UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS

23RD ANNUAL STUDENT RESEARCH FORUM

AND

STUDENT RESEARCH AWARDS CONVOCATION

GRADUATE SCHOOL

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JANUARY 21, 2009
ANSCHUTZ MEDICAL CAMPUS
RESEARCH COMPLEX
2ND FLOOR ATRIUM

23rd ANNUAL
UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS
STUDENT RESEARCH FORUM

Wednesday, January 21, 2009

Poster Session
1:00-3:30 pm

Awards Convocation
4:00 pm
RC2 Room 2100

ANSCHUTZ MEDICAL CAMPUS
RESEARCH COMPLEX
2ND FLOOR ATRIUM

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ABSTRACTS

ADAMS, SA

MEDICAL SCRENNING EXAM STUDY: AN OPPORTUNITY TO MEASURE HEALTH OUTCOMES AND PATIENT SATISFACTION. SA Adams, (MD, SOM), M Valley, and B Evans, School of Medicine, University of Colorado, Denver, CO.

Emergency Departments (EDs) have become the largest providers of non-emergency care to persons who are insured or uninsured. In response, several hospitals have implemented medical screening exams (MSE) to detect and refer patients seeking ED care for non-emergent problems. We studied health outcomes and satisfaction among non-emergent patients who were screened out of the ED.

A telephone survey was completed by a convenience sample of patients who were screened out of an urban, academic ED during a 4-month period. Eligible participants included non-urgent patients with normal vital signs who presented to the ED with one of five pre-selected chief complaints (toothache, rash or skin problem, back pain, cough, cold or bronchitis, or extremity problem). Participants were called 4 to 6 days after being discharged from the ED. Questions addressed the symptom improvement or resolution, satisfaction with the screening process, ability of screened out patients to contact a primary care provider or clinic, and ED recidivism.

Of 320 known eligible patients, 162 (51%) were asked to participate in the study, and 57 consented (18%). Of those who consented, 46 (14% of the total eligible population) completed the questionnaire. Respondents' age ranged from 20-64 years old, 59% were men, and 63% were uninsured. The study showed that 43% (95%CI 29-58%) reported improvement of their symptoms, 39% (95%CI 25-53%)were unchanged, and 17% (95%CI 6-28%) had worsened. Regarding their ED visit, 50% (95%CI, 36-64) were satisfied, 24% (95%CI 12-36%) were neutral and 26% (95%CI 13-39) were unsatisfied. In addition, 41% (95%CI 27-56%) accessed a community health provider, and 6% (95%CI 0-14%) returned to an ED within 6 days for a different complaint.

Although the data suggests half the participants who where screened out of the ED had symptom resolution and were able to access community health care, the study's low enrollment rate severely limited our ability to generalize to the larger population of individuals who are screened out of EDs. This demonstrates the difficulty in studying a patient population who is reluctant to consent to follow-up. The safety and efficacy of MSE programs have yet to be assessed successfully. Other methods of accessing this population and incentives for participation should be considered.

ADWAN, TS

THE ROLE OF A C-TERMINAL PROLINE –RICH MOTIF IN TYROSINE PHOSPHORYLATION AND NUCLEAR IMPORT OF PROTEIN KINASE C DELTA. TS Adwan, (Ph.D., GS), SD Lennox, JG Parvani and ME Reyland. Department of Craniofacial Biology, University of Colorado Denver and AMC, Denver, CO

Inappropriate activation or inactivation of apoptosis is a hallmark of a myriad of disorders including cancer and auto-immunity. Our lab has demonstrated an essential role for protein kinase C delta (PKCδ), a serine/threonine kinase, in genotoxin-induced apoptosis. We have shown that nuclear localization of PKCδ is required for its pro-apoptotic function, and requires both a C-terminal Nuclear Localization Siganl (NLS) and phosphorylation at Y64 and Y155, in the regulatory domain of PKCδ (δRD). This suggests that tyrosine phosphorylation in response to apoptotic inducers, facilitates NLS dependent nuclear import of PKCδ. Sequence analysis has revealed a single putative SH3 domain ligand (PxxP) which overlaps the NLS. We hypothesize that in response to apoptotic stimuli, a SH3 domain containing tyrosine kinase (TK) binds to the PxxP site and phosphorylates Y64 and Y155 in the δRD, which results in a conformational change that allows access of nuclear import machinery to the NLS and nuclear import of PKCδ. To determine if PKCδ interacts with SH3 domains of known proteins, we screened SH3 domain arrays for binding to His-PKCδ. We show that PKCδ interacts with the SH3 domains of Lck, Lyn, Yes1, Fyn, PLCγ, Tec, Src, Abl, Hck and Itk. Using GST-SH3 fusion peptides of Lyn, Fyn, Hck, c-Src, Abl and PLCγ we were able to verify the binding of PKCδ to these SH3 domains in a GST pull-down assay. In addition, we show that endogenous PKCδ co-immunoprecipitates with Src, c-Abl and Lyn from Parotid C5 cells. To determine the role of the PxxP motif in nuclear import of PKCδ, we generated GFP tagged Proline (P) to Alanine (A) mutants of the PxxP motif and analyzed their nuclear localization by fluorescence microscopy. Our data indicate that the AxxP and the PxxA mutants accumulate in the nucleus even in the absence of etoposide treatment.

Interestingly, mutation of both of the prolines to alanines as in the AxxA mutant results in a reduced nuclear accumulation of PKCδ, presumably due to loss of NLS function, since the PxxP motif overlaps the NLS. Analysis of Y64 and Y155 phosphorylation in these mutants suggest that the PxxP motif plays a role in tyrosine phosphorylation at these residues. In addition, we show that the AxxA mutant have reduced phosphorylation at T505 in the activation loop, S642 in the turn motif, and S662 in the hydrophobic motif of PKCδ. Consistent with loss of phosphorylation at these sites, the AxxA mutation results in loss of the kinase activity of PKCδ. Our studies provide insight into the mechanism regulating the nuclear import of PKCδ which in turn regulates the pro-apoptotic function of this ubiquitous kinase.

ALLINGTON, TM

TRANSFORMING GROWTH FACTOR-BETA SUPPRESSES BREAST CANCER PROGRESSION THROUGH ACTIVATION OF ABL KINASE. TM Allington, (Ph.D., GS), AJ Galliher-Beckley, and WP Schiemann, Department of Pharmacology, University of Colorado, Aurora, CO.

