively. Ventricular RNA was harvested, and differences in transcription factor expression were identified using a high-throughput qRT-PCR screen, with primers for over 1,800 known and putative transcription factors.

We confirmed hypertrophy with analyses of the heart weight to tibial length ratio, which was increased 28% in TAC mice over sham ($p < 0.05$) and 32% in swimming mice over controls ($p < 0.05$). We identified 59 transcription factors whose expression levels were significantly changed (fold change > 2, $p < 0.05$) in the swimming model and 99 that were significantly changed in the TAC model. Of these, 15 transcription factors were altered in both pathologic and physiologic hypertrophy. Eight transcription factors were then selected for further studies, based on parameters such as fold change, p-value, and expression levels in the heart, as well as on reproducibility in a separate cohort of mice.

These preliminary data suggest different patterns of regulation for pathologic and physiologic hypertrophy, and also suggest a role for transcription factors in the development of cardiac hypertrophy. The identification of key transcription factors that regulate the hypertrophic response could provide important insights into the mechanisms involved in both the development of hypertrophy and the progression from hypertrophy to heart failure.

**Maternal Alcohol Consumption and Risk for Oral Clefts: A Meta-Analysis**

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Oral clefts are among the most common congenital anomalies, occurring in 1 per 600-700 live births. Alcohol, a known teratogen, has shown adverse effects on the growth and mental development of the fetus, with the severity of the effects dependent on the timing of exposure and amount of alcohol consumed. It is not clear if there is an associated risk between fetal alcohol exposure and major congenital malformations, such as oral clefts. Studies have produced mixed results, making it difficult to draw conclusions regarding the effect of alcohol, particularly binge drinking, on risk of cleft palate (CP) or cleft lip with or without cleft palate (CLP).

Evidence from these studies remains equivocal, since effect estimates were modest, lacked a clear trend across exposure categories, and were inconsistent with respect to the different categories of oral clefts. We undertook this meta-analysis to test the hypothesis that nonsyndromic oral cleft birth prevalences are different for those whose mothers consumed alcohol during pregnancy and for those whose mothers did not.

Human studies published in English were identified through MEDLINE, bibliography reviews, and contacting experts in the field. Within strata of case-control studies, CLP, CP, and all clefts, respectively, were analyzed using either a fixed or random effects model, as appropriate. We assessed for publication bias using Begg and Mazumdar's rank correlation and Egger's regression-based tests.

Nineteen case-control studies were assessed, and their odds ratios were combined. We assessed any alcohol intake vs. no alcohol intake, as well as binge drinking (≥5 drinks at one sitting) vs. no alcohol intake. The results of our analysis shed light on the controversy surrounding the relationship between alcohol intake during pregnancy and the risk for oral clefts.

**History of Heart Disease in the Developing World**

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Between 1950 and 1990 heart disease became a leading cause of death in nearly every country in the world. While the increased epidemiologic prominence of heart disease in developed world has been well documented and studied, much less attention has been given to heart disease in developing countries. This study is an attempt to develop a chronology of the epidemiology of heart disease in the developing world, determining at what point in time cardiovascular disease became a primary contributor to the global burden of disease.

A preliminary literature review revealed a mortality database maintained by the World Health Organization. Twelve countries were selected for analysis based on historical completeness of the record and geographical representation. The twelve countries analyzed were Argentina, Chile, Cuba, Egypt, El Salvador, Hong Kong SAR, Kuwait, Mauritius, Mexico, Philippines, Sri Lanka, and Thailand. The data was extracted using FileMaker Pro 10 and analyzed with Microsoft Excel.

The study revealed that in every country analyzed cardiovascular disease became the leading cause of death by 1980. With the exception of Mexico, the Philippines, and Thailand every country made this transition before 1970. The study revealed that while each country approached this epidemiologic transition in a unique manner, they generally followed one of two patterns. Cuba, Mauritius, and the Philippines are examples of countries in which death rates for cardiovascular diseases increased at the same time that deaths attributable to communicable diseases declined. Argentina, Chile, Egypt, Hong Kong, Mexico, Sri Lanka, and Thailand are examples of countries in which the death rates due to cardiovascular disease remained relatively constant while death rates for communicable diseases declined. In every country analyzed the percentage of total deaths attributable to cardiovascular diseases increased during the second half of the twentieth century.

Analysis of the mortality data for twelve countries in the developing world provides a historical perspective on the increased prominence on cardiovascular disease in the developing world. Cardiovascular disease has existed as the leading cause of death in these countries well before it was recognized by the global health field. Additionally, in many countries death rates due to cardiovascular disease have been surprisingly stable and it was the decline of other infectious diseases that allowed for heart diseases to be the primary contributor to the burden of disease.

**Macromastia in Adolescence: a Prospective Look at the Physical and Psychological Impact**

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In macromastia, the female breast grows disproportionately large compared to the rest of the body. Macromastia is common in adolescents and has significant physical and psychological consequences. As adolescence is a period of rapid physical and emotional development, controversy surrounds the treatment of macromastia in teens. No research has explored the consequences of macromastia for adolescents using validated measures.

The Eating Attitudes Test 26 (EAT 26), Rosenberg Self-Esteem Scale (RSES), and Short Form 36 (SF 36) assess disordered eating, self-esteem, and health-related quality of life, respectively, and have been validated in adolescent populations. The Breast-Related Symptoms Questionnaire (BRSQ) evaluates the physical symptoms of macromastia. We hypothesized that adolescent girls with macromastia would have more disordered eating, less self-esteem, and lower health-related quality of life than previously studied adolescent populations, and that they would have significant physical symptoms related to macromastia. To test these hypotheses, adolescent girls ages 12 to 20 (mean±SD, 17.1±1.7 years) diagnosed with macromastia (n=28) completed the EAT 26, RSES, SF 36, and BRSQ, and the results were compared with published norms.

Compared with norms, adolescent girls with macromastia did not have significantly different scores on the EAT 26 ($p>0.05$). However, girls with macromastia did have significantly higher scores on the BRSQ than adult women and higher scores on RSES than a published comparison (BRSQ 40.7±3.9 vs. 33.13±0.91, RSES 30.0±5.7 vs. 26.8±5.0, $p<0.05$). Girls with macromastia scored lower on the physical functioning, role physical, bodily pain, general health, and social functioning scales of the SF 36 than norms (PF 71.1±25.2 vs. 96.6±9.3, RP 74.6±26.1 vs. 89.3±25.6, BP 52.6±26.7 vs. 79.2±17.8, GH 65.2±21.3 vs. 83.0±12.7, SF 72.3±29.9 vs. 84.4±16.2, $p<0.05$). Age was not correlated with any of the survey measures. BMI percentile for age category (normal vs. overweight vs. obese) was negatively correlated with only the SF 36 role physical score ($r=-0.40$, $p<0.05$).

These data indicate that adolescent girls with macromastia experience significant physical and social consequences, especially decreased quality of life due to physical symptoms. The results, except for the SF 36 role physical score, are independent of association with age and BMI category, suggesting that the negative consequences of macromastia persist throughout adolescence and are specific to macromastia. Although adolescents have considerable physical symptoms, adult women with macromastia have worse breast symptoms than adolescent girls, suggesting that the physical consequences only worsen over time and that early intervention may be beneficial.

**Health Insurance Status and Upper Extremity Elective Surgery: Is There an Association?**

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Following the diagnosis of an orthopedic injury such as a rotator cuff tear, a decision often must be made between conservative and elective surgical treatment. There is evidence that health insurance status may influence this decision; however, the rates of elective orthopedic surgery and the period between first office visit and surgery in a patient population with a variety of health insurance providers, including government-subsidized health care plans created by the 2006 Massachusetts Health Care Reform Act, have not been explored.

The purpose of this study was to determine whether the operative rates and the time between first office visit and surgery among patients treated at a single academic center for orthopedic injuries that warrant elective surgery varied according to health insurance status. We hypothesized that the operative rates would be lower among the uninsured and the period between first visit and surgery would be longer among the uninsured.

We identified 5,748 patients from administrative database who received a primary diagnosis of one of twenty upper extremity orthopedic injuries between 1/1/03 and 6/30/09. Patients were assigned one of the following insurance categories: Insured (private insurance, Workers' Compensation, Champus/Tricare [military], Medicare, Medicaid, and government-subsidized health care plan) and uninsured (self-pay, and Health Safety Net). International Classification of Diseases, 9$^{th}$ edition (ICD-9) codes and Current Procedures & Terminology (CPT) codes were used to identify patients who received surgery.

The operative rates were: 45% among patients with Workers' Compensation, 36% among patients with Chapmus/Tricare, 34% among patients with Medicaid, 29% among patients with government-subsidized health care plans, 28% among patients with private insurance, 27% among self-pay patients, and 13% among patients covered under the Health Safety Net. We anticipate that adjustment for nature of injury, age, and gender may alter the operative rates. We anticipate variation among different insurance categories in the period between first office visit and surgery, but not necessarily following the same trend as the operative rates.

Our results support the hypothesis that operative rates are lower among the uninsured population. Importantly, patients who received care under the Health Safety Net received elective surgery at less than half the rate of patients who were insured.

**Mandibular Advancement Splint Therapy to Treat Obstructive Sleep Apnea**

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Obstructive sleep apnea (OSA) is a major public health problem that imposes considerable morbidity around the world. The current gold standard of treatment is Continuous Positive Airway Pressure (CPAP). However, this treatment is often suboptimal due to highly variable patient adherence. Mandibular Advancement Splint (MAS) treatment is a viable alternative because it is well-tolerated, but is not efficacious in all patients. Studies suggest that 60-70% of patients achieve a good clinical response, but there is difficulty in predicting its efficacy in the individual patient. While there is some evidence to suggest that craniofacial factors may have an influence of treatment response, the details remain unclear.

We looked at various craniofacial measurements and anatomical landmarks to explore their relationship with MAS treatment response. Specifically, we sought to correlate these features with a reduction in sleep apnea events, which is measured by the apnea/hypopnea index (AHI). We hope that we may identify a relationship that may be used to predict the efficacy of MAS treatment in a clinical setting. We predict that maxillary and/or mandibular constriction will negatively influence the treatment response to an MAS device.

Seventy OSA patients were recruited from an academic sleep centre for the study, from which we used a sample size of n=55 for general analysis, and we obtained additional tongue measurements from 31 patients. Patients were adults (age >18 yrs), without other sleep disorders or serious active comorbid medical or psychiatric disorders or taking hyponotics sedative. Patients needed to be suitable for a mandibular advancement splint (*i.e.* in good dental health, have a minimum of 10 teeth per dental arch, and absence of periodontal disease or tempormandibular joint dysfunction). Baseline dental investigations were made prior to MAS treatment. Dental impressions were taken for the fabrication of the oral appliance and lateral skull (cephalometric) radiographs were performed in the orthodontics department of the dental hospital. We will use these measures to investigate the relationship of facial shape to bones and soft tissue.

Dolphin Imaging software was used for standard cephalometric x-ray analysis to derive linear and angular measurements. ImageJ was used in addition for tongue area measurements. Calipers, a depth gauge, and ruler were used on the dental plaster casts to document inter-tooth distances as well as the length and depth of the dental arches. Data were compiled into an excel document for statistical analysis. We plan to do perform T-tests and Chi-squared tests to ascertain a relationship between the measurements and treatment efficacy.

We await post-treatment polysomnography data on a small subset of patient, after which detailed statistical analyses will be conducted.

**Mapping of Lingual Taste Bud Sensitivity Fields**

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*Objective:* The purpose of this study was to determine whether the glossopharyngeal nerve (CN IX) supplies taste afferents in more anteriorly rather than solely on the posterior third of the tongue, as commonly believed. The objective of this project was to map the sensitivity of the tongue to chemical and electrical stimuli in individuals with normal taste function, as well as in individuals whose taste function has been potentially compromised as a result of middle ear surgery, as well as to better understand the lingual distributions of sensitivity to chemical and electrical stimuli in normal and pathological states. The goal of this research has been to better understand the normal and pathological distribution of lingual taste sensitivity by deliberate application of techniques that allow for stimuli of defined spatial and temporal extant.

*Hypothesis:* We hypothesize that the glossopharyngeal nerve (CN IX) supplies taste afferents in anterior portions of the tongue rather than solely on the posterior third of the tongue, as commonly believed. *Methods:* Electrogustometry (EGM) and chemosensory taste tests have been given to subjects whose chorda tympani nerve had been unilaterally damaged due to middle ear surgery. Lingual sensitivity on the side in which the operation was performed was compared to that of the side which remained unaffected by surgery. For the electrogustometry test, R-index values were calculated for 16 different loci on the tongue and percentages of correct responses for the chemosensory task were also recorded.

*Results:* When compared to the normal subjects, subjects who have had middle ear surgery, had a lower number of correct responses on the side of the tongue in which they had chorda tympani nerve damage in both the electrogustometry as well as the chemogustometry tests. More interestingly, at certain loci on the side where the chorda tympani was damaged, these patients were still able to distinguish taste stimuli accurately, above a "chance" percentage.

*Conclusions:* Chorda tympani nerve damage results in decreased taste function in the anterior two thirds of the tongue. Additionally, although more patients must be tested, above "chance" percentages in patients with unilateral chorda tympani nerve damage may suggest the possibility that the glossopharyngeal nerve is playing a role in taste sensation at more anterior regions of the tongue. In order to obtain more significant conclusions, more subjects with chorda tympani nerve damage would be needed to enroll for further studies.

**Effectiveness of Different Methods of Information Presentation in Oral Health Education in Peruvian Pediatric Population**

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Our goal was to determine whether different methods of information presentation in oral health education to parents and caretakers of Peruvian children from ages 3 to 6 years yields different levels of improvement in DMFT and plaque index scores. We then determined which of these methods, if any, have the highest benefit in improving oral health conditions of these children. Firstly, a baseline DMFT and plaque score was determined. Then, the different methods of oral health information presentation were provided to 7 different randomized groups. Finally, the DMFT and plaque scores was recorded after a 1 month period to assess the changes in oral health status of these children and to determine if a correlation exists between the oral health improvements and the different methods of oral health education.