In normal mammary tissues, the cytokine transforming growth factor-β (TGF-β) acts to suppress tumorigenesis and block mammary epithelial cell (MEC) motility, invasion, and metastasis. However, as premalignant MECs progress, they subvert the tumor suppressive effects of TGF-β and convert this cytokine to a promoter of invasion and metastasis, a process termed the "TGF-β Paradox." Localized breast cancer has a 5-year survival rate of 98%; however, if the disease metastasizes to distant areas of the body, the 5-year survival rate plummets to 26%. Hence, preventing TGF-β stimulation of breast cancer invasion and metastasis will significantly improve the survival rates of patients afflicted with this deadly disease.

TGF-β regulates normal MEC morphology and their interactions with the microenvironment by altering expression of integrins and matrix-metalloproteinases (MMPs), and by modulating the stability of adherens junctions. Similar to TGF-β, activation of the protein tyrosine kinase Abl also plays an essential role in regulating epithelial tissue development, and in organizing their cytoskeletal architecture. We hypothesized Abl as an important mediator of tumor suppression by TGF-β, doing so through its ability to maintain normal MEC tissue architecture. We tested this hypothesis by manipulating Abl expression and function in normal and malignant MECs, which showed that Abl does indeed function within the TGF-□ signaling system to *(i)* prevent TGF-□ stimulation of EMT and invasion in MECs by stabilizing their adherens junctions, and by reducing their synthesis and secretion of MMPs; and *(ii)* promote TGF-□ stimulation of normal MEC acini architectures and morphologies in 3D-cultures. More importantly, constitutively-active Abl dramatically reduced the growth and pulmonary metastasis of 4T1 tumors produced in mice. Significantly, these findings highlight an important function for Abl in balancing the tumor suppressing and promoting activities of TGF-□ in normal and malignant MECs. Moreover, our results offer new insights into why Gleevec monotherapy promotes disease progression in breast cancer patients, thereby underscoring the need to enhance our understanding of how Abl suppresses breast cancer development and progression.

[Susan G. Komen BCTR0706967 to W.P.S., DOD Predoctoral Fellowship BC083323 to T.A.]

ATWOOD, BF

EVALUATION of PULSE OXIMETRY and the PULMONARY EMBOLISM SEVERITY INDEX for RISK ASSESSMENT in PULMONARY EMBOLISM PATIENTS in the EMERGENCY DEPARTMENT

Benjamin Atwood(M.D., SOM), Kristen Nordenholz, MD, Jordan Ryan and Kennon Heard, MD.
UC Denver, School of Medicine, Department of Surgery, Division of Emergency Medicine

Background: Risk stratification of patients diagnosed with pulmonary embolism (PE) is important to determine appropriate medical management. **Objectives:** We attempted to assess the performance of 2 previously published risk stratification tools in identifying in-hospital morbidity and mortality in Emergency Department (ED) patients diagnosed with PE in Denver. We assessed a specific pulse oximetry cutoff (1) and the Pulmonary Embolism Severity Index (PESI) (2,3). **Methods:** Electronic medical records of all patients diagnosed with PE in the ED from June 2004 to June 2008 were retrospectively searched by trained researchers for triage vital signs, prior co-morbidities, adverse short term outcomes requiring hospital interventions (defined as respiratory failure, hypotension requiring pressors, and hemodynamic impairment requiring thrombolytics) and lastly death. We applied the risk stratifying models to the ED PE patients to classify them as high or low risk for morbidity or

mortality. The performance of these classifications was then assessed using actual patient complications and outcomes. This study was IRB approved. **Results:** 168 PE patients were identified from the ED. The overall rate of adverse outcomes was 7.1% (12/168), including a 3.0 % mortality rate. A pulse oximetry cutoff of 92.5% saturation would have classified 64.9% (109/168) patients as low risk, of which 3.7% (4/109) had an adverse outcome, and 35.1% (59 /168) patients as high risk, of which 13.6% (8/59) had an adverse outcome. The PESI would have classified 54.2% (91/168) patients as low risk, of which 2.2% (2/91) required hospitalization procedures and 0 died. The remaining 45.8% (77/168) were classified as high risk, with an adverse outcome rate of 13.0% (10/77). We found a PESI cutoff of II to have a sensitivity of 83% (95%CI= 52-98%), specificity of 57% (49-65%), negative predictive value of 98% (92-100%). A Pulse oximetry cutoff of 92.5% oxygen saturation had a sensitivity of 64% (31-89%) and specificity of 67% (59-74%) for adverse events. **Conclusion:** Our data indicates the Pulmonary Embolism Severity Index is potentially a reliable and practical identifier of low-risk patients with PE (who are potential candidates for less costly, non- monitored therapy.)

BADTKE, MM

PROGESTERONE RECEPTORS ATTENTUATE TAXANE-INDUCED CELL DEATH IN BREAST CANCER CELLS BY ALTERING SPINDLE ASSEMBLY CHECKPOINT GENES

MM Badtke, (Ph.D., GS), P Jambal, WW Dye, KB Horwitz and BM Jacobsen

Department of Medicine, University of Colorado Denver – Anschutz Medical Campus, Aurora, CO, USA 80045

Introduction: The taxanes, paclitaxel (Px) and docetaxel (Dx), are among the most effective treatments for advanced breast cancer. Taxanes regulate genes in many cancers, however, little is known about genes regulated by taxanes in breast cancers. Also, while taxanes are clearly effective for advanced breast cancers, it is unclear whether response to taxanes is influenced by presence of estrogen (ER) or progesterone (PR) receptors in tumors. Some studies indicate that taxanes are less effective in ER+ than in ER- disease. Others indicate that hormone receptors do not affect taxane responsiveness. In our ER+, PR+ breast cancer models, presence of unliganded PRs attenuates taxane-induced apoptosis.

Study Objectives: To 1) to identify the genes regulated by taxanes in breast cancers, 2) define molecular mechanisms by which PR suppress tumor response to taxanes.

Methods: To determine mechanisms of PR mediated resistance to taxane-induced apoptosis, an Affymetrix U133 plus 2 microarray was performed in ER+ breast cancer cells engineered to inducibly express PR. Cells lacking or expressing PR for 48h, were treated with vehicle, Px or Dx for 24h. Statistical analyses with Partek Statistical Suite and pathway analysis with Genespring and Ingenuity were performed.

Results: While Px and Dx regulate many genes in common, some gene subsets are uniquely regulated by each taxane. There is also a complex relationship between taxane and PR regulated genes with some gene subsets regulated in the same direction, and others in the opposite direction, by taxanes *vs.* PR. A key gene pathway regulated by both taxanes controls apoptosis and this pathway is modified by PRs. Additionally, unliganded PRs regulate multiple cell cycle and spindle assembly checkpoint (SAC) genes that amplify or oppose the effects of taxanes. As a result, the simple presence of PRs, even without progestins, modifies the ability of both Px and Dx to regulate gene expression. For example, taxanes increase SAC genes, while PRs decrease them. Moreover, PRs increase taxane-induced multinucleation; an anomaly linked to apoptosis resistance.

Conclusions: In summary, we show that PRs attenuate taxane-induced apoptosis by counter-regulating key taxane controlled genes and by increasing multinucleation. We propose that combination therapy with antiprogestins may improve taxane responsiveness of breast cancers.

BEAUDOIN, ME

TRANSLATIONAL REGULATION OF AMYLOID PRECURSOR PROTEIN. ME Beaudoin, (Ph.D., GS), VJ Poirel, and LA Krushel, Neuroscience Program, School of Medicine, University of Colorado, Denver, CO.

Increased expression of amyloid precursor protein (APP) and its cleavage by beta- and gamma-secretases into an insoluble 40-42 amino acid Amyloid Beta (A-beta) peptide is thought to contribute to Alzheimers Disease (AD). Thus, regulating APP protein expression levels may be one approach to ameliorate the disease. APP mRNA has previously been shown to be associated with polysomes even during mitosis, when cap-dependent translation is largely inhibited (Qin and Sarnow, JBC 279:13721-8).

We previously confirmed that the APP 5' leader contains an Internal Ribosomal Entry Site (IRES) whose activity cannot be attributed to cryptic promoter activity or cryptic splicing. We showed this using dicistronic and monocistronic luciferase reporter assays containing the APP 5' leader intercistronically.

We now report that by knocking down cap-dependent translation for 72 hours using siRNA against eukaryotic initiation factor 4E (eIF4E) in rat neural C6 cells, basal APP expression is not affected, nor is endogenous APP expression affected by inhibition of the mTOR pathway using rapamycin. Cycloheximide treatment also shows that the endogenous protein half-life of full length APP is ~30 minutes.

Recently, we also identified La/SSB as a potential IRES transacting factor (ITAF) for the APP IRES, using a candidate-based approach in which we examined the 5' leader sequence for known ITAF protein binding sites. By filter-binding analysis with recombinant La/SSB, we found La/SSB to bind the APP 5' leader with an affinity of ~33 nM. Furthermore, a ~ 80% knockdown of endogenous La/SSB in C6 cells led to a 50% decrease in endogenous APP synthesis. Also, APP IRES activity was decreased by ~30 % as shown using dicistronic luciferase reporter constructs. Taken together, these data suggest that La/SSB is necessary but not sufficient for endogenous APP synthesis and APP IRES activity.

The present research is informative for the AD research community, as a role for La/SSB protein in the synthesis of APP has not previously been characterized, and may present a potential target for drug development in the future. Furthermore, this research may lead to correlational bioinformatics analysis of La/SSB protein expression in AD patients of varying ages of onset.

BELAY, B

TRAUMA EXPOSURE AND COPING MECHANISM IN ERITREAN REFUGEES: AN EXPLORATORY STUDY B Belay, (M.D., MS), D Savin, Department of Psychiatry, University of Colorado, Denver, CO.

Purpose of Study: East Africa is a region of tremendous political and social upheaval. Refugees from this region have resettled in many countries, including the United States. Despite recognition of the enormous mental health needs in East African refugees, there is little literature formally assessing the degree of trauma exposure or the types of coping strategies employed by this group. This lack of knowledge may be partially attributable to the lack of culturally valid assessment tools. The purpose of this pilot-study is to identify traumatic life events in Eritrean refugees and explore what adaptations of existing measures are necessary to identify coping strategies employed by this population.

Methods Used: Open-ended interviews were conducted with 10 Eritrean refugees and asylees between the ages of 23 and 47 who migrated to the United States after 1998 (7 Males and 3 females). The Horn of Africa Needs Assessment was used by integrating questions from the Ways of Coping Checklist (WCC), Coping Orientation to Problems Experienced (COPE) and the Connor-Davidson Resilience Scale (CDRS). Additional questions included in the interview reflected domains pertinent to the Eritrean history and traditions projected to give insights on culturally relevant coping strategies. Resulting transcription of the interviews were analyzed and types of trauma and coping strategies categorized.

Summary of Results: Types of traumatic stressors identified included combat-related trauma, physical torture, detention, sexual harassment, natural disasters and culture shock. Identified coping strategies included faith and religion, family and community support, positive reinterpretation, use of herbs and alcohol as well as various spiritual practices deeply rooted in the Eritrean culture.

Conclusions: Trauma exposure was common and severe in this population. General categories of coping mechanisms identified in Eritrean refugees were similar to coping strategies recognized in other regions of the world. The use of specific herbs, traditional ceremonies and certain spiritual rituals were unique to this population. Future studies or clinical work with this or similar populations may benefit by integration of these newly identified strategies.

BERENZ, AJ

UNDERSTANDING THE ROLE OF B CELLS IN HUMAN DEMYELINATING DISEASES. AJ Berenz (MD, SOM), GP Owens, JL Bennett. Department of Neurology, University of Colorado, Denver, CO.