Our hypothesis was that different methods of information presentation in oral health education to parents of Peruvian children will yield different levels of improvement in decayed, missing and filled teeth (DMFT) and plaque index scores. My fellow classmate Vikrum Singh Nanda and I worked jointly to test this hypothesis in a dental clinic at the Universidad Peruana Cayetano Heredia. In the pilot study, we used validated a questionnaire to evaluate the degree of knowledge of oral health and prevention techniques of parents and caretakers of school children between 3 to 6 years at the Schools of Alegria and Fe. We then divided the parents and caretakers of these children into 7 groups randomly. Then we used oral instruction, video, and brochures for the three different means of educating the parents and caretakers. The information content for each group was the same. Each group was then assessed on their ability to retain the information by completing a questionnaire. Due to difficulties discussed in detail in our final report our results were products of our pilot study. These results are in the process of being analyzed and recorded.

This investigation was aimed at a Peruvian sample population. The results of our investigation process have led us to believe that it would be a promising investigation to apply to a US population. Pediatric oral health is often overlooked in the United States and education regarding the importance of early prevention would help instill positive oral health habits at early ages in the pediatric population. This method of education would have lasting effects on the oral health of our future generation.

**Directed Differentiation of Embryonic Stem Cells Using Tissue-Specific microRNAs**

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Embryonic stem (ES) cells have attracted much attention for their ability to differentiate, or convert, into all the cell types of the body. Researchers in the field of ES cells hope to utilize this property (pluripotency) to produce specific cell types for use in treating and studying human disease. With recent advances in the production of inducible pluripotent stem cells ("embryonic stem cell-like" cells that eliminate the need for actual embryos), research has become focused on developing new methods for directing the differentiation of such cells into particular cell types capable of carrying out specific functions within the body.

Meanwhile, research at the boundary of microRNAs (short ribonucleic acid molecules) and ES cells has demonstrated a role for microRNAs in regulating the expression of genes that control ES cell pluripotency and differentiation. Furthermore, certain microRNAs are highly expressed in differentiated cell types, such as hepatocytes and neurons. Thus, we hypothesized that the introduction of tissue-specific microRNAs into differentiating ES cells could guide their conversion into particular cell types. In addition, we hypothesized that let-7, a microRNA whose expression is increased in differentiated cell types, could be used to accelerate embryonic stem cell differentiation.

Using information gathered from literature review and our own microarray data, we generated a list of microRNAs highly expressed in hepatocytes and neurons. We created plasmids that highly express these microRNAs (hepatocytes: miR-122; neuron: miR-9-1, miR-124-1, miR-128) and transiently transfected them into embryonic stem cells. The ES cells were grown in LIF-free media for their differentiation. At days 5 and 10, the transfected ES cells were harvested. mRNA isolated from the cells was analyzed by qRT-PCR to determine if there was an increase in hepatocyte or neuronal markers. Analysis of the qRT-PCR data showed that there was no statistically significant increase in tissue-specific mRNA transcripts with microRNA overexpression.

Our second investigation concerned the use of let-7 microRNA in accelerating ES cell differentiation. We introduced let-7 expression plasmid modified so that the let-7 precursor RNAs can be efficiently processed in ES cells. Then, we used qRT-PCR to measure markers of ES cell pluripotency and differentiation. Despite high levels of let-7 expression, our results indicated that there was no statistically significant enhanced rate of differentiation.

Though microRNAs may play essential roles in ES cell function, pluripotency, and differentiation, their overexpression alone in monolayer-cultured ES cells may not be sufficient to affect the pace or direction of ES cell differentiation.

**Traditional & Western Approaches to Fracture Care in the Developing World**

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**Background:** Musculoskeletal morbidity is the leading cause of disability in the developing world. In countries such as Nigeria, where there is a shortage of surgeons formally trained in fracture care, many of the injured seek care from traditional bonesetters.

**Method:** We conducted a qualitative study of fracture care in two settings in Enugu, Nigeria: The National Orthopaedic Hospital Enugu (NOHE) and a traditional bonesetter practice. Primary assessment measures at the NOHE included evaluations of the structure and process of fracture care according to the Orthopaedic Trauma Association's *Level 1 Trauma Center Requirements.* Further, we conducted semi-structured interviews of patients and hospital staff. We also assessed fracture care at a traditional bonesetter practice. We observed traditional care and interviewed bonesetters and patrons of the practice.

**Results:** The NOHE provides quality fracture care with Orthopaedic techniques and tools comparable to those found in developing nations. However, the hospital is volume overloaded and deficient in certain areas of care including pre-and post hospital care, diagnostic services and specialized support staff. Interviews of patients and physicians identified widespread use of bonesetters as well as a number of resultant complications from traditional care. At the bonesetter, we observed that fracture care was directed by an explicit algorithm for closed reduction, non-weight bearing and then gradual weight bearing – all directed without radiographs or other tests. Reasons cited for going to the bonesetter included: Word of mouth, strong belief in bonesetter potency, cost of hospital care and fear of implants.

**Conclusion:** There is no codified standard of Orthopaedic care in the developing world. The NOHE does not qualify for certification as a Level 1 Trauma Center; however, the hospital does provide quality care. Assessments of the NOHE suggest a need for quality guidelines tailored toward centers in developing countries. Bonesetters are utilized by Nigerians for a variety of reasons. There is a tension between Western and indigenous practices. We propose that bonesetters be taught certain injury management techniques and also be incorporated into the healthcare scheme in Nigeria. Bonesetters fill a void created by the severe lack of surgeons and further; bonesetters are primarily located in rural areas where they best care for underserved communities. In an integrated scheme, bonesetters manage fractures for which they can achieve acceptable outcomes, referring others to local hospitals. An integrated model of care is applicable not only in Nigeria but in all developing countries where bonesetters perform a large proportion of fracture care.

**Using Cell-Phone Based Clinical Decision Making Algorithms with Rural Health Promoters: Results from Preliminary Field Trials**

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The purpose of this project was to assess the feasibility and utility of two cell-phone based clinical decision-making algorithms designed to improve care and outcomes for children with diarrhea and adults with epilepsy for health promoters located in rural Chiapas, Mexico. Over the seven weeks of the project eight health promoters ( three in the diarrhea algorithm alone and five in both diarrhea and epilepsy algorithms) from six different communities in the Siltepec region were trained in conjunction with the local non-governmental association EAPSEC (Equipo de Apoyo en Salud y Educacion Comunitario, or "Group of Support in Health and Community Education"). Each training session included basic health information relating to the topics of the algorithms (childhood diarrhea and management of adult epilepsy), instruction in basic use of cellular phones, and practice using both paper form and cell phone based algorithms with fictitious and real patients (who received consult with doctor after use of algorithm, thereby ensuring highest level of care). Individual focused interviews were also conducted with each trainee to understand their thoughts on the advantages and disadvantages for using this type of technology in their practice setting as health promoters, as well as their suggestions for improving the algorithms and training.

Overall promoters had positive opinions of both algorithms and indicated preference for cell phone form over paper version. Various cultural and programmatic issues were identified as barriers that should be addressed in the revision of algorithms and training curriculum: for diarrhea, understanding that dehydration is the grave aspect of diarrhea in children, local use of laxatives to treat diarrhea, preference of community members to receive care (including pre-made packets of oral rehydration solution) from government clinic in cases of childhood diarrhea, and overuse of antibiotics; for epilepsy, lack of understanding and acceptance of mental illness and chronic diseases, programmatic focus that does not include home visits and patient follow-up, and irregular access to medicines from local government clinics. Cases in which the algorithm did not yield the same recommendation as the doctor were noted and will also be used to revise algorithm content. These results suggest that further steps should be taken to implement the revised cell phone algorithms and training curriculum in order to perform more rigorous evaluations to address questions such as health promoter opinions of the technology over longer time of use, opinions of patients and community members, and impact on health outcomes.

**Postprandial Energy Availability with Three Popular Diets during Weight Maintenance after Weight Loss**

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Maintenance of weight loss is notoriously difficult, and diets used for prevention of weight regain vary widely in macronutrient composition and glycemic load. The effect of diet composition on postprandial metabolic fuel availability may influence hunger and, in the long term, body weight. In the context of a larger ongoing investigation, the current study compared the effect of three popular diets on postprandial metabolism and hunger.

Participants were 8 (4 male) overweight or obese (mean BMI 33.2, SD 9.3 kg/m2) young adults (mean age 30.5, SD 6.5 yr). The intervention consisted of a 2-phase feeding trial. In the first phase, participants were fed standard hypocaloric diets to produce 10-15 percent weight loss in approximately 10 weeks. The second phase involved isocaloric feeding of low fat (LF), low glycemic index (LGI) or very low carbohydrate (VLC) diets, each administered for 1 month in a randomized cross-over fashion. Participants were studied before and every 30 minutes for 5 hours after test meals consumed at breakfast.

Endpoints included circulating (plasma or serum) concentrations of glucose, free fatty acids (FFA), and beta-hydroxybutyrate (BHB), energy availability (EA, comprising the combined energy density of glucose, FFA and BHB) and hormones; metabolic rate by indirect calorimetry; and subjective reports. EA was calculated assuming a mean fatty chain length of 17. Data were analyzed by repeated-measures ANOVA allowing for random variability in individual response. Contrasts were constructed from the ANOVA to compare EA among diets in the early (30-150 min) and late (180-300 min) postprandial periods.

Dietary composition had a significant effect (diet-time interaction) for glucose ($p=0.0001$), FFA ($p=0.0002$), BHB ($p<0.0001$), insulin ($p<0.0001$), glucagon ($p=0.045$), metabolic rate ($p<0.0001$), and RQ ($p<0.0001$), but not for cortisol ($p=0.72$), epinephrine ($p=0.39$), or hunger ($p=0.96$). EA differed negligibly among the 3 diets during the early postprandial period ($p=0.99$) but significantly in the later period ($p<0.0001$), being lower on the LF diet (mean 4.03, SEM 0.03 kcal/L) than on the LGI (4.52, 0.12 kcal/L) or VLC (4.65, 0.08 kcal/L) diets. The LGI-VLC difference was non-significant ($p=0.24$).

As previously hypothesized, a LF diet appears to limit EA in the late postprandial period compared to LGI or VLC diets after weight loss. Additional work is needed to examine the implications of these findings to body weight regulation and risk for weight regain.

**CT perfusion (CTP) can predict clinical outcome in acute stroke patients with aphasia**

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Purpose: Aphasia is present in 25% of acute stroke patients. Predicting speech recovery after stroke remains challenging. Previous studies have shown the association of certain factors with improvement of aphasia following stroke, including patients' age, type of aphasia, and infarct size. Recent PET and fMRI studies have also tried to predict aphasia outcome, but these imaging modalities are not widely available in clinical settings.

The results of CT perfusion (CTP) studies are promising for improved determination of acute brain perfusion deficit and differentiation of "infarct core" from "penumbra." Moreover, availability and feasibility of CT in emergency settings makes CTP a good alternative to MR-perfusion. The purpose of our study was to construct a multivariate model for prediction of clinical outcome in stroke patients with aphasia using admission CTP. The study was designed to identify optimal cerebral areas as well as CTP parameters that can predict early improvement of speech in acute stroke patients presenting with aphasia.

Results: In our cohort of 58 patients; 45 had speech difficulty at time of admission to MGH. Of these, 12 had early improvement of speech according to discharge notes and NIHSS. A multivariate logistic regression using different CTP parameters separately showed that only the rCBF and rMTT values of Brodmann's area(BA) 37 can independently predict an early improvement of speech in aphasic patients. Indeed, the mean rCBF value of BA 37 was significantly higher in patients with improvement compared to those who had persistent/progressive aphasia (0.92±0.04 vs. 0.72±0.04, $p<0.05$). Moreover, the mean rMTT value of the same brain region was comparably lower in patients who had a clinically detectable improvement of speech (1.12±0.04 vs. 1.42±0.10, $p<0.05$).

Receiver Operating Characteristic (ROC) curves were constructed for rCBF and rMTT values in BA 37. The area under the ROC curve for rCBF and rMTT was 0.784 ($p<0.01$) and 0.741 ($p<0.01$), respectively. Based on these models, using admission CTP maps in a stroke patient presenting with aphasia, a rCBF value >0.97 in BA 37 predicts early improvement of speech (literally by time of discharge) with 54.5% sensitivity and 92.3% specificity, whereas a rMTT value <1.33 in the same brain area is predictive of improvement with ~100 % sensitivity and 55.5% specificity.

Conclusion: We show that the combination of rCBF and rMTT values at BA 37 can predict outcome and guide treatment, based on the admission CTP imaging data.

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**The Perinatal and Neonatal Clinical History Form (PANC) Project Colombia 2009**

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With increasing concerns about the vertical transmission of HIV and Syphilis in Latin America, it becomes pivotal to evaluate the effectiveness of the current system for gathering information related to these diseases. Currently, in most of Latin America, perinatal information is collected using the Historia Clínica Perinatal (HCP), a form developed by the Latin American Center for Perinatology (CLAP). With this in mind, the Perinatal and Neonatal Clinical History Form (PANC) Project was created. The project aims at characterizing the current use of the HCP, identifying any weaknesses of the HCP as a tool for HIV and syphilis information gathering, and collecting suggestions for the form's improvement.

Under the umbrella of the PANC Project, Mr. Patiño conducted 30 semi-structured interviews in the city of Cali, with personnel involved in maternal and perinatal health at all levels of the health care system. Among those interviewed were officials from Cali's and Valle del Cauca's health secretariats, physicians, nurses, and staff from three public hospitals, as well as personnel from private insurance companies. Information from the interviews was used to draw flow charts of the current use of the HCP in the different hospitals and of the flow of pregnant women infected with HIV and/or syphilis. Additionally, many barriers for the proper use of the HCP and the prevention of vertical transmission of HIV and syphilis in Colombia were identified.