The pathogenesis of the human CNS inflammatory demyelinating diseases multiple sclerosis (MS) and neuromyelitis optica (NMO) are poorly understood. In MS, the persistence of CSF intrathecal IgG synthesis, oligoclonal bands and B cell clonal expansion are hallmarks of disease suggesting a possible role for antibodies (Ab)

in disease pathogenesis. This view is supported by lesion pathology and the recent efficacy of the anti-B cell drug, rituximab in the treatment of relapsing-remitting MS. Although CSF oligoclonal bands are not observed in all NMO patients, serum IgG binds the aquaporin-4 (AQP-4) water channel of astrocytes that are abundant in the perivessel and subpial areas of the brain and screening of NMO-IgG for AQP-4 immunoreactivity is now a diagnostic criterion for disease. To study the role of CNS antibodies in demyelinating disease, FACS sorting and single cell PCR have been used to identify and analyze clonally expanded plasma cell populations recovered from MS and NMO CSF. A distinct benefit of single-cell PCR is the ability to generate recombinant antibodies (rAbs) that duplicate the *in vivo* specificities of plasma cell clones. To develop an immunoassay for the analysis of NMO rAbs, the Aquaporin-4 (AQP-4) gene was amplified from fetal astrocyte cells and cloned into the PCEP-4 expression vector. Additionally, MS CSF-derived bivalent human IgG1 rAbs were produced in mammalian tissue culture cells and assayed by Western blotting for binding to purified MS and normal white matter proteins and to proteins resolved from multiple active MS lesions. A total of 19 rAbs generated from 2 different MS patients were assayed for binding to purified proteins; however, none of the rAbs demonstrated binding to either MS or normal white matter myelin. Additionally, none of the screened rAbs bound with sufficient affinity to proteins from resolved active MS lesions or to matched control tissue. Our data shows that rAbs generated from single MS CSF plasma cells do not bind to purified myelin proteins and that other cryptic autoantigens or foreign pathogens should be considered as antigenic targets.

BISHOP, J

A ROLE FOR HAND2 IN CELL SURVIVAL DURING FACIAL DEVELOPMENT. Jonathan Bishop (DDS, SODM), Katherine Kuhn, Crystal Woods and David E. Clouthier, Department of Craniofacial Biology, School of Dental Medicine, University of Colorado Denver, Aurora, CO.

The development of craniofacial bone and cartilage is a unique aspect of skeletogenesis. In contrast to the mesodermal origin of bone and cartilage throughout the body and skull vault, craniofacial structures are derived from cranial neural crest cells (NCCs). These cells reside in pharyngeal arches, transient structures from which craniofacial structures arise. One factor directing NCC development in the first pharyngeal arch is the transcription factor Hand2. Targeted inactivation of *Hand2* in mice results in almost complete NCC apoptosis (cell death) within the pharyngeal arches by embryonic day (E)10.0, though this finding has resulted in a long-standing controversy, as cell death could either be due to a direct requirement for Hand2 in NCC survival or result from decreased embryo viability (*Hand2*-null embryos die by E10.5 from vascular failure). **Objective:** To determine whether loss of *Hand2* specifically in NCCs results in wide spread apoptosis and changes in proliferation within the pharyngeal arches of mouse embryos. **Methods:** To address the role of Hand2 in NCC survival, we used Cre-*lox*P technology to create *Hand2*$^{fl/fl}$*;Wnt1-Cre* mouse embryos, in which *Hand2* is inactivated specifically in NCCs. Embryos were then collected at E10.5, E11.5 and E12.5, embedded in paraffin and serially sectioned. Matching consecutive sections were then examined for apoptosis (TUNEL analysis), proliferation (EdU incorporation) and general histology (hematoxylin and eosin staining). **Results:** We find that apoptosis within the mandibular arch of *Hand2* mutants is elevated 4 fold at E10.5, with regional elevation at both E11.5 and E12.5. In contrast, cell proliferation is less affected. **Conclusion:** These findings illustrate that loss of Hand2 does result in a moderate increase in arch apoptosis and suggests that absence of Hand2 leads to incorrect patterning and hence cell death. However, Hand2 is not an overall cell survival signal for all NCCs within the pharyngeal arches. Support: NIH/NIDCR DE14181.

BROCKER, C

ALDH7A1 IS A NOVEL ALDEHYDE DEHYDROGENASE THAT PROTECTS AGAINST OXIDATIVE DAMAGE. C Brocker[1] (Ph.D., GS), N Lassen[1], T Estey[1], T Chavakis[2] and V Vasiliou[1]. [1]Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO, [2]Experimental Immunology Branch, National Cancer Center, National Institutes of Health, Bethesda, MD.

The human aldehyde dehydrogenase (ALDH) superfamily contains 19 enzymes involved in the NAD(P)+-dependent oxidation of endogenous and exogenous aldehydes. ALDH7A1 was first identified as an osmotic stress-induced protein in plants. In humans, mutations in *ALDH7A1* are responsible for Pyridoxine-Dependent Epilepsy (PDE). The aim of this study was to characterize the biochemical properties, subcellular localization and tissue distribution of mammalian ALDH7A1. Human ALDH7A1 protein, baculovirus expressed and affinity purified, was

found to be catalytically active with various aldehyde substrates. Results from Western blot analyses and IHC revealed high expression of ALDH7A1 protein in kidney, liver, brain, and tongue. ALDH7A1 protein was found in the cytosol, nucleus and mitochondria. Confocal microscopy of HUVEC cells indicated primarily nuclear localization. Through sequence analysis and RT-PCR we identified a second novel ALDH7A1 transcriptional variant, which was found to contain a mitochondrial leader sequence. RT-PCR revealed ALDH7A1 transcript variants are differentially expressed in a tissue specific manner. Confocal microscopy verified mitochondrial localization in transfected CHO cells. In addition, quantitative RT-PCR indicates significant up-regulation of ALDH7A1 in ALDH2 knockout mice. Finally, ALDH7A1 protects against osmotic stress-induced apoptosis. In conclusion, ALDH7A1 is a novel ALDH found in the cytosol, nucleus and mitochondria, which along with its substrate specificities suggest potentially tissue- and cell-specific biological roles against oxidative damage. (Grant support: EY11490).

BROOKENS, AE

A SURVEY OF TUBERCULOSIS PATIENT ATTITUDES TOWARDS AN INGESTIBLE ELECTRONIC TREATMENT MONITOR. AE Brookens, (M.D., SOM), RW Belknap & RR Reves, Denver Metro Tuberculosis Clinic, Denver Public Health Department.

Purpose of Study: Approximately one-third of patients exhibit poor adherence with medical treatment, and health care providers are unable to accurately predict which patients will have difficulty with adherence to a treatment regimen. Directly observed therapy (DOT) for active tuberculosis (TB) treatment provides timely monitoring of adherence, improves treatment outcomes when combined with patient-centered approaches and is effective in preventing acquired drug-resistance. However, requiring all patients to come to clinic or an outreach worker to locate the patient in the field for DOT multiple times per week is costly and time-consuming for both patients and clinic staff. Proteus Biomedical, Inc. is a company currently developing an electronic monitoring system whereby ingestions of medications containing a mico-chip label are electronically detected on the skin surface, providing a record of each ingested dose.

Methods: Over a one-month period, we surveyed clinic patients to assess the acceptability of an alternative technical monitoring system for TB treatment (using the Proteus system) compared to DOT. A survey was developed incoporating likert scale responses, open ended and yes/no questions. Active and latent tuberculosis patients above 18 years were invited to participate during regular clinic hours.

Results: 21 patients with TB and 9 with latent infection (LTBI) were interviewed. Similar to the clinic population, 80% were foreign-born (9 countries) including 30% Latino. 100% believed standard DOT to be inconvenient, uncomfortable or both. Regarding the distance monitoring device, patients believed it could improve monitoring (76%) and improve their health outcome (67%), but 52% felt the monitoring technology was worrisome and invasive. 95% of active TB patients thought the clinic should offer this monitoring alternative, and 71% would personally consider using it. When questioned about participating in research to evaluate the monitoring system, 59% of TB patients said yes in the 12 initial interviews, increasing to 89% (N=9) after technical language in the interview was refined and simplified (overall 64%).

Conclusions: The majority of a culturally diverse population of patients being treated for TB and latent TB infection in a public health setting described DOT as uncomfortable and inconvenient. Patients highly accepted the concept of using an ingestible electronic system to monitor self-administration. The majority of patients were also willing to participate in research to evaluate the electronic monitoring system, particularly when described in non-technical language. The ingestible electronic monitoring system may offer a way to rapidly identify TB patients who exhibit poor adherence to self-administered TB treatment and permit more focused public health outreach activities instead of universal DOT.

BROWN, K

THE ROLE OF BRAIN WATER DIFFUSION IN ACUTE MOUNTAIN SICKNESS (AMS). K Brown (MD, SOM), A Subhudi, V Browne, S Altobelli, A Caprihan, and R Roach, Altitude Research Center, University of Colorado, Denver, CO.

The pathophysiology of Acute Mountain Sickness (AMS) remains unexplained. In high altitude cerebral edema, considered a late-form of AMS, there is marked vasogenic edema. Considerable controversy currently surrounds the role of brain water diffusion in the pathophysiology of AMS. Two recent papers suggest that restricted diffusion may predominate in AMS, but vasogenic edema would be associated with unrestricted diffusion. We sought to clarify the role of water diffusion by applying diffusion tensor imaging (DTI) before and after high altitude

exposure. Methods: We exposed 22 healthy volunteers to simulated high altitude in a hypobaric chamber (Pb=430mmHg) for 10 hours. 14 of the 22 developed AMS, with a Lake Louise AMS score of greater than or equal to 3. Each volunteer completed 3-Tesla MRI while breathing hypoxic gas before (PRE) and after (POST) altitude exposure. From DTI we computed fractional anisotropy (FA) and mean diffusivity (MD). Results: In AMS we observed lower FA and elevated MD in white matter, both pre and post altitude, however this pattern was also present in the group that didn't get sick at altitude. This pattern suggests a breakdown in cellular integrity with increasing unrestricted water diffusion. The most significant result was the difference in baseline MD and FA values between the group that developed AMS and the group that didn't develop AMS. Discussion: In AMS, DTI revealed greater unrestricted water diffusion, both at PRE and POST altitude. This finding is suggestive of vasogenic edema, but the group differences were small, and did not increase as symptoms worsened from PRE to POST. Equally important, no evidence was obtained to support a role for restricted diffusion (intracellular edema). The difference at baseline between groups suggests that there is either a genetic difference between groups, present regardless of the hypoxic stress, or there is a difference in their reactions to acute hypoxic exposure. The root of this difference cannot be determined with the current study because we did not perform baseline MRI scans at normoxia on any of the volunteers.

CAPORASO, JG

SEQUENCE COOCCURRENCE AND COVARIATION SUGGEST SPECIFIC PHYSICAL INTERACTIONS BETWEEN TYPE VI SECRETION SYSTEM COMPONENTS

J. Gregory Caporaso (Ph.D., GS), Amy Dear, Larry Hunter, Marcelo Sousa, and Rob Knight
Department of Biochemistry and Molecular Genetics, University of Colorado Denver

The Type VI Secretion System (T6SS) is a recently discovered protein complex used by pathogenic bacteria to transfer effector proteins from the pathogen's cytosol to the host's cytosol. Twenty-four Salmonella typhimurium proteins are potentially involved in the T6SS apparatus, but the specific protein-protein interactions important for complex formation are currently unknown. A brute-force approach to identify the interactions biochemically would involve testing 276 pairs of proteins for physical interactions: an expensive and time-consuming task. We have identified proteins that cooccur in bacterial gene clusters, and applied joint-entropy-normalized mutual information (NMI) to detect covarying positions between multiple sequence alignments of cooccurring proteins. Covariation between proteins is taken as evidence of physical interaction between those proteins, and covariation results are therefore applied to guide the biochemical analysis of the T6SS apparatus by predicting the pairs of proteins most likely to interact.

Of the twenty-four Salmonella typhimurium proteins, thirteen were found to frequently cooccur in bacterial gene clusters. Our covariation analysis focused on these thirteen proteins, and predicted sixteen pairs of proteins (involving ten of the thirteen cooccurring proteins) to physically interact. Of these sixteen predictions, one has been biochemically confirmed; an additional six appear to be correct based on current models of the T6SS; eight involved predicted cytoplasmic proteins that have not been previously characterized; and one prediction is inconsistent with the current model. This work has shed light on the organization of the T6SS complex, and all sixteen predictions are currently being evaluated biochemically. Our approach to identifying protein-protein interactions on the basis of cooccurrence and covariation is easily expandable to other sets of proteins, and therefore has potential to greatly reduce the cost and labor involved in protein-protein interaction studies.

CHEVALLIER, KM

RAGE EXPRESSION IN A RAT MODEL OF DIABETES: DIFFERENTIAL EXPRESSION IN DIABETIC VS. NON-DIABETIC RAT BONE SAMPLES. KM Chevallier, (M.D., SOM), M Levy and Y Bogert, Department of Renal Medicine, KB King, Department of Orthopaedics, University of Colorado, Denver, CO.