The interviews also shed light on the current state of the use of the HCP. Although never officially adopted by the health care establishment, the HCP is used almost universally in public hospitals throughout the country, and most private health care providers have based their clinical histories on the HCP variables. However, the role of the HCP as a tool for history taking has predominated over its role as an epidemiological data collection tool. One of the aims of CLAP was that data bases were built with information from the HCP at the hospital, local, regional and national levels, but in Colombia, no such data bases exist.

In conjunction with researchers who conducted similar interviews in the Dominican Republic and Peru, a report comparing the state of the HCP and prevention of vertical transmission of HIV and syphilis across countries will be prepared for CLAP. We hope this report can inform the development of future HCP forms and protocols for their use, and that this in turn contributes to better epidemiological data that can inform the efforts to prevent the vertical transmission of HIV and syphilis.

**Hypoxic Regulation of Mature Osteoclasts**

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Remodeling is the process of creating new bone and removing old bone. These two opposing processes work together to maintain bone homeostasis, which is critical for skeletal integrity and bone health. Cells known as osteoclasts are responsible for the removal of mineralized bone.

Osteoclasts are multinucleated giant cells with a defining characteristic of digesting bone. Formation of osteoclasts is the result of the fusion of multiple monocytes/macrophages. This process is induced in a laboratory setting by adding RANKL to a mouse macrophage cell line (RAW 264.7). Osteoclasts degrade bone by secreting proteases into an acidic compartment on the bone surface.

The importance of osteoclast function is demonstrated in a number of pathologic conditions including osteoporosis, osteopetrosis and periodontitis.

Hypoxia, or oxygen deprivation, occurs in human bone and may be a key regulator of osteoclast activity. Osteoclast stimulation by hypoxic stress in bone marrow culture models has been reported; however, there is still much to be elucidated about the chronology and mechanism of this phenomenon.

The objective of this project was to develop *in vitro* methods for isolating osteoclasts at the mature multinucleated stage where the resorptive activity could then be compared in hypoxic and normoxic conditions. Isolating mature osteoclasts allowed the effects of hypoxia to be studied in a narrower window of development. We hypothesized that the mature osteoclasts in hypoxic conditions will have increased resportive activity compared to those in normoxic conditions.

To observe these differences in resorptive activity, RAW cells were cultured in tissue flasks and then transferred to collagen coated plates where RANKL was added, causing differentiation into osteoclasts. After the osteoclasts formed, collagenase was added to dissolve the collagen and "lift" the osteoclasts. These osteoclasts were then plated onto calciumphosphate mineral coated wells, which simulated the mineral surfaces of bone. These plates were subsequently placed in normoxic (20% oxygen, v/v) and hypoxic (5%, v/v) conditions at 37° C. After 48 hours the cells were fixed and microscopically photographed. Using a stitching program, images were assembled in a collage for each well. These composite images made it evident that greater resorption occurred under hypoxia (observationally noted by increased total area of resportion). The fact that resorption occurred, also showed that the collagenase method used in this experiment was successful in transferring mature osteoclasts without hindering their resorptive capacity.

**Needs Assessment and Asset Map of Women in Siltepec, Chiapas: A Photovoice Project**

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Background: Chiapas, located along Mexico's Guatemalan border, reports the worst health indicators in the country, including the highest infant, child, and maternal mortalities. Almost half of its citizens lack basic necessities such as running water, flooring, and drainage systems. Further, women in Chiapas experience increased barriers to health care and public services, poorer outcomes, and inferior care compared to men, as well as frequently lacking access to basic services such as transportation, education, and health information.

Goals: This project sought to empower women in isolated rural communities in Siltepec, Chiapas to analyze, document, and present the challenges they face as women and individuals through photography, group discussions, and personal interviews. It also formed discussion and activity groups which provide a supportive environment for the generation of specific goals and a framework for future action.

Methods: The project included 6-11 self-selected women (ages 18->75) in each of five rural communities and spanned five days. The first day consisted of an informational meeting where we explained the project structure, goals, and philosophy and invited women to participate. The second day, we engaged self-selected participants in a group discussion of local women's problems, opportunities and resources. We also provided digital cameras for their use in the project and gave relevant training. Each participant then spent approximately one day photographing things that were important, helpful, or difficult in her life. Then, we visited her in her home to discuss (on audiotape) her pictures, reasons for taking them, and perspective on women's problems and resources in the community. On the final day, we met with the group again to display participants' pictures and discuss emerging themes and ideas for future action.

Results/Conclusions: The photos and recordings generated during the project will be used to produce participant-edited short videos addressing the problems, resources, and ideas identified by the women. These videos will serve to refine the goals of political and health-care activism in the region and to build solidarity and support between women across local communities, while allowing the participants to control their individual and community representation. Themes will include land ownership and use, household resources and decision-making, illness, domestic violence, alcoholism, migration, education, sense of community, upward mobility, natural resources, and role/obligations of the government. All photos were also printed and returned to the participants.

**Identification of Direct miR-26a mRNA Targets in Glioblastoma**

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**Hypothesis:** Glioblastoma is one of the most aggressive cancers of the primary central nervous system. To date, much of the molecular biology of glioblastomas remains an enigma. Of microRNAs have been implicated as an influential molecular determinant in many cancers including glioblastoma. Of particular interest is miR-26a, which is amplified in glioblastoma, and its expression promotes tumor growth by altering the expression of PTEN and RB1. We hypothesized that miR-26a accomplishes this task by directly targeting the 3'-UTR of mRNAs for PTEN, RB1, and MEKK2 to depress translation. Additionally, we hypothesize that astrocytes overexpressing miR-26a will generate glioblastomas when injected intracranially into mice.

**Methods:** PCR was used to generate fusion constructs between enhanced green fluorescent protein (GFP) and the 3'-UTR of the PTEN, RB1, and MEKK2 mRNAs. These constructs were then co-expressed with miR-26a or a control microRNA in human 293T cells, and the effects of miR-26a on expression of the fluorescent protein were assessed qualitatively via fluorescence microscopy. For quantitiative data, the 3'-UTR of RB1 and MEKK2 were fused to a luciferase reporter. Using a luminometer, the ability of miR-26a to decrease the expression of RB1 and MEKK2 was assessed. Finally, miR-26a was overexpressed in astrocytes that also expressed luciferase. These astrocytes were then transplanted intracranially into nude mice to determine whether miR-26a can transform cells to generate a tumor

**Results:** It was found miR-26a binds to the 3'-UTR of PTEN, RB1, and MEKK2, subsequently repressing the activity of all three. PTEN, RB1, and MEKK2 had a reduction in GFP expression of ~0.05 ($P < 0.031$), ~0.02 ($P < 0.038$), and ~0.085 ($P<0.0001$), respectively. The luciferase reporter experiments showed a 20% and 38% reduction for RB1 and MEKK2, respectively. The intracranial transplantation showed significant tumor growth speed and size.

**Conclusion:** miR-26a directly binds and represses the translation of PTEN, RB1, and MEKK2. This inhibition accounts for increase cell cycle progression, cell growth, and anti-apoptotic properties. miR-26a also showed significant tumorgenic ability when over expressed in astrocytes.

**Characterizing Disease-Associated Genomic Loci Utilizing Human Embryonic Stem Cells**

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A complex interplay of environmental, lifestyle, and genetic factors contribute to the pathogenesis of chronic diseases such as cardiovascular disease and diabetes. Genome-wide association studies have identified common genetic variants significantly associated with myocardial infarction (MI) and type II diabetes (DM2), creating promising opportunities to elucidate novel molecular mechanisms underlying the development of these diseases. Translating genetic associations into molecular insight can be a substantial challenge, particularly when the disease-associated loci are within intergenic regions and the identity of a causal DNA variant is unclear.

Common genetic variants within the human chromosome 9p21.3 locus are reproducibly associated with MI and DM2, independently. The mechanisms by which these disease-associated variants – located many kilobases away from any known genes – influence risk of the respective diseases remains entirely unknown. A suitable model system is needed in which genetic and functional analyses of the 9p21.3 locus and other disease-associated loci can be conveniently conducted. Ideally, the effects of a disease-associated genetic variant should be precisely characterized by controlled experiments in a relevant human cell type.

We have developed a system in which to directly interrogate the genetic effects of variants within the 9p21.3 locus utilizing human embryonic stem (hES) cells. MI- and DM2-associated regions within the 9p21.3 locus were targeted by homologous recombination in hES cells using constructs designed to knockout the region or replace it with a homologous region containing specific genetic variants. In this fashion a series of hES cell lines containing genetic modifications within 9p21.3 were established and will be differentiated *in vitro* into adipocytes, vascular endothelial cells, and other cell types of interest. In future experiments, the effects of these modifications on local and global gene expression will be assessed. This system may allow for the definitive identification of causal genetic variants within the disease-associated 9p21.3 locus and lead to the discovery of novel genes and mechanisms that contribute to the pathogenesis of MI or DM2.

**The effect of SRD5a1/2 genetic variants on the TMPRSS2:ERG fusion in prostate cancer progression**

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BACKGROUND And SIGNIFICANCE: Prostate cancer (PCa) is the most commonly diagnosed cancer and second-leading cause of cancer-related death among American men. Enhanced awareness of the underlying genetics and pathophysiology of PCa will lead to both earlier detection and improved chemotherapies. The recently discovered sex-hormone regulated *TMPRSS2:ERG* fusion, which is found in about 40% of all PCa cases, has been associated with more severe PCa progression. An understanding of the underlying mechanisms leading to fusion-positive tumors could lead to improved patient outcomes. Since chemotherapeutic agents that inhibit 5-alpha-reductase, which is encoded by the *SRD5a1* and *SRD5a2* genes, have proven effective in the treatment of certain cases of PCa, further analysis of the interaction between 5-alpha-reductase and the *TMPRSS2:ERG* fusion is warranted.

SPECIFIC AIMS: The aim of this study is to determine the effect of *SRD5a1/2* genetic variants on PCa progression in both fusion-positive and fusion-negative tumors.

HYPOTHESIS: We hypothesize that *SRD5a1/2* genetic variants will result in altered gene products that will hinder prostatic tumorigenesis.

METHODOLOGY/EXPERIMENTAL DESIGN: This prospective cohort study will utilize two cohorts of men from the Physicians' Health Study and the Health Professionals Follow-Up Study. Approximately 1,500 subjects with PCa will be analyzed for the *TMPRSS2:ERG* fusion using a modified FISH assay. DNA extracts from cases will be genotyped to elucidate the presence of SNPs in the 5-alpha-reductase genes.

ATA ANALYSIS: Statistical analysis will analyze PCa progression, fusion status, and *SRD5a1/2* mutation rate with a Cox proportional hazards model. Confounding and chance are anticipated to be kept at a minimum since we will be collecting data from subjects from the Physicians' Health Study and the Health Professionals Follow-Up Study in a blinded fashion.

RESULTS: Preliminary results from 400 analyzed specimens indicate that there is not a statistically significant association between *SRD5a1/2* SNPs and fusion-positive and fusion-negative tumors. However, the SNP AG rs1651071 of the *SRD5a1* gene (HR=2.17 (0.96-4.90) and the CT rs3731586S NP of the *SRD5a2* gene (HR=0.42 (0.17-1.05)) could prove significant once all cases have been analyzed.

CONCLUSION: Successful troubleshooting and continued research through FISH analysis on the remaining 1,100 cases is underway to ascertain whether or not genetic variation in the *SRD5a1/2* hinders tumor growth in fusion-positive tumors. However, preliminary analysis indicates that individual SNPs for the *SRD5a1/2* genes might differentially influence PCa progression in fusion-positive and fusion-negative tumors. Therefore, PCa progression rates may vary depending on the specific mutation present within that tumor.

**Role of TNF-α in Immune Response to Scarification with Vaccinia Virus**

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Following skin injury or infection, the epidermis releases large quantities of proinflammatory cytokines that initiate the intricate cascade, including interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α). These "primary cytokines" signal through the NF-κB signaling cascade, which activates genes critical to cutaneous inflammation including those responsible for other cytokines, chemokines, and adhesion molecules.

Vaccinia virus (VV), the virus used to immunize against smallpox, has emerged as a model to study antiviral response in the skin and more specifically, inflammation following viral challenge. Transepidermal inoculation of VV is through scarification using a multipuncture technique administered on the deltoid or triceps muscles. Atopic skin disease and immunocompromised patients are at risk for complications including accidental infection, erythema multiforme and even postvaccinial encephalitis. Because such patients have imbalances in cytokines including IL-1 and TNF-α, studying the role of these cytokines may explain these complications. The goal of this project was to study to what extent TNF-α is critical in the skin's ability to fend off infection and generate long-term immunity against viruses.

We hypothesized that TNF-α, like IL-1, is necessary for the recruitment of immune cells and subsequent long-term immunity against VV following scarification. The specific aims were: to compare the T cell response in TNF-α receptor (TNF-R) knockout mice versus wild-type mice, IL-1α overexpressing mice, and IL-1R knockout mice following scarification and to examine the production of VV-specific antibodies in TNF-R knockout mice following scarification.

To quantify the VV-reactive T cells response, we examined interferon-γ production using enzyme linked immune spot (ELISPOT) assay. We also did real-time PCR to examine the viral load in the inoculated skin, adjacent skin, ear, inguinal lymph node, spleen, liver and lung. To assess long-term VV immunity, serum antibody levels were measured weekly for six weeks. We also examined IL-2 and IL-10 levels to understand the global immune response.