Post-operative total knee replacement outcomes in diabetic patients at the Denver VA are significantly poorer than outcomes in non-diabetic patients. Studies assessing the role of obesity, tissue healing, and infection rates have not demonstrated an underlying mechanism for this differential in outcomes. The outcomes differential may be related to metabolic differences in the chondocytes and osteocytes between the two populations. Advanced glycosylation endproducts (AGE) are found in collagen, and when present in excess amounts, may make the tissue stiff and brittle. The activation of AGE receptors (RAGE) has been shown to

increase matrix metalloproteinase (MMP) activity in vitro. If diabetic joint tissues are shown to have higher levels of AGE-RAGE expression, this difference might serve to explain the differential outcomes in diabetic and non-diabetic patients.

Five rats with Streptozotocin-induced beta cell destruction were used as a model of diabetes. In combination with three age-matched controls, the rat model was used to determine relative levels of RAGE expression in the bones diabetic versus non-diabetic animals. For each rat, the left tibia was harvested, flash-frozen, and powderized with a Satorius Mikro-Dismembrator S. The samples were lysed in a guanidine lysis buffer with protease inhibitors and dialyzed against a 10,000 MWCO membrane Pierce. 15 μg of each sample was loaded into an acrylamide gel (10% resolving with 4% stacking). Gels were transferred onto a PVDF membrane using a discontinuous transfer buffer with a Bio-Rad Trans-Blot SD Semi-Dry Transfer cell. RAGE was detected with an R&D Systems rat anti-mouse/rat RAGE primary antibody and HRP-conjugated goat anti-rat IgG secondary antibody Thermo-Scientific Immuno-Pure. The membrane was developed with Pierce Super Signal West Dura for HRP and imaged with Kodak Imaging Station software used to record images and assess the relative intensities of the bands.

On visual inspection each sample appeared to have between 3 and 4 bands ranging from 50 to 25 kD. However, only certain bands were detectable by the imaging software. Bands of 50kD size are believed to represent the RAGE protein and bands of smaller sizes (44kD, 35kD, 25kD) are believed to represent RAGE isoforms. Of the three control subjects, only one had a detectable band at 50kD. Of the five diabetic subjects, four had a detectable band at 50kD. All eight subjects had detectable bands in the region of 25kD. To our knowledge, this is the first report of RAGE in rat bone, and it appears there is more RAGE expression in STZ-treated rats. As in human bone, RAGE isoforms exist and merit further investigation.

CLARK, SL

PEDIATRIC CARDIAC RETRANSPLANTATION. SL Clark, (M.D., UCHSC); MB Mitchell, M.D. The Children's Hospital, Denver, CO

Background: There is a critical shortage of donor hearts and their use should be carefully monitored to maximize the effectiveness of this treatment option. Experience with cardiac retransplantation in pediatrics is limited and the sparse amount of literature exploring this topic is often inconclusive. **Objective:** This study aims to evaluate outcomes of pediatric cardiac retransplantation in a major center for pediatric cardiac care. **Methods:** Since 1990 a total of 327 heart transplants have been performed at The Children's Hospital in Denver, CO. Among them 21 patients received cardiac retransplantation. Pre-operative, operative and post-operative data on demographics, morbidity, short and long term survival were collected from 1990 to present. **Results:** The most frequent diagnosis for patients undergoing retransplant surgery was chronic rejection (9), followed by coronary artery disease (6). The mean time interval between primary transplantation and retransplantation was 2.2 days in the acute and 6.3 years in the chronic retransplant group. Actuarial survival rates at 1, 3, and 5 years after cardiac retransplantation were 57, 51, and 48%, respectively. Major causes of death were acute and chronic rejection, infection and sepsis. **Conclusions:** Retransplantation in a pediatric population is a viable form of treatment in patients with primary graft failure. Additional studies need to be conducted to appropriately assess the use of donor hearts in retransplantation.

COFFEY, D

EXPANSION OF A VARIABLE NUMBER TANDEM REPEAT DOES NOT ALTER THE PROCESSING OF MICRORNA-137. D Coffey (M.D., SOM), S Robinson, C Amato, W Robinson, Division of Medical Oncology, University of Colorado Denver.

microRNAs (miRNAs) are a class of non-coding RNA that negatively regulate gene expression by binding to the 3' untranslated region of messenger RNA. We recently showed that miRNA-137 might function as a tumor suppressor by inhibiting the expression of the melanoma oncogene microphthalmia-associated transcription factor (MITF). We also identified a variable number tandem repeat (VNTR) upstream of the precursor miRNA-137 in melanoma cell lines and peripheral blood samples from melanoma patients. We found that a melanoma cell line with twelve VNTRs has increased MITF protein expression as compared to a cell line with three VNTRs. We hypothesized that an expansion of this VNTR may alter the enzymatic processing of miRNA-137 by Drosha and DGCR8. To test this hypothesis, we preformed an *in vitro* miRNA processing reaction using purified Drosha and DGCR8 proteins and primary miRNA-137 transcripts with three or twelve VNTRs. We analyzed the processed

transcripts by bioanalyzer chip and direct sequencing. Our preliminary data suggests there is no significant difference between the processing of the primary miRNA-137 transcript with three or twelve VNTRs. Since this data does not support our hypothesis, we are now investigating alterative mechanisms by which the VNTRs may alter the function of miRNA-137. One possibility is that since the VNTRs are rich in CpG dinucleotides they may become hypermethylated in melanoma cells resulting in epigenetic silencing of miRNA-137.

COOK, S and DUNN, JH

FAMILIAL IDIOPATHIC SCOLIOSIS IN MALES: LOCALIZATION TO CHROMOSOME 22q

Authors: Shane Cook[1] (MD, SOM), Jeffrey Holter Dunn[1] (MD, SOM), Nancy Hadley-Miller[1], Kandice Swindle[1], Beth Marosy[2], Christina Justice[3], Alexander F Wilson[3]
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2. Center for Inherited Disease Research, Johns Hopkins Medical Institution, Baltimore, MD.
3. Genometrics Section, National Human Genome Research Institute, Baltimore, MD.

Purpose of Study: Familial idiopathic scoliosis (FIS) is a structural, fixed lateral curve of the spine of ≥10 degrees that occurs in otherwise normal individuals and demonstrates heterogeneic etiology. One strategy for identifying causative genes in heterogeneic disorders is to isolate subgroups of affected individuals. Studies throughout the literature show differences in scoliosis between males and females. Male spines are clinically found to be more rigid, have a higher prevalence of thoracic curves, and have curve progression that often persists into late adolescence, beyond that of females. Thus, the goal of this study is to examine genetic etiology in males with a scoliotic curvature of ≥30 degrees.