We found that TNF-R deficient mice had comparable levels of IFN-gamma production at day 7, but at days 14 and 28 in both spleen and inguinal lymph node, IFN-gamma production was significantly higher in TNF-R KO mice than Wt mice. RT-PCR revealed that TNF-R KO mice have significantly higher levels of virus at the inoculated site and adjacent skin than Wt mice, demonstrating that TNF-R KO mice have an impaired ability to clear the viral infection. TNF-R KO mice also have decreased antibody production compared to Wt mice.

**Children with Intellectual Disability in Residential Care in Israel**

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In this project, we piloted a cross-sectional survey of nurse and physician health care providers regarding the individual and health-related characteristics of children and adolescents with serious intellectual disability (ID) living in residential care centers in Israel. The Office of the Medical Director of the Ministry of Social Affairs conducts an annual survey (since 1998) of its residential care centers. Of about 6,000 persons with ID, approximately 1,000 were children based on data from 1999-2004. While in 1999, children comprised 18.1% of the total residential care population, in 2004 this population decreased to 14.4%. Besides these relative percentages, little information is known about this population of children living in residential care facilities. The goal of this pilot project was to create and administer a questionnaire to survey nurse and physician providers of 120 children with intellectual disability (ID) living in St. Vincent and Aleh residential care centers in Jerusalem, Israel.

The questionnaire is divided into six sections: resident and caregiver information, quality of life, communication, behaviors, medical history, and medical care. The questionnaire includes the following subsections: information on the age, gender and level of intellectual disability of the child or adolescent, role of the caregiver and hours of daily interaction with the child, length of stay at the site, frequency of family visits, communication abilities of the child, quality of life of the child, a full medical history including medications, review of systems, recent treatments and hospitalizations, a full psychiatric history, and a review of the treatments and their frequency received at the site.

We collected data on 120 children and demonstrated that our pilot survey was successfully able to collect the extensive health care and health care utilization data regarding these children. Future work will refine the survey for future administration within Israel and adapt it for use in other countries.

**Health and Social Assessment of the Punjab Rural Water Supply and Sanitation Project.**

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Residents of the northwestern Indian state of Punjab rely heavily on groundwater for daily consumption. Overutilization of the same ground water by Punjab's agricultural sector, a driver of India's green revolution, has contributed to contamination and rapid depletion of water tables. In 2006 the Government of Punjab launched the World Bank funded Punjab Rural Water Supply and Sanitation project (PRWSS) to improve water supply in over 4000 villages.

Marking a transition from previous "supply-driven" projects, PRWSS emphasizes decentralization of operations, maintenance, and funding to the village level. To be eligible for PRWSS, villages are required to form a Water Supply and Sanitation Committee, finance 5-10% of construction costs and 100% of operational and maintenance costs. Fee reductions have been allocated to "scheduled caste" (SC) populations, underserved villages, and villages with large SC populations. The purpose of this study was to assess PRWSS using household surveys and detailed microbiological analysis of water samples.

Household surveys (n=250) exploring topics of water quality, access, costs, sanitation, participatory development, and health were administered in the poor sectors of six Punjabi villages. Villages were categorized into Group 1: having constructed and initiated schemes or constructed schemes nearing initiation or Group 2: not anticipating schemes.

In Group 1 villages, 60% (120/199) of surveyed households had bought into the scheme. Of the households deemed poor, using a proxy of socioeconomic status, only 28% (16/58) had bought into the scheme with 38/42 remaining households citing costs as the limiting factor. Only 7% (4/58) of poor households were aware of the Village Water and Sanitation Committee compared to 21% (41/192) of other households.

Microbiological analysis of water samples collected from Group 1 villages revealed 8/10 handpump samples were non-potable. Additionally, handpump water samples revealed fecal coliform, *Clostridium sp.*, *Listeria sp.*, and other contamination.

Findings from this limited study suggest that the PRWSS is effectively reaching a large rural population. Yet, prohibitively high user expenses may be excluding the poorest populations. Similarly, excluded population showed low rates of awareness regarding the water scheme, suggesting detachment from the participatory development process. Water sample analysis suggests that excluded populations resort to the consumption of handpump water often unfit for consumption. While the PRWSS project currently provides subsidies, additional efforts may be needed to reach the poorest populations. Further analysis of PRWSS and similarly structured water supply schemes is warranted.

**Genetic Mechanisms Underlying Lung Hypoplasia in Congenital Diaphragmatic Hernia**

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Developmental blocks which cause lung hypoplasia are associated with respiratory distress at birth. Lung hypoplasia occurs in congenital diaphragmatic hernia and is associated with high mortality and morbidity risk. Knowledge of the genetic and/or molecular abnormalities underlying these disease processes is limited. Recently, micoRNAs (miRNA) have gained recognition for their importance in regulating genes during development. MicroRNAs regulate specific genes by binding the 3'UTR of messenger RNA (mRNA) of targets and preventing translation.

In order to survey which miRNAs are important in lung formation, our group ran miRNA expression microarrays in duplicate to compare microRNA expression profiles in the pseudoglandular (8 week old samples provided by Dr. R. Choy, Hong Kong) and canalicular phases (18-20 week pooled samples from Stratagene) of human fetal lung development. Among the most differentially expressed was microRNA 449a. Prediction algorithms, protein-protein interaction networks, disease databases, and literature review were used to identify MYCN as a possible target of miRNA 449a. MCYN has been reported to cause severe lung hypoplasia when knocked out or overexpressed in transgenic mice. I designed an assay to test direct MYCN 3'UTR suppression by miRNA 449a. The assay is based on transfection of a plasmid construct in which the luciferase gene was inserted upstream of the MYCN 3'UTR, thus the luciferase mRNA is subject to the same miRNA regulatory cues as the MYCN gene. Co-transfection of this plasmid with a scrambled miRNA or with miRNA 449a demonstrated miRNA 449a specific inhibition of luciferase activity indicating that MYCN mRNA is regulated by miRNA 449a. Further, I deleted the two predicted binding sites for miRNA 449a which were identified by Targetscan within the MYCN 3'UTR. Co-Transfection with this plasmid showed that suppression of luciferase was abated. These data indicate a very specific regulation of MCYN transcripts mediated by miRNA 449a binding to precise sites in the MYCN 3'UTR. The control of MYCN expression by miRNA 449a in the embryonic lung likely plays a critical role in development. Disruption of this regulation may cause human disease such as lung hypoplasia. Characterization of the miRNA 449a/MYCN interaction and associated pathways may provide new targets for therapy to prevent or treat lung hypoplasia.

**Pneumatic Compression Treatment for Upper Extremity Acquired Lymphedema**

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Lymphedema is incurable, chronic, progressive swelling from injury or abnormal development of the peripheral lymphatic system. Complications include infection, functional disability, psychosocial morbidity, skin changes, and rarely malignant degeneration. First-line management is compression with static garments or pneumatic devices. No consensus exists on which device works best or whether pneumatic or static pressure is superior. The purpose of this study is to determine the best compression treatment for lymphedema. We hypothesize that pneumatic compression with peristaltic pulse is the most effective compression modality at reducing limb volume and improving quality of life.

In order to test this hypothesis, we are conducting a blinded, prospective, randomized-controlled trial will comparing different pneumatic compression devices to a combination of stockings, exercise and elevation (combination management). Patients with unilateral, upper-extremity lymphedema are included in the study and are randomized to one of five groups: 1) combination management, 2) non-sequential, non-gradient pump, 3) sequential, non-gradient pump, 4) sequential, gradient pump, or 5) peristaltic pulse pump. Treatment lasts 6 months, and patients then are crossed over to or continued on peristaltic pulse compression for one additional month. Extremity volume, infection rate, and quality of life (using a validated survey) are measured prior to treatment and one, three, six, and seven months after intervention. The study is powered to 12 patients per group to detect at least a five percent volume difference. Target recruitment is 75 patients to account for a 25% drop-out rate. Compliance is documented with patient log-books; completion of two-thirds of sessions is required for continued participation.

The study is currently underway with four patients enrolled. Data will be reviewed for safety and outcome analysis when half of participating patients complete three months of therapy. Outcomes will be compared across groups using ANOVA. No conclusion can be determined at this time as the study is still in the early recruitment phase.

**Identification of Genetic Variation in Autism and Comorbid Epilepsy Using Massively Parallel Sequencing**

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Autism is a heterogeneous neurodevelopmental disorder characterized by communication and language deficits, impairments in social interaction, and stereotyped, repetitive behavior. While autism is highly heritable, the genetic basis of nonsyndromic autism is poorly understood, owing to genetic, allelic, and phenotypic heterogeneity. However, identification of rare genetic variants through candidate gene resequencing has provided important insights. While next-generation sequencing technologies have facilitated such efforts, the throughput at which candidate regions are captured and the number of samples that can be simultaneously sequenced represent bottlenecks. Additionally, reduction of phenotypic heterogeneity has aided in the identification of susceptibility loci in autism. Accordingly, we analyzed a population enriched for autistic individuals with comorbid seizures to identify rare variants contributing to that phenotype.

In an initial study, we resequenced 94 individuals, 68 of whom have had ≥ one seizure, across exons from 4 genes: *contactin-associated protein like 1 (CNTNAP1), CNTNAP2, CNTNAP4,* and *RELN*, on the Illumina Genome Analyzer (GA). We used multiplex PCR for amplifying candidate regions, and further increased throughput using bar-coded resequencing, which involves adding a DNA barcode to each individual's DNA and enables pooled sequencing. We screened 16 controls from the National Institute of Mental Health (NIMH) and ~270-350 controls from the 1000 Genomes Project. Variants were subject to validation by Sanger sequencing. In a larger-scale study, we sought to increase throughput even further, by replacing multiplex PCR with microarray-based capture, which involves capturing candidate regions using complementary probes on the array. We prepared bar-coded samples from 60 trios (mother, father, and affected child) and 95 NIMH control samples. Genes belonging to the same pathway or are homologous to genes previously implicated in autism and/or comorbid epilepsy were chosen as candidates for resequencing.

We identified 8 unique nonsynonymous variants in the 94 cases across the 4 genes. Only variants in *CNTNAP2* and *CNTNAP4* were found at a higher frequency in cases vs. controls. The frequency of unique *CNTNAP2* missense variants was 2.95- and 3.01-fold greater in autistic individuals with seizures than in the general autism population and controls, respectively. Results from the larger-scale trio study are pending.

We found modest support for enrichment of unique *CNTNAP2* missense variants in autistic individuals with seizures, suggesting that rare variants in *CNTNAP2* may contribute to the seizure phenotype in autism. Our work signifies the utility of multiplexed next-generation sequencing and illustrates the value of reducing heterogeneity in dissecting the genetic basis of complex traits.

**Development of a Novel Chemical Cross-Link for Peptide Stabilization**

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The versatile surface-recognition properties of peptides make them quite attractive as potential scaffolds for drug development. However, peptides suffer from several major drawbacks such as poor cell membrane permeability, susceptibility to proteolysis, and inability to maintain a proper three-dimensional structure when removed from the context of a protein. As a result, there has always been a significant interest in designing rigidified peptides that might overcome these drawbacks, increasing their potential for use as therapeutical agents and genetic tools.

This summer, we designed and tested a novel chemical cross-link for the stabilization of α-helical peptides. A short peptide fragment (16 amino acids) of the protein called Mastermind-Like (MAML) was used as a scaffold for installing the cross-link and evaluating its efficacy. The following steps were taken to prepare a cross-linked MAML peptide: 1) unnatural amino acids were synthesized in a five-step route from commercially available Schöllkopf chiral auxiliary, 2) a MAML peptide, incorporating two of the unnatural amino acids in its sequence, was synthesized using solid-phase peptide synthesis, 3) the unnatural amino acids were chemically joined via a [3+2] Huisgen cycloaddition reaction, more commonly known as the "click" reaction, to create a triazole ring.

In the physiological setting, MAML is an α-helical protein that binds to CSL and the intracellular domain of Notch, taking part in a pathway that is heavily implicated in cancer and various developmental disorders. Thus, a chemically cross-linked MAML peptide has the potential to serve as a selective inhibitor of the Notch pathway if the cross-link stabilizes its proper three-dimensional structure (allowing it to bind to and sequester CSL and the intracellular domain of Notch). A well-designed cross-link can also make the peptide more cell permeable and less prone to proteolysis.

Several versions of the chemically cross-linked MAML peptide were obtained by varying the length of the cross-link and position of the triazole ring in the cross-link. Following synthesis and purification, each peptide was evaluated for its α-helical content using a circular dichroism spectrometer. In one case, a cross-linked MAML peptide was found to have enhanced helical content (>70%) versus the wild-type MAML peptide (23%). These results support the notion that our newly designed cross-link has the potential to stabilize an α-helix, thus conferring a natural three-dimensional conformation upon the MAML peptide. The cross-linked MAML peptides are currently being evaluated using cell permeability and functional binding assays.

**The Balance of Care between Communicable and Non-communicable Disease in Jamaica**

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Development Assistance for Health (DAH) has increased four-fold in almost two decades, comprising an increasingly significant portion of national spending on health. Oscillations in donor funding between disease-specific interventions and the promotion of (selective) primary healthcare have prompted shifts in funding at the level of national governments. Accordingly, this creates dual interests as government attempts to appease the public (by reducing the overall disease burden) and donors (by measurable reductions in disease-specific burden). Furthermore, the impact of these global resource flows into local internal markets remains unknown, and it has the potential to indelibly influence the long-term strength and capacity of health systems.

This study explores the interplay between development aid for health and health system strength in the Jamaican national health care system. Highlighting patterns of service provision, financing arrangements, and human resources, this study examines population health delivery models in cardiovascular disease and HIV patient populations. This study employs a multidisciplinary approach, utilizing in-depth interviews, archival research, and ethnographic observation as the main methods of data collection.

Traditionally, health care provision and financing are triangulated as a relationship—an exchange of resources—between the individual, third-party purchaser (government or insurer) and the provider, and these relationships define the financing, allocation, and delivery of care. In Jamaica (similar to other lower and middle income countries), the role of the third-party insurer is limited and the government and the individual are the primary purchasers of care. Indeed, Jamaica has a long-standing history of development aid for health. Consequently, this simplified model of provision and financing is complicated by forces external to local markets; donor assistance critically impacts allocation and delivery of care.

**Evaluation of Intern and Resident Physician Efficiency and Satisfaction with a Comprehensive Hand-Off Program in Pediatric Units**

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The implementation of resident work hours restrictions by the Accreditation Council for Graduate Medical Education in 2003 has resulted in increasing rates of transfer of clinical responsibility, or "hand-offs," among medical providers. Communication errors are a contributing cause of approximately two out of every three reported medical errors, and hand-offs have been identified as a vulnerable source of this communication failure. However, standardized training for hand-offs are infrequent and rarely instituted in residency programs. Furthermore, despite the known potential of technology, such as computerized order entry, to reduce serious medical errors and adverse events, few hand-off programs import medical information directly from the patient record. Objectively collected information is particularly needed to evaluate the use of computerized hand-off tools and hand-off training in the field of pediatrics, where the patient population is particularly vulnerable to serious errors and may especially benefit from such programs.

We implemented a Comprehensive Hand-off Program (CHP), consisting of training in standardized verbal hand-off techniques and a computerized hand-off tool, for resident-physicians in select inpatient units at Children's Hospital Boston (CHB). Our aims were (1) to determine whether CHP implementation would lead to reduction in time spent gathering and signing out data, as measured through characterization of time spent by interns and residents per shift, and (2) to determine whether CHP implementation would result in improved overall resident satisfaction with sign-out. We began a prospective controlled pre-post intervention study to assess the impact of the CHP on two general pediatric units at CHB in July 2009. I measured intern and resident workflow patterns and satisfaction for 7 weeks before CHP implementation.

The remainder of the study, including data from the post-CHP implementation period, will be completed on January 2010. Resident workflow patterns and satisfaction will be measured in the context of a larger study that will collect data examining the impact of the CHP over a 3 month period following a 3 month period of baseline data collection. Additional aims of the study include the impact of the CHP on medical error rates and verbal and written physician communication.

**Proteomic Insights into Chronic Prostatitis/Chronic Pelvic Pain Syndrome Etiology**

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Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a common disease in men, the etiology and optimal treatment of which are poorly understood. Symptoms of CP/CPPS include pain in the prostate, perineum or urethra, increased urinary frequency and urgency, and sexual dysfunction. CP/CPPS has been associated with a drastically reduced quality of life in affected men. Research in recent years has indicated that CP/CPPS may be a manifestation of systemic disease involving systemic inflammation, over-activation of the sympathetic nervous system and endocrine imbalance. The goal of this study is to identify biomarkers and biological processes involved in the pathogenesis of CP/CPPS that will provide a roadmap for a better understanding of the disease and better treatment options for patients.

The primary objective of this ongoing study is to identify molecules in the serum, urine, and expressed prostatic secretion (EPS) of men with CP/CPPS that serve as biomarkers of the disease. We hypothesize that there are protein and metabolite biomarkers in these biological fluids that are differentially expressed in the diseased population and that these will help provide insight into the disease process. Our follow-up aims include analysis of pathways and networks that are implicated by these biomarkers, and correlation of these biomolecular networks with epidemiologic risk factors.

We have generated proteomic data using post-prostatic massage urine and EPS samples. These data were collected using gel electrophoresis and subsequent analysis of differentially expressed proteins using mass spectrometry. Proteins scoring high in the mass spec analysis were confirmed using a Western blot and quantitatively analyzed by dot blot. Ingenuity Pathway Analysis? software was used to identify molecular networks and biological processes based on protein identity. We are currently developing a new proteomics protocol that circumvents the conventional gel electrophoresis step in order to validate previous biomarkers and study new samples.

Each Western and dot-blot analysis has yielded proteins that show differential expression between the 100 CP/CPPS patients and 100 healthy asymptomatic controls. From the post-prostatic massage urine samples we have obtained six top candidates, including several different types of proteases. The analysis of EPS samples revealed molecular networks suggestive of metabolic, endocrine, inflammatory, and several other conditions.

Initial results suggest that there are biomarkers that can help elucidate the etiology of CP/CPPS. Several of the processes purportedly involved in the disease have been suggested by the proteomic data analysis. The development of our new proteomics protocol shows promise in building upon these results.

**Achieving Vision 2020 in the Andes:**
**Preliminary outcomes from a national cataract elimination program**

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**Objective**: To access visual outcomes of patients receiving free cataract surgery under a newly created national cataract elimination program of the Peruvian Ministry of Health in comparison to traditional fee-for-service cataract surgeries performed at a major tertiary eye hospital in northern Peru.

**Design**: Retrospective, comparative, consecutive review.

**Methods:** Patients: All (n=187) patients receiving free extracapsular cataract extraction (ECCE) compared to all patients *(*n=68) paying in the highest charge tiers to receive ECCE surgery at the Regional Institute of Ophthalmology, Trujillo, La Libertad, Peru, during the first 12 months of inception (August 2008-July 2009) of the Peruvian National Cataract Elimination Plan.

**Main outcome measures:** Uncorrected, Pinhole, and Best Corrected Visual Acuity and surgical complication rate.

**Results:** At one month followup, both groups had similar uncorrected, post-operative visual acuities (p=.22). Surgery performed by supervised, final year residents conferred no appreciable risk of poor visual outcome or complication incidence. Women enrolled in the National Plan were 2.18 times less likely ($p<.05$) to have a WHO "Good" outcome (uncorrected visual acuity (UCVA), $\geq$ 20/60 ). Patients 75 and older were 2.2 times more likely ($p<.05$) to have a WHO "Poor" outcome (UCVA <20/200); patients receiving surgery performed at community clinics were 4.1x more likely ($p<.05$). Patients receiving surgery under the National Campaign had higher rates of OCTET Grade II intra-operative adverse events ($p<.01$). 10% of patients who received surgery under the National Plan had suspected, but due to dense cataracts unconfirmed, retinal co-morbidities; these patients were 4.5x more likely ($p<.05$) to have a post-operative visual acuity worse than 20/200. Additionally, visually compromising vitreo-retinal disease was discovered post-operatively in 6% of National Campaign patients. Only 24% of National Campaign patients achieved an UCVA 20/60 or better.

**Discussion:** Neither the WHO's nor the Peruvian Ministry of Health's benchmarks for surgical cataract intervention, that 85% of eyes achieve 20/60 vision and 75% of eyes achieve 20/50 vision postoperatively, respectively, were met. Earlier diagnosis and more aggressive public health campaigning aimed at encouraging the visually impaired to present sooner will likely improve the sensitivity with which patients more likely to benefit from surgery are selected and improve outcomes of the National Cataract Elimination Plan. Further study is needed to assess the causality of underutilization of surgical services by patients with visual acuities closer to the National Cataract Elimination Program's visual acuity threshold criteria (20/200).

**Emotional and Behavioral Issues in Siblings of Children with Autism in Mumbai, India**

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Autism is a disorder of development that affects many children and their families. It is identified by three characteristics: impaired social interactions, impaired communication and stereotyped or repetitive behaviors. Caring for an autistic child can have an impact on the whole family. It is also thought that given the altered social interactions due to autism and the importance of the sibling relationship in social development, siblings of autistic children may also develop different behavioral and emotional characteristics. In addition, in developing nations such as India, there is a lack of resources and community understanding of developmental disabilities like autism.

The purpose of this study was to address whether among a population of children in India, siblings of children with autism have increased behavioral or emotional problems, and if so, whether these issues are correlated with any socio-economic, psychosocial or social support factors.

All participating families had children previously diagnosed with autism. The primary caregiver was asked to complete three questionnaires. The General Health Questionnaire evaluated the mental health of the primary caregiver, the Child Behavior Checklist evaluated the behavioral and emotional issues of the typically developing sibling, and the additional questionnaire elicited socio-economic and social support data. Participating families were recruited from the patient population of Ummeed, an organization in Mumbai, India that provides integrated care for children with developmental disabilities, including autism spectrum disorders. As this was the continuation of an ongoing study, 9 additional questionnaires were collected, bringing the total completed to 50, which are currently being analyzed.

**Prevalence and Characterization of Familial Myeloma**

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Multiple myeloma (MM) is an incurable cancer of the mature plasma cells that accounts for approximately 10% of all hematologic cancers. In the United States, approximately 19,920 new cases and 10,690 deaths are expected in 2008, accounting for 2% of deaths due to all cancers and 20% of deaths from hematologic malignancies.

Multiple myeloma can evolve from an asymptomatic premalignant stage of clonal plasma cell proliferation, monoclonal gammopathy of unknown significance (MGUS). MGUS is present in more than 3% of the population above age 50 and progresses to myeloma or a related malignancy at a rate of 1% per year. The etiology of MGUS and the reasons why some MGUS progress to myeloma is uncertain.

The causes of multiple myeloma are unknown. As with other cancers, the risk of developing MM is 2-4-fold higher in patients with a first-degree relative with the disease. A few multigenerational MM/MGUS families have been reported, but only a small number of families have been examined in detail. Among MM patients, a small, but unknown fraction is familial. Studying familial MM may provide insights into the pathogenesis, and ultimately, the control, prevention and treatment of MM and related disorders. Therefore, we aimed to estimate the prevalence of familial MM in a specialty clinic population and collect preliminary data on other malignancies or immune disorders among kindreds with familial MM. We hypothesized that a familial form of multiple myeloma exists and that among families in which multiple cases of MM and MGUS occur, there is at least one shared cancer-susceptibility locus.

Patients were recruited at the Jerome Lipper Center for Multiple Myeloma at Dana-Farber Cancer Institute. Participants filled out a questionnaire presenting questions on demographics, past medical history, relevant social history and occupational exposure, and family history and were interviewed for a more extensive review of family history. Participants were classified as "high risk" if they met certain criteria for family history of MM, including 1 or more relatives diagnosed with myeloma, MGUS, lymphoma, a neoplastic blood disorder, cancer diagnosed under the age of 45, any childhood cancer, and/or prostate cancer in multiple relatives, with at least one diagnosed before age 50. DNA samples were collected from "high risk" participants for future genetic analyses.

As of early August, 32 participants were enrolled in the study. 14 (44%) participants were classified as "high risk." Of these 14, 7 participants had a relative with myeloma and 1 had a relative with MGUS. The remaining 18 (56%) participants did not meet criteria for family history of myeloma. The data, while preliminary, shows that families with multiple cases of myeloma exist. Participants will continue to be enrolled in the study and collection of preliminary data among kindreds with familial myeloma has begun. In the long-term, we aim to conduct genetic analyses toward identification of myeloma susceptibility genes toward development of novel targets. Identification of at-risk family members may lead to risk reduction interventions, aimed at delaying and ultimately preventing clinically significant MM.

**Optimal Viewing Parameters for**
**Myocardial Stress Perfusion Computed Tomography**

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Myocardial stress computed tomography perfusion (CTP) has comparable diagnostic accuracy for detecting myocardial perfusion defects when compared to single-photon emission computed tomography (SPECT). However, the optimal diagnostic viewing parameters and post-processing techniques for CTP are unknown. We sought to compare the diagnostic accuracy of different post-processing techniques, cardiac phases and slice thicknesses for the detection of angiography-proven defects.

A stress and rest dual-source cardiac CTP protocol was performed using adenosine. Ten subjects with invasive-angiography proven significant stenosis (>70% by quantitative coronary angiography-QCA) were selected, as well as five normal controls (negative SPECT & QCA). Short-axis left ventricular stress CTP images were reconstructed with 2 processing techniques (Minimum Intensity Projection - MinIP, Average Multiplanar Reconstruction - MPR) and 3 slice thicknesses (1, 3 and 8 mm), and 2 phases (35% systolic and 65 % diastolic). The 180 resulting pairs of stress images were then randomized and interpreted by independent blinded readers. QCA was considered the reference standard.

The CTP parameters of 8 mm thick MPR reconstructions showed the highest per-patient sensitivity (95.6%, 95% CI: 81-99%), followed by 3 mm MinIP (90.0%, 95% CI: 76 – 98%). The lowest sensitivity was 1 mm MPR (75%, 95% CI: 64-88%). Per-vessel sensitivity followed this same trend: with highest sensitivity of 8 mm MPR (84.6%, 95% CI: 67.3%-94.8%) followed by 3 mm MinIP (73%, 95% CI: 55.3-86.9%), and lowest sensitivity for 1 mm MPR (65.4%, 95% CI: 47.6-80.8%). A comparison between systolic and diastolic phase images showed higher sensitivity for diastolic images (98.3%, 95% CI: 93.4-99.9%), but higher specificity for systolic images (70%, 95% CI: 53.9-83.1%) on a per-patient basis. The results of both reconstruction parameter/type and phase of the cardiac cycle correlated to reader confidence (highest reader confidence for diastolic 8 mm thick MPR images).

Adjustment of certain reconstruction and viewing parameters during CTP interpretation can increase the diagnostic accuracy for the detection of hemodynamically significant stenosis by QCA. The optimal reconstruction parameters were 8 mm thick MPR, diastolic phase images.

**Establishing Community-based Perinatal HIV/AIDS Services in Provincia Peravia, Dominican Republic**

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Although HIV prevalence in the Dominican Republic hovers at roughly 1 percent, the proportion of pregnant women who are HIV-positive is several-fold higher, as high as 8 percent. This disproportionate burden demonstrates opportunities to improve HIV/AIDS care for expectant mothers and to prevent vertical transmission to their children.

In 2000, the Dominican Ministry of Health established *El Programa National de la Reducción de la Transmisión Vertical del VIH* (PNRTV, or the National Program for Reducing the Vertical Transmission of HIV). The PNRTV offers services including HIV testing, HIV counseling, optional cesarean sections, antiretroviral treatment for HIV-positive mothers and their newborns, and access to infant formula. Analysis by the Ministry, however, concluded that low availability of voluntary counseling sessions and inadequate HIV rapid tests have constrained program implementation. Further, PNRTV services remain centralized in hospitals, decreasing service accessibility to Dominicans who do not seek hospital-based care.