Methods: Blood samples were obtained and genomic DNA was extracted from a large study sample of families with two or more individuals affected with FIS (202 families: 1198 individuals). All individuals underwent genomic screening using a modified Weber 9 marker set. Subsequent model-independent linkage analysis on clinical subgroups was accomplished using SIBPAL. A subgroup of males diagnosed with ≥30 idiopathic spinal curvature in adolescence was identified. Results from this subgroup of 241 individuals from 31 families are reported.

Summary of Results: Analyses revealed significant results (≥2 adjacent markers $p<0.005$) in regions of chromosomes 2 and 22. The area on chromosome 2 spans 48 Mb; the area on chromosome 22 spans 13 Mb. Very significant p-values of 4.25×10^{-9} and 4×10^{-4} were found for markers on chromosome 22q.

Conclusions: Isolation of this subgroup of patients reveals an area of interest on chromosome 22. Of particular interest is the deletion at 22q11.2 associated with velo-cardio-facial syndrome, which is implicated in musculoskeletal disorders including scoliosis. Several other candidate genes in this area have been cited for their influence on phenotypic appearance of bone abnormalities and/or scoliosis. Future goals include the finer mapping of this area of chromosome 22q through intragenic SNP analyses.

COOPER, JE

COMPLEMENT-INDEPENDENT KILLING OF OPSONIZED PNEUMOCOCCUS BY STIMULATED HUMAN NEUTROPHILS.

JE Cooper (MD/PhD, GS), CE Fasching, A Johnson, **Error! Bookmark not defined.**, Department of Microbiology, Mucosal and Vaccine Research Colorado (MAVRC), University of Colorado Denver, Denver, CO.

The most prevalent diseases elicited by *Streptococcus pneumoniae* are rooted in the mucosa, particularly the lung. Prevention has focused on development and use of vaccines that generate antibodies to the bacterial capsule because pneumococci can be opsonized by antibodies with complement for phagocytosis and killing. However, levels of complement in the upper and lower respiratory tract are low. We hypothesize that activation of neutrophils and alveolar macrophages can be elicited by pathogen-induced local inflammation, abrogating the need for high levels of complement to effect IgA- and IgG-dependent killing in the lung. Among multiple cell surface markers monitored during activation of neutrophils by a panel of cytokines and pneumococcal cell wall, CD11b (part of complement receptor 3) and CD62L (L-selectin) consistently showed the greatest changes with activation in response to IL-8, TNF□, C5a, and the bacterial product, fMLP (formyl-methionine-leucine-phenylalanine). Increases in surface CD11b and decreases in CD62L expression on human neutrophils following activation, correlated with oxidation of dihydrorhodamine, an effect associated with generation of oxygen radicals. Pretreatment of neutrophils with TNF□ or C5a supports killing antibody opsonized bacteria in the absence of complement in a dose-dependent manner. Pretreatment with fMLP also supports neutrophil binding/uptake of FITC-labeled bacteria opsonized with type-specific antibody. These data provide proof of principle that neutrophils recruited to a site of early pneumcoccal infection and inflammation may be activated and primed for phagocytosis

and killing of S. pneumoniae by local cytokines or isolated complement components. These data highlight the need to generate sufficient levels of capsule-specific antibodies in the lung and to understand their mechanism of action under local conditions.

DUNDAS, LE

THE ROLES OF HYPOXIA-INDUCIBLE FACTOR-1 (HIF1) AND HYPOXIA-INDUCIBLE FACTOR-2 (HIF2) IN HEAD AND NECK CANCER. LA Dundas, (D.D.S., SOD), CJ Hu, M Pawlus, E Kay, Department of Craniofacial Biology, University of Colorado, Denver, CO.

Hypoxia-inducible factors HIF1 and HIF2 play important roles in the growth of several solid tumors including liver and kidney. However, the expression status and function of HIF1 and HIF2 in head and neck (HN) cancer is still not clear. The goals of this study are to determine if HIF1 and HIF2 are expressed in HN cancer cells, and to determine whether HIF1 and HIF2 have different functions. We hypothesized that HN cancer cells express both HIF1 and HIF2. Furthermore, HIF1 and HIF2 have distinct roles in HN cancer progression due to a significant sequence difference in their transactivation domains. Using quantitative PCR (Q-PCR), we determined that both HIF1 and HIF2 mRNA are expressed in all HN cancer lines we analyzed. Interestingly, we found that HIF1 and HIF2 mRNA expression was induced by hypoxia in UMSCC22B and HN-31 cell lines. This is a novel finding since HIF1 and HIF2 mRNA have not been reported as hypoxia-induced previously. Consistent with HIF mRNA data, we detected HIF1 and HIF2 proteins in all HN cells cultured under hypoxia using western blot analysis. We then successfully knocked down the levels of HIF1 or HIF2 mRNA in HN-31 cells using siRNA technology. Interestingly, using HIF knock-down cells, we found that HIF1 was required for hypoxic induction of phosphoglucokinase (PGK), a kinase critically important in glucose metabolism while both HIF1 and HIF2 likely activate adrenomedullin (ADM), a gene involved in blood dilation and blood vessel growth. From these studies, we concluded that HN cancer cells express both HIF1 and HIF2. Importantly, some HN cancers exhibit hypoxia induction of HIF1 and HIF2 mRNA. Our preliminary data also supports a distinct role of HIF1 and HIF2 in gene regulation and HN cancer progression.

FARABAUGH, SM

THE ROLE OF EYA AS COFACTORS OF SIX1 IN HUMAN BREAST CANCER. SM Farabaugh (Ph.D., GS), AL Welm, and HL Ford, Department of Biochemistry and Molecular Genetics/Division of Obstetrics and Gynecology, University of Colorado, Denver, CO.