To address these issues, the non-profit Infante Sano seeks to offer rapid HIV testing and counseling services in two communities served by organizational clinics bear the city of Baní. Infante Sano is a Dominican Republic-based NGO focused on the health of mothers and children. By offering community-based services, the organization hopes to extend the continuum of HIV/AIDS care to the local level, increasing both test uptake and successful communication of results.

In preparation for program implementation, Infante Sano sought to analyze the state of existing hospital-based PNRTV services and also to develop community-based educational materials to promote rapid HIV testing. Hospital services were documented through key informant interviews with staff members in the hospital's Integrated HIV/AIDS Service, including counselors, nurse, and physician. In addition, group pre-test counseling sessions were observed directly for multiple sessions (n=6). Preparations for community-based education through group presentations and community health workers included development of an informational brochure and hour-long educational module on HIV/AIDS and the rapid HIV test.

Analysis of the hospital-based counseling curriculum revealed opportunities for increased harm-reduction education on both sexual and intravenous transmission of HIV. Further, privacy concerns associated with the disclosure of personally identifying information were identified in hospital procedures. Understanding of counseling, testing, and clinical care protocols of the hospital will be used to tailor community-based services and provide smooth referrals to PNRTV services. Remaining challenges include: (1) optimizing community-based educational and testing protocols, (2) commencing delivery of community-based services, (3) addressing the concerns in hospital procedures, and (4) ensuring the integration of community- and hospital-based services.

**Using NKG2D/Fc Fusion Protein to Modulate Immune-mediated Tumor Destruction Against a Broad Spectrum of Cancer Types**

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Cancer is a heterogeneous disease, encompassing many different tissue types and tumors that respond differently to therapies. Currently, there are very few unifying treatments for all types of cancer. Despite this hurdle, one commonality across all tumors is DNA damage and activation of DNA damage repair pathways. These pathways induce expression of various "distress" ligands, which signal to the immune system that the cell is undergoing malignant transformation. These ligands have been found in diverse malignancies, including lung, colon, prostate, and breast cancers, as well as melanomas, leukemias, myeloma, and gliomas. Normally, natural killer (NK) cells are able to recognize these distress ligands through a receptor called NKG2D. This receptor binds to the ligands (also called NKG2D ligands, NKG2DLs) that are expressed on the tumor cell surface, resulting in NK cell-mediated destruction of the tumor cell.

Unfortunately, tumor cells have evolved mechanisms to escape NKG2D-mediated lysis. Many tumors cleave the NKG2DLs from the tumor cell surface, creating soluble NKG2DLs (sNKG2DLs) that are released into the blood. These soluble ligands bind to NKG2D on the NK cell surface and cause internalization and degradation of the NKG2D receptor. Accordingly, the NK cells no longer recognize and destroy the tumor cells, allowing the tumor to proliferate and metastasize unchecked.

This study aims to use the NKG2D receptor's intrinsic ability to bind NKG2DLs expressed by many tumor types, and use that to generate an immune response against tumor cells in murine models. We created an NKG2D/Fc fusion protein, which fuses the extracellular domain of the murine NKG2D molecule to the Fc region of an activating IgG molecule. Since many different cancer types express NKG2DLs, we hypothesized that NKG2D/Fc will target a broad variety of tumor types for immune-mediated destruction.

Using the YAC-1 leukemia cell line, we found that NKG2D/Fc fusion protein activates many aspects of the immune system *in vitro*, including complement dependent lysis, macrophage antibody-dependent cellular cytotoxicity (ADCC), and dendritic cell cross-presentation. Additionally, NKG2D/Fc potently destroys a variety of different tumor types from diverse cellular origins, including lung cancer, renal cell carcinoma, myeloma, sarcoma, colorectal cancer, and leukemia. Importantly, NKG2D/Fc did not destroy normal tissues; rather, it selectively destroyed malignant cells.

These data suggest that NKG2D/Fc mediates immune destruction against a broad spectrum of tumor types *in vitro*. NKG2D/Fc thus may prove useful in treating a wide variety of different malignancies by stimulating the immune system to destroy tumor cells throughout the body.

**The TLR9 Ligand CpG Promotes Cutaneous Expression of IFN-γ the Skin in a Mouse Model of Atopic Dermatitis (AD)**

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Rationale: Acute AD skin lesions express Th2 cytokines and to a lesser extent Th17 cytokines, while chronic AD lesions express the Th1 cytokine IFN-γ. We examined whether exposure to bacterial/viral DNA elicits a Th1 response in Th2 dominated allergic skin inflammation.

Methods: Dorsal skin of BALB/c mice was shaved and tape stripped then subjected to three one-week applications of ovalbumin (OVA). CpG was applied together with OVA either during the last week of antigen exposure or throughout the three weekly cycles of epicutaneus sensitization in a mouse model of AD. Skin histology was assessed by H&E staining and immunohistochemistry, cytokine mRNA expression by quantitative RT-PCR and splenocyte production of cytokines by ELISA.

Results: CpG application during the last week sensitization resulted in decreased local expression of IL-4 and IL-13 and increased expression of IFN-γ. CpG application with OVA in all three sensitization cycles increased epidermal thickening, dermal infiltration with $CD4^+$ cells and eosinophils, local expression of IFN-γ, IL-4, and IL-17 mRNA and production of these cytokines by OVA-stimulated splenocytes.

Conclusion: TLR9 ligands may promote Th2-> Th1 conversion in AD skin lesions, whereas introduction of antigen concomitantly with CpG may potentiate local and systemic IL-4, IL-17 and IFN-γ responses. These findings are important when considering CpG therapy in AD.

**Microinjection of the $GABA_A$ Antagonist Bicuculline to the Pontine Reticular Formation of the C57BL/6J Mouse Blocked the Increase in Wakefulness and Decrease in Sleep Caused by the $GABA_A$ Agonist Muscimol**

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The pontine reticular formation (PRF) is located underneath the cerebellum in the brain stem and is crucial for generating rapid eye movement (REM) sleep and regulating the sleep wake cycle. Direct injection of $GABA_A$ receptor agonists into the PRF causes a significant increase in wakefulness and a decrease in sleep in both cat and rat. The reverse effect is seen with injection of $GABA_A$ receptor antagonists in the same animal models. However, to date the role of $GABA_A$ receptors in regulating sleep in the mouse has not been completely elucidated. Recent data show that microinjection of the $GABA_A$ receptor agonist muscimol to the PRF of C57BL/6J (B6) mice increases wakefulness in a concentration dependent manner. To further demonstrate that GABA is a mediator of the sleep wake cycle in the PRF, this study tested the hypothesis that coadministration of the $GABA_A$ receptor antagonist bicuculline with muscimol to the PRF blocks the increase in wakefulness and decrease in sleep caused by muscimol alone.

Adult male B6 mice (n=6) were implanted with a guide tube cannulas aimed for the PRF, three electroencephalogram (EEG) electrodes, and two electromyogram (EMG) electrodes. Each mouse received randomized microinjections of 0 mM muscimol (saline control), 1 mM muscimol, and 1 mM muscimol with 0.1 mM bicuculline. EEG and EMG signals were recorded for 4 hours post injection. The recorded signals were scored in 10 second intervals for states of wakefulness, non-REM (NREM), or REM sleep. All data were analyzed using one way analysis of variance and Tukey-Kramer post hoc comparisons test. All microinjection sites were localized histologically to the PRF.

Microinjection of muscimol significantly ($p<.001$) increased the amount of wakefulness (muscimol: 86.3% control: 70.8%), decreased the amount of NREM sleep (muscimol: 12.7%, control: 24.5%), and decreased the amount of REM sleep (muscimol: 1.1%, control: 4.7%) as compared to control. Muscimol also significantly ($p<.05$) increased the latency to onset of NREM sleep (muscimol: 64.6 min, control: 12.3 min). Coadministration of bicuculline with muscimol restored the amount of time spent in wakefulness (coadministration: 68.4%), NREM sleep (coadministration: 26.8%), REM sleep (coadministration: 4.8%), and the latency to onset of NREM (coadministration: 19.0 min) to values that were not significantly different from control values. These data support the conclusion that $GABA_A$ receptors in the PRF of the B6 mouse promote wakefulness and are involved in the regulation of the sleep-wake cycle.

**Development of a Microfluidic Device for Diagnosing Multidrug-Resistant Tuberculosis**

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Tuberculosis (TB) remains one of the most devastating infectious diseases in the world. Each year approximately nine million people develop active TB and approximately two million of them succumb to the disease. The TB epidemic is compounded by an increase in multidrug-resistant (MDR) strains of tuberculosis, which result in higher mortality and are more difficult to treat than drug susceptible strains.

The countries most affected by TB face a number of obstacles to TB control, perhaps the most pressing of which is the lack of a simple, rapid, and accurate diagnostic test for active TB. In part, this problem stems from the fact that the initial screening test for active TB remains sputum smear microscopy, a technique which is reliable only in relatively advanced disease. Smear microscopy can be supplemented by routine culture methods in advanced laboratories; however, culture is slow and currently serves only a tiny fraction of patients with active disease.

In order to reach a larger fraction of patients infected with active tuberculosis, a diagnostic device will need to be available at the point-of-care (POC). Microfluidic chips are appealing POC devices due to their small size, ability to integrate all steps of the diagnostic into a single cartridge, and because they do not require an expert operator. One critical aspect of many microfluidic POC diagnostics is the ability to isolate a specific living organism from its surroundings. Therefore, the focus of this project is to identify or produce a compound that binds to *Mycobacterium tuberculosis (Mtb)* with sufficient sensitivity and specificity for use in a microfluidic diagnostic device.

We found that existing binding molecules for *Mtb* had inadequate sensitivity and/or specificity. For example, a lipobiotin compound captured a related bacterium, *Mycobacterium smegmatis,* with a capture efficiency of only 1.25%; it also captured *M.smeg* no better than it bound to *Escherichia coli.* Given the failure to find a suitable existing compound, we then attempted to identify *Mtb* antigens that could be used to generate a suitable anti-*Mtb* antibody by biotinylating cell surface proteins. While this protocol is still being refined, we have demonstrated an ability to isolate numerous known cell surface proteins.

These data indicate that existing compounds for capturing *Mtb* are inadequate and that it will be necessary to develop a protocol for creating a novel capture agent. Future work will focus on refining this procedure and creating the binding agent.

**Do Weight Concerns Predict Smoking Cessation Program Outcome in Women Engaged in Exercise Program**

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Background: Despite overwhelming and highly publicized evidence of the adverse effects of tobacco use, the prevalence of smoking in adolescent girls and young women has steadily increased in the last few decades. Previous research indicates that women have more difficulty quitting smoking than do men. One argument for this divergence is that concerns over post-cessation weight gain interfere with successful smoking cessation and that many women use cigarettes as a weight-control tool. However, the relationship between weight concerns and smoking cessation outcomes has not consistently been observed.

Aims: Our first aim was to determine whether the magnitude of concerns about weight gain predicts a) pretreatment attrition and b) early versus late relapse. The second aim examined the characteristics of women with varying degrees of weight concerns.

Methods: A total of 300 female subjects ages 18-55 completed baseline questionnaires including demographic information, depression, smoking history and quitting motivation, and were enrolled in a 15-week aid-to-cessation program combining an exercise regimen and nicotine replacement therapy. A 6-item survey addressing weight concerns, weight, and fitness level were also assessed at baseline. Biochemically verified continued abstinence was assessed for 1-year.

Results: Neither univariate nor Cox regressions indicated weight concerns to be predictive of pretreatment attrition. Confounders such as depression, fitness level, body mass index (BMI), or exercise frequency could not explain this finding. Women who had lower levels of depressive symptoms, better baseline fitness level, and lower BMI reported the lowest levels of weight concerns (ps $<.05$), while women who smoked the greatest number of cigarettes per day had the highest levels of concerns ($p= .05$).

Conclusions: While our data does not support the notion of weight concerns impeding smoking cessation, it elucidates the existence of a subgroup of women who have a cluster of prospective risk factors (weight concerns, depression, poor fitness level, and high tobacco intake) for a failed quit attempt and tobacco-related morbidity.

**Analysis of rare variants within *DISC1* for association with schizophrenia and bipolar disorder**

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Both schizophrenia and bipolar disorder are common neuropsychiatric disorder, with schizophrenia characterized by hallucinations, delusions, and cognitive defects and bipolar disorder characterized by mood symptoms ranging from mania to severe depression. Genetic factors are likely to play a significant role in the etiology of both diseases. One of the first breakthroughs in understanding the genetic architecture underlying these diseases came in 1990 with the identification of the *Disrupted-in-Schizophrenia-1* (*DISC1*) gene in a Scottish family with a number of major psychiatric illnesses, such as schizophrenia, bipolar disorder, and major depression. Functional studies of the protein encoded by the *DISC1* gene (DISC1) have revealed a critical role for DISC1 in a variety of aspects of neurodevelopment. Numerous genetic analyses have been undertaken to elucidate a role for common *DISC1* variants in the genetic risk for schizophrenia and bipolar disorder in the general population, but there has been no obvious variant or gene region that has demonstrated a clear and consistent association with disease. There is accumulating evidence, however, that ultra-rare variants may contribute significantly to the genetic risk of psychiatric diseases, such as schizophrenia. In this study, we sequenced the exons, adjacent intronic regions, and the untranslated region (UTR) of *DISC1* in a cohort of 719 individuals composed of 188 schizophrenia cases, 165 schizophrenia controls, 185 bipolar disorder cases, 181 bipolar disorder controls to discover rare coding and non-coding variants. These variants were then genotyped in a large case-control cohort of 17,155 Individuals (4607 schizophrenia cases, 4,466 bipolar disorder cases, and 8082 controls). Preliminary evidence indicated that in the combined data set (bipolar disorder and schizophrenia cases vs. controls), the most significant finding was a rare intronic variant (minor allele frequency = 0.2%) with a *P*-value of $2.5 \times 10^{-5}$ (odds ratio (OR) = 2.96). This intronic variant was also the most significant finding in the bipolar data set (bipolar disorder cases vs. controls) with a *P*-value of $2.1 \times 10^{-5}$ (OR = 3.21). No single variant demonstrated significant association ($P \leq 0.5$) in the schizophrenia data set (schizophrenia cases vs. controls). These preliminary findings suggest that rare variants within *DISC1* are likely to contribute significantly to the genetic risk of bipolar disorder but not schizophrenia.

**Transneuronal analysis of the auditory reflex pathways using pseudorabies virus**

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The middle ear muscle (MEM) acoustic reflex is a major efferent system to the auditory periphery and is thought to protect the ear from acoustic injury and reduce the masking effects of background noise on understanding speech. Sound presented to one ear can trigger the contraction of the MEMs in the same ear (uncrossed reflex) and opposite ear (crossed reflex). While the afferent pathway, passing from nerve fibers in the cochlea to the cochlear nucleus, and the efferent pathway, passing from motoneurons in the brainstem to the MEMs, have been well described, the central pathways, called interneurons, that originate in the cochlear nucleus and project to the MEM motoneurons have not been identified. The aim of this study was to localize and characterize the interneurons of the stapedius and tensor tympani reflex in rats using Bartha pseudorabies virus (PRV). PRV is a retrograde neurotropic viral tracer that labels neurons in a trans-synaptic fashion. Time-graded survival experiments and immunohistochemistry for both pathways revealed labeling of MEM motoneurons, superior olivary complex (SOC) neurons, and cochlear nucleus neurons. Specifically, we demonstrate PRV-labeled neurons in the anteroventral cochlear nucleus that comprise reflex arc of the tensor tympani, and, for the first time, parallel circuitry for the stapedius reflex pathway that appears to proceed through the posteroventral cochlear nucleus.

**Self-Report Measures Of Adherence To Antiretroviral Therapy In Rural Rwanda**

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Background: Non-adherence to antiretroviral therapy (ART) is a strong predictor of progression to AIDS and mortality, and systematic adherence monitoring may outperform CD4 count change in predicting viral suppression. Thus, accurate adherence assessment tools are critical for targeting adherence support activities and improving treatment outcomes. The Center for Adherence Support Evaluation (CASE) Adherence Index, a simple three question measure, has not been validated in a resource-limited setting.

Objective: To compare the utility of several adherence assessment tools, including the CASE Index, in a resource-limited setting.

Methods: We prospectively measured adherence among patients in rural Rwanda 3 months after initiating ART. Patients received either a standard clinic-based care delivery model or a community-based model, which included directly observed therapy and socioeconomic support. A modified version of the CASE Index and a visual analog scale (VAS) were compared to standard four-day recall. Sensitivity and specificity were calculated using four-day recall as a standard. T-tests were used to assess differences in change in CD4 count from baseline to 6 months.

Results: Patients in the community-based model (N=307) were similar to those in the clinic-based model (N=306) with respect to gender (58 versus 65 percent female, p=0.06) and age (mean: 39 versus 38 years, p=0.69). A modified CASE score of 10 or greater predicted 95 percent adherence by four-day recall with a sensitivity and specificity of 93.2 and 62.4 percent, respectively. Participants with a CASE score of 10 or greater also achieved a 172 cell/$mm^3$ mean increase in CD4 count over 6 months, compared to a 150 cell/$mm^3$ increase for those with scores less than 10 (p=0.34). 95 percent adherence by VAS was not associated with a significant difference in CD4 change (155 vs 143 cells/$mm^3$, p=0.44). In contrast, adherent patients, with no reported missed doses on four-day recall, experienced a greater six-month increase in CD4 count than non-adherent patients when assessed by four-day recall (176 versus 117 cells/$mm^3$, p=0.01).

Discussion: In this preliminary analysis, four-day recall was a better predictor of immunologic outcome than either the CASE or VAS. The validity of the CASE Index as a tool for assessing ART adherence in rural African settings will be further assessed using unannounced pill counts as a reference standard, and viral load as a clinical outcome.

**Novel Missense Mutation Of The GPR56 Gene In A Bilateral Frontoparietal Polymicrogyria Patient**

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Bilateral Frontoparietal Polymicrogyria (BFPP) is a rare form of congenital brain malformation. Affected individuals develop seizures at an early age, mental retardation, language impairment, and gait difficulty. GPR56 has been identified as a causative gene of BFPP, but functions of the gene product remain elusive. The protein product of GPR56 is a G-protein-coupled receptor which autoproteolyzes itself to the N- and C-terminus domains. Both of the two termini have been known to be expressed on the cell surface.

A 3-year-old boy who was born to first cousin Yemini parents in the Netherlands presented with developmental delay and seizures for a clinical genetic assessment. The patient was diagnosed with BFPP according to his brain MRI imaging results. The sequence analysis of his DNA revealed a 1730G>A substitution in exon 11 of the GPR56 gene, leading to an E496K change in the C-terminus of the GPR56 protein. Two mutations – R565W and L640R – in the C-terminus domain of the protein have been reported to cause BFPP. But the E496K mutation has not been documented to play a role in BFPP.

We investigated the trafficking of the novel mutant protein in comparison with the wild-type and the two previously confirmed C-terminus mutant proteins to determine whether the E496K mutation compromises proper functions of the protein by misplacing the membrane protein. Specifically, HEK293T cells transiently transfected with the wild type, E496K, R565W, or L640R GPR56 gene were lyzed and western blotted with the probes detecting the N- or C-terminus. In addition, expression patterns of the protein in the cells were analyzed by immunohistochemistry and cell surface protein biotinylation.

The western blot of the cell lysate of the cells transfected with wild type GPR56 revealed two N-terminal fragments at 60kDa and 80kDa and one C-terminal fragment at 22kDa. The same results were obtained for L640R. However, only one N-terminal fragment at 60kDa and a faint C-terminus band at 22kDa were observed in the western blot of E490W and R565W. No discernible differences of the N- and C-terminus distribution patterns between the wild type and mutant proteins were observed. Western blot analysis of the biotinylated proteins detected the C-terminus at 22kDa in the wild-type and L640R proteins, but no C-terminus was detected in the E496K and R565W mutants.

These results indicate that the E496K mutant is trafficked differently from the wild-type protein and the novel mutant's C-terminus is not expressed on the cell surface. Such misplacement would certainly expect to compromise functions of the GPR56 protein, leading to BFPP.

**The Effect of HIV-Status on Social Factors, Access to Oral Health Care, and Oral Hygiene Practices in Port Elizabeth, South Africa**

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HIV is one of the most important health concerns in South Africa. Additionally, HIV status may be linked to factors such as language, employment, and socioeconomic status. Although, oral manifestations, orofacial pain, tooth loss, and loss of function affect 36% of South Africans, less than 10% of the population utilizes public oral health services. The high prevalence of oral health related problems (>43% of men and women) in the Eastern Cape, coupled with a low rate of dental visits demonstrate that there are barriers in access to care. However, studies have shown the general population to practice regular oral hygiene.

We aimed to understand the differences in oral health behaviors of HIV+ patients, compared to patients with HIV-negative status, being treated at the Port Elizabeth Health Complex (PEHC) in the Eastern Cape. HIV status may have a negative effect on these outcomes, such that there are differences between HIV+ and HIV- patients' social factors, access to oral health care, and oral hygiene practices. A total of 159 patients at five clinics and hospitals were surveyed, of those, 121 patients were HIV+ and 38 patients were HIV-. The tools used were a demographic survey, the Association of State and Territorial Dental Directors Access to Care Survey, and an oral hygiene practices survey and was given in English, Xhosa, and Afrikaans.

Preliminary findings showed unemployment rates to be 61.16% among HIV+ patients and 34.21% among HIV- patients and most patients to be non-English speaking (91.74% in HIV+ patients, 94.74% in HIV- patients). More patients with HIV (27.12%) said that there was a time when they couldn't get dental care (emergency or check-up) they needed, compared to HIV- patients (17.86%). Affordability was the most common barrier to access to oral health care, a finding nearly doubled in HIV+ patients (37.19%) compared to HIV- (18.42%). Other common barriers were pain that was tolerable, lack of medical aid, and long waiting times in the clinic. Most patients said they brushed at least twice a day in both groups (74.38% of HIV+, and 60.52% of HIV-). The majority of patients in both groups said they never flossed (65.83% of HIV+, 63.18% of HIV-) or used mouthwash (58.33% of HIV+, 44.74% of HIV-), which may be due differing cultural practices. T-tests will be done to show significance differences between the two groups and perhaps look at other variables that affect access to oral health care and/or oral hygiene practices.

**Change of Gingival Margin after Gingival Retraction in Different Biotypes and Tooth-types**

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In restorative dentistry, gingival retraction is necessary to expose the margin of the tooth preparation. Currently, the gingival cord placement technique is one of the most popular retraction methods, but the technique is often associated with gingival recession, a pathological erosion of the surrounding gum tissue. This recession, in turn, can subsequently lead to hypersensitivity, root caries, and aesthetic compromises. However, how gingival cord placement can lead to gingival recession remains unclear, and to our knowledge no comprehensive study has addressed this issue.

Gingival retraction technique is not standardized among dental care professionals, and factors such as gingival cord type and size, chemicals infused into the cords, and cord displacement time and force all contribute to variability. These specifications are often chosen at the clinician's discretion and preference. Therefore, identifying associations between gingival recession and one or more of these technique specifications may potentially identify optimal gingival retraction procedure guidelines.

Previous studies have shown that gingival recession is highly correlated with the shape of the anterior teeth and the individual's periodontal thickness: patients with tapered anterior teeth or thinly scalloped gingiva are more susceptible to gingival recession. We hypothesize that varying the gingival cord sizes will only affect the gingival margin of individuals with a thin periodontal biotype.

A total of 20 medically and periodontically healthy test subjects will be divided into two groups: thin periodontal biotype and thick periodontal biotype. Gingival margin changes in each test subject will be measured longitudinally for six months after cords of varying sizes are placed. Two methods for measuring gingival margin changes will be used: direct measurements using a periodontal probe and a novel photographic approach. The photographic approach was validated using 100 photographs of four different standard mouth casts taken with a Canon SLR camera. Surprisingly, our preliminary data suggest that the photographic approach is more accurate than the standard direct measurement method.

Once the gingival biotype and retraction cord size have been assessed for their association with permanent gingival recession, this study will potentially elucidate proper recommendations for gingival retraction technique.

**Characterization of 3-dimensional Mitral Valve Annulus Geometry in Patients with Ventricular Septal Defects**

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The normal mitral valve (MV) annulus is shaped like a 3D saddle with an annular-height-to-commissural-width-ratio (AHCWR) conserved at around 20%, the conformation yielding lowest leaflet stress. However, in children with Ventricular Septal Defects (VSDs), the left ventricle (LV) dilates and ultimately hypertrophies to compensate for the increased hemodynamic load, deforming the MV. Knowing the exact 3D geometric changes in abnormal MVs can inform clinical judgment ("at what stage of deformity does a child develop mitral regurgitation?") and surgical interventions ("when and how should we best fix a child's MV?"). In this study, we hypothesize that the MV saddle is flattened in VSD children.

Study candidates were selected from the complete Children's Hospital cardiac surgical database. The entry criteria were children who (1) were 0-7 years-old at time of surgery, (2) underwent full surgical closure of VSDs, (3) had surgery after 2002 (when 3D echocardiograms (3D-Echo) became available), and (4) had undergone 3D-Echo before surgery. The elimination criteria were (1) no other associated pathologies besides VSD, (2) visualization of the entire MV in the 3D-Echo, and (3) clear long-axis view of MV leaflets. We converted the 3D-Echo images into a 3D numerical graphical plot using automated segmentation algorithms, and quantified the MV saddle geometry using planarity (average distance of points from a best-fit plane divided by the annular height) and AHCWR as primary and secondary end-points respectively.

We found 16 patients fitting our entry criteria and only 1 after elimination. The patient was an 8-year-old female who weighed 23.4 kg at surgery. She was compared against a de-identified healthy adult control. The ave_dist/AHs were 22.72% (normal) and 22.34% (VSD), and the AHCWRs were 16.17% (normal) and 10.43% (VSD). The 2D projection areas were 7.3179 $cm^2$ (normal) and 5.2397 $cm^2$ (VSD).

To summarize, these measurements are in line with our hypothesis that the annulus flattens (lower planarity and AHCWR) in children with VSD, according to a sample size of 1. Our methods of analysis are feasible for a large-scale study, and we can further investigate our hypothesis and show statistical significance by prospectively collecting more specific data (conforming to our eligibility criteria) from a larger population. For future studies, this methodology can also be used as a platform to answer further questions: (1) Is this geometric change permanent or is it reversed after surgery? (2) How long after surgery is the shape re-established? (3) How does the MV change in other pathologies?

**The effects of different mouthrinses on the color stability of denture base polymers**

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Color stability is one of the most important clinical properties for all dental materials since color changes can be an intelligible indicator of aging or deterioration. Discolorations of acrylic resins used in fabricating denture bases result in esthetic and functional problems including patient dissatisfaction and additional expense for replacement. Discolorations are particularly problematic when dental restorations are subjected to prolonged exposure to colorants during lengthy treatment.

Extrinsic staining of teeth has emerged as an unpleasant effect of some common brands of mouthrinses. However because mouthwash is an effective method for controlling caries, gingivitis, and bad breathe, it is commonly used by denture wearers. Furthermore mouthwashes may contain ingredients that control xerostomia or dry mouth, another common side effect of dentures. Although studies have shown that frequent use of mouthrinse may exert detrimental effects on oral and dental tissues, studies about their effects on esthetic restorative denture materials are limited.