The Six1 homeodomain-containing transcription factor stimulates the proliferation and survival of progenitor cells and plays a role in the epithelial to mesenchymal transition (EMT) during development. Using cell culture and mouse models, we have shown that, when expressed out of context in adult cells, Six1 not only promotes proliferation and survival, contributing to tumorigenesis, but also upregulates the TGFβ pathway bringing about an EMT-like transformation, and promoting metastasis. Most importantly, reducing Six1 levels decreases cancer cell proliferation and metastases in several models of cancer, strongly suggesting that Six1 will make an excellent cancer therapy target. As Six1 has no known intrinsic activation or repression domains, cofactors are required for its regulation. The Eya family (Eya1-4) of coactivators play important roles in Six1-mediated transcriptional activation during development and the Eya-Six1 interaction is lost in several diseases. Eya and Six1 are overexpressed in the same cancers, suggesting that Six1 may require Eya for transcriptional activation not only in development, but also in cancer. Here we show that while Six1 correlates with shortened time to metastasis in breast cancer patient tumors, presence of both Eya2 and Six1 together further reduces time to metastasis as well as significantly correlates with decreased survival. Additionally, decreasing Eya2 levels in MCF7 cells overexpressing Six1 reverses Six1-induced upregulation of the TGFβ pathway and most Six1-induced EMT phenotypes, suggesting that Eya2 is required for the ability of Six1 to mediate tumor progression. As Eya2 appears to be required for Six1 activity in breast cancers and as Six1 and Eya are not normally expressed in most adult tissues, we believe that targeting Eya phosphatase activity or the Six1-Eya interaction with small molecule inhibitors could result in new chemotherapeutics for breast cancer patients.

FLAKE, C

PERIODONTAL HEALTH STATUS OF ADOLESCENTS WITH TYPE 1 DIABETES. C Flake (DDS SODM), E Morrato, L Johnson, F Bishop, V Orlando, SODM

According to recent research conducted at Columbia Dental School, there is some indication that periodontal disease, including attachment loss of gingival tissue around the tooth may begin in childhood for adolescents with diabetes. The present cross-sectional epidemiologic study is designed to determine the periodontal health status, oral hygiene behavior, and oral health knowledge, HbA1c, micro-vascular, and serologic inflammatory markers in adolescents (12-19 years old) with type 1 diabetes compared to non-diabetic adolescents. It is hoped that this will aid us in devising methods of improving the periodontal health of adolescents with type 1 diabetes. Study patients are selected from the clinical population of the Barbara Davis Center for Childhood Diabetes. Control patients are selected from family and friends of the faculty and staff of the Barbara Davis Center and the University of Colorado Health Sciences Center. Patients participate in a series of vascular tests, provide a sample for baseline HbA1c and inflammatory markers, and are given a periodontal exam (plaque and gingival indices, pocket depth, and measures of overall periodontal health) and questionnaires regarding their oral hygiene habits and nutritional diet. The results will be analyzed to compare the periodontal health of adolescents with type 1 diabetes to adolescents without the disease. Preliminary results will be available in July 2009, and we anticipate finding increased risk factors for cardiovascular disease including, elevated HbA1c and periodontal attachment and bone loss, correlated with more advanced periodontal disease in the adolescents with type 1 diabetes.

GRENOBLE, J

INTERNATIONAL MEDICAL VOLUNTEER WORK: IMPROVING AWARENESS AND SUSTAINABILITY. J Grenoble, (M.D., MS); T Ning, M.D. Global Health Track, School of Medicine, University of Colorado, Denver, CO.

Short-term international medical volunteer work can potentially improve access to care for underserved populations, and develop the skills and awareness of medical professionals. However, such work has been criticized as being self-serving, ineffective, and inappropriate, raising unmet expectations, and imposing burdens on local facilities. In response to many of these challenges, The San Lucas Mission, in San Lucas Toliman, Guatemala felt a need to better integrate visiting health volunteers into a sustainable healthcare system. The purpose of the study was to identify the challenges faced by the health volunteer program of the Mission, and to propose potential solutions. The study aimed to improve the cultural/situational awareness of health volunteers, and to better integrate them into the existing healthcare system.

Methods included observation and informal interviews with volunteers, local health promoters and physicians, community members, and Parrish staff.

My work identified the following concerns in the healthcare program: (1) lack of awareness of health volunteers regarding the Parrish philosophy, the existing healthcare system, and Mayan culture; (2) lack of appropriate skills of health volunteers, especially in language and pharmaceutical distribution; (3) failure of medical volunteers to address root causes of health problems; (4) lack of communication between the different areas of the healthcare system; and (5) structural/organizational issues with the health promoter program.

Based on these results, I recommended the creation of specific requirements for future medical volunteer groups regarding language, group size, physician:student ratio, medication organization, and work with health promoters. I also recommended division of groups between acute care and preventive care; further development of the health promoter program, and centralization of scheduling and communication within the healthcare system. Finally, I created an orientation document to improve the awareness and preparation of future volunteers. Future research should address the role of volunteers in prevention and the development of sustainable health promoter programs. The challenges faced by the San Lucas Mission are not unique, and this experience suggests the need for global guidelines for short term medical volunteer work.

GUESE, ME

CHRONIC TOBACCO USE AND P50 SENSORY GATING. ME Guese (BA SOM), LF Martin, R Freedman, A Olincy, Department of Psychiatry University of Colorado, Denver, CO.

Purpose: Individuals with schizophrenia and some of their family members have the diminished ability to suppress the auditory evoked potential to the second of two clicks. This is called impaired P50 sensory gating. The importance of this finding is that impaired sensory gating is associated with impaired attention and treatment of this abnormality could improve some of the symptoms of schizophrenia. Acute nicotine administration improves P50 sensory gating deficits in the nonsmoking family members of individuals with schizophrenia and in individuals with schizophrenia who abstain from nicotine overnight. However, tachyphylaxis may limit the ability of nicotine

to reverse P50 sensory gating deficits on a chronic basis. The purpose of this study is to see if individuals who identify themselves as smokers have different P50 sensory gating levels than those who are nonsmokers.
Methods: Subjects for this study participated in a larger study, The Consortium on the Genetics of Schizophrenia (COGS). The P50 auditory potential was measured for 899 subjects. 310 were controls, 170 were individuals who had a diagnosis of schizophrenia as determined by the Diagnostic Interview for Genetic Studies criteria, and 409 were family members of schizophrenic individuals. Smoking status (smoker or non smoker) was established by self report.
Results: Forty-two (14%) of the controls, 76 (45%) of the individuals with schizophrenia, and 59 (14%) of the family members reported they were smokers. As a group, individuals with schizophrenia had a higher mean P50 ratio than controls (0.63 $\pm$ 0.478 vs. 0.36 $\pm$ 0.310). Family