This study aims to evaluate the color stability of heat-polymerized polymethyl methacrylate (PMMA) acrylic denture bases (Lucitone 199, Diamond D, and Fricke HI-I) submerged in three different types of mouthwash (Listerine, Biotene, and Listerine without alcohol). The polymer used for most denture base acrylic resins consists of polymethyl methacrylate (PMMA) because of its reliance on simple processing equipment. Even so, there are significant differences in chemistry among denture materials based on PMMA chemistry. These denture base materials should have good esthetics such that they match the natural appearance of gingival soft tissue. Thus we hypothesize that there will not be a significant color change in the denture bases even after lengthy mouthwash treatment.

Seventy-five denture base pieces (twenty-five pieces for each different denture base acrylic) were fabricated into 20mm x 20mm x 2mm rectangular boxes. Each piece was placed into individual 50 milliliter conical tubes. The tubes were then filled with a 15 milliliter solution of mouthwash. Each denture acrylic had five samples submerged in each mouthwash for seven days. This is the equivalent of using mouthwash for 30 seconds, twice a day for 27.6 years. Color changes (delta E) were measured every other day using a Crystaleye spectrophotometer. Data was analyzed by ANOVA (alpha = 0.05). Results are pending.

**A prospective study of IL-6, TNF-αR2, and CRP levels and the risk of colorectal cancer in men**

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The links between carcinogenesis and inflammation originated as early as Rudolf Virchow's observation in 1863 that cancerous tissues contained immune system cells and have been substantially corroborated by increasing knowledge from the last twenty years of the inflammatory microenvironments of malignant tissues. A key in the development of colorectal cancer, which involves more than 150,000 new cases and 55,000 deaths each year, is inflammation.

While the mechanisms and pathways by which inflammation can lead to cancer are still being elucidated, the goal of our project was to determine whether there is an association between blood levels of three inflammatory biomarkers (specifically interleukin-6, C - reactive protein, and tumor necrosis factor alpha receptor 2) and the risk for colorectal cancer. Very few studies up to this point have simultaneously investigated the role of inflammatory biomarkers as predictors of colorectal cancer development.

Our study was a nested prospective case-control study within the Physicians' Health Study (PHS 1), a double-blind, randomized, and placebo-controlled trial of aspirin and β-carotene for the prevention of cardiovascular disease and cancer that was started in 1982 through Channing Laboratory.

While our original hypothesis stated that men with higher plasma levels of CRP, IL-6, and TNF-aR2 would have higher risks for developing colorectal cancer than those with lower levels, preliminary data has revealed that of the three inflammatory biomarkers, only IL-6 may have statistically significant results that support the hypothesis after all relevant confounding variables have been controlled for. When the higher quintiles of biomarker serum levels are compared to the lower (and thus baseline) quintiles, there seems to be a threshold effect. As levels for three biomarkers increase, the risk for developing cancer also increases to a certain point and then levels off. However, most relative risks values, though above 1.0, had confidence intervals that included 1.0 in its range. The study was analyzed with the SAS 9.1 statistical package by the SAS Institute.

Hopefully, statistically significant results from our study could have important implications for both prevention and treatment of colorectal cancer in the future. Even if the biomarkers are poor predictors of colorectal cancer risk, the study will at least further the understanding of the disease, raise interesting questions, and illuminate future paths for research.

**Massachusetts Health Reform and Disparities in Coverage, Access and Health Status**

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Context: Massachusetts health reform legislation has achieved near-universal insurance coverage since its enactment in 2006, yet less is known about the legislation's effect on access to care and health status, particularly for disadvantaged populations.

Objective: To examine whether this legislation was associated with changes in health coverage, financial barriers to care, access to care, after accounting for temporal trends in the region. Since racial/ ethnic minorities and low-income groups are over-represented among the uninsured, we also explored the effects of health reform on disparities in coverage, access, and health status.

Design, Setting, and Patients: Data from the 2006-2008 Behavioral Risk Factor Surveillance Survey for adults aged 18-64. To control for trends unrelated to reform, we used a differences-in-differences method to compare adults in Massachusetts (n=36,505) to those in all other New England states (n=63,263).

Main Outcome Measures: Self-report of health insurance coverage, inability to obtain care due to cost, access to a personal doctor, and self-reported health status.

Results: Overall, health insurance coverage in Massachusetts rose from 94.7% in 2006 to 97.7% in 2008, whereas coverage in New England remained around 92% ($p < 0.001$ for difference-in-difference). While cost-related barriers were reduced in Massachusetts, there were no improvements in access to a personal doctor, or health status. Although there were improvements in coverage and cost-related barriers for some disadvantaged groups relative to trends in New England, there was no narrowing of disparities in large part because of comparable or larger improvements among whites and the non-poor.

Conclusions: During the initial years following health reform in Massachusetts, access to a personal physician and health status did not improve despite improvements in coverage and financial barriers to care. Achieving equity in health and healthcare may require additional focused intervention.

**Characterizing the Role of Cornea Resident Immune Cells in the Adenovirus 37 Keratitis Mouse Model**

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Epidemic keratoconjunctivitis (EKC), a common ocular adenovirus syndrome induced by human adenoviruses Ad8, Ad19, and Ad37, is characterized by inflammation of the subepithelial cornea. The role of cornea resident immune cells in this inflammatory response has not been well characterized due to lack of a suitable animal model for adenoviral ocular infection.

Recently, our lab developed a keratitis mouse model with successful infection of the cornea stroma using microinjection of Ad37. With this model, we aimed to characterize the responses of resident bone marrow-derived cells to Ad37 cornea infection by noting changes in distribution, morphology, and maturation.

Ad37 virus or fluorescently-labeled Ad37 (Cy3 dye) was injected into the cornea stroma of mice. Control corneas were untouched or injected with PBS. Mice were sacrificed at 30 min, 4h, 16h, and 24h post-injection. Corneas were dissected and fixed in 4% paraformaldehyde. Corneas were stained with fluorescent antibodies specific for cell surface markers CD11b (leukocyte), CD11c (dendritic cell), CD86 (maturation), and F4/80 (macrophage). Corneas were whole-mounted on glass slides and examined under confocal microscope. To validate confocal data, corneas were subjected to flow cytometry using CD86 and CD11c fluorescent antibodies at 16h.

Untouched corneas revealed large populations of round and spindle-shaped CD11b+ and CD11c+ cells in the anterior stroma; smaller populations were observed in the posterior. The density of CD11b+ and CD11c+ cells decreased from the periphery towards the center. Star-shaped CD86+ cells were observed only in the periphery, suggesting the localization of immature dendritic cells or macrophages (CD11b+CD86-) to the center. At 30 min post-injection, Cy3-Ad37 was visible within CD11b+/-, CD11c+/-, and CD86+/- cells, but not F4/80+. As early as 4h, upregulation of CD86+ cells was observed in the periphery indicating the maturation of resident immune cells. These CD86+ cells also appeared larger with a greater number of extensions. No notable changes were observed for CD11b. At 16h, CD86+ cells expanded into paracentral regions. Most notably, a significant influx of small, multi-lobed, CD11b+CD11c-CD86- leukocytes (presumably neutrophils) were observed throughout the stroma. The observation of CD86+ upregulation and neutrophil infiltration was validated by flow cytometry.

These data suggest that the acute maturation of resident immune cells in the cornea may play an important role in the downstream inflammatory events responsible for the clinical presentation of EKC. Moreover, Ad37 appears capable of infecting certain resident bone marrow-derived cornea cells using a receptor or molecular pathway not expressed on F4/80+ macrophages.

**The Lateralization of Trigeminal Pain Processing in the Brain**

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The social cost of pain is ubiquitous and staggering, thus further understanding of pain processing in the central nervous system could lead to more effective treatment and rehabilitation of the millions of patients with chronic and acute pain. One often overlooked aspect is the asymmetry of pain, which has potential clinical significance for understanding the underlying neurobiology and co-morbid changes. Experiments in healthy subjects have demonstrated higher thresholds to induced pain on the right side. Chronic pain, particularly in depressed patients, has been shown to be more often on the left side.

This project attempts to find biological support for and insight into lateralization of nociception (the neural encoding of pain) in the trigeminal system through functional magnetic resonance imaging (fMRI). Five right-handed subjects (3 male: 2 females, mean 22.6 yr, SD 1.29) have completed the study thus far. We applied heat stimuli at one of three different stimulus levels (44?C, 46?C, and 48?C) to the cheeks, innervated by the maxillary branch of the trigeminal nerve. For comparison, the thumbs were also stimulated (48?C) separate from the face. Each heat stimulus was applied to the left, right, or both sides of the body in a randomized fashion. fMRI scans were performed using a 3 Tesla scanner to identify regions activated. After each scan, subjects rated each stimulus?s pain intensity and unpleasantness on a computerized visual analog scale (VAS). The amount of activation on the left and right sides will be compared.

Previous positron emission tomography (PET) and fMRI studies have explored the possibility of right lateralized activation during pain perception. These regions will be evaluated in terms of the lateralization of nociceptive processing within the trigeminal system, in contrast to previous studies that have evaluated the upper limb. Given that pain ascends through different pathways in the case of the face (trigeminothalamic tract) and body (spinothalamic tract), similar activation of the cortex cannot be assumed.

Furthermore, unilateral stimulation has yet to be compared with bilateral stimulation to evaluate hemispheric dominance of pain-related activation. Through greater understanding of the role of brain lateralization in pain processing, we hope that better treatments for chronic pain will be formulated. Prelimary data analysis indicates greater overall cortical activation with right-sided stimuli compared to left-sided, particularly in contralateral sensorimotor cortex. Also, the amygdala and insula show greater right hemisphere activation during bilateral stimulation.

**Dental Implant Survival in Patients with Periodontal Disease**

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Periodontal disease is a leading cause of tooth loss in adults. Dental implants are a widely accepted therapy for tooth replacement in healthy adults. Although the use of dental implants to replace missing teeth in patients with periodontal disease has become routine in some practices, few longitudinal studies have evaluated the long-term survival of dental implants in periodontally-compromised patients. The purpose of the present retrospective study was to compare the survival and success of solid screw implants in patients with varying degrees of periodontal disease.

One hundred seventy-two patients (mean age: 45+/- 11 years) (55% women, 45% men) with chronic periodontal disease were recruited for the study from a cohort of 392 patients who completed cause-specific treatment which could include scaling and root planing in the periodontal program at the University of Berne, Switzerland between 1978-2002. To be eligible for the study, patients were required to have completed baseline and follow-up evaluations (including two sets of radiographs and periodontal examinations). As determined from radiographs, Level 1 disease is defined as >/= 3mm proximal attachment loss in 2 or more teeth; Level 2 =/> 5mm attachment loss in >/=30% of teeth. The mean duration from the completion of treatment to re-evaluation was 11.3 +/- 4.9 years. Kaplan-Meier failure function was used to analyze time to implant failure. Chi squared test was used to test for an association between peri-implantitis and the practice setting patients used for routine care following treatment.

One hundred sixty-five dental implants were placed in 172 patients. Four implants were lost during the initial 2.5 years of follow-up; and two additional implants were lost during the subsequent 7.5-12.5 years of follow-up prior to re-evaluation. The cumulative incidence of implant survival was (166/172) = 96% after up to 15 yrs follow-up. The mean failure rate was .0046 implants per year [95% CI] [.002-.01]. At reevaluation, 22% of implants showed evidence of peri-implantitis defined as > 5mm probing depth and bleeding on probing. Patients followed at the university after completing therapy were somewhat less likely to experience peri-implantitis (17.6%) compared to patients followed in a private office (30.2%) although the difference was not statistically significant (p =.057). Implant loss occurred in 4/40 of the dental implants affected by peri-implantitis.

In summary, these data suggest long-term dental implant success rate exceeding 95% in patients with chronic advanced periodontitis. Peri-implantitis affected implant survival in ~10% of affected implants.

**A Retrospective Radiographic Study on Dental Implant Survival Rates for Periodontal Residents at HSDM**

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Dental implants are a restorative device used as a long-term option to replace teeth that have been lost. The implants are surgically inserted into the bone of the jaw, and with proper oral care can last permanently. However, implant failure is an event that does occasionally arise. The purpose of this study is to determine whether the success rate of dental implants placed by periodontology residents at the Harvard School of Dental Medicine (HSDM) meets, exceeds, or falls short of the published standards of success.

Through the selection of 156 patients who received implants by a HSDM periodontology resident during the 2003-2006 calendar years, analysis of the patients' charts and radiographs took place to determine whether each implant can be considered a success or failure according to Albrektsson's criteria of implant success and the aesthetic approval of the patient. Specific health criteria of the patient, the level of experience of the periodontal resident, and the materials used in the placement of the implant were recorded to determine through multivariate analysis if any of these factors play a role in the success or failure of an implant.

The hypothesis of this study is that the success rates of implants placed by periodontology residents at the Harvard School of Dental Medicine exceed the published levels of expected success. If the HSDM periodontology success rate exceeds the published standards, then the curriculum and training model utilized at Harvard can be offered as a template for other dental programs to follow. Should no significant difference between HSDM and published success rates exist, then new innovations can take place to ensure that Harvard will continue to meet and eventually exceed the accepted standards. If the HSDM success rate falls below the published guidelines, then further research can examine possible interventions that may be needed to develop new implant placement techniques as well as identify previously undefined risk factors with the goal of improving implant success rates of the periodontology program.

The results of this study are significant, as the conclusions reached can have a meaningful impact on future periodontology training methods at the Harvard School of Dental Medicine. With data collection completed during the month of August 2009, analysis of the data has yet to be completed, and conclusions remain to be drawn. Statistical analysis is underway, and examination of radiographs is set to begin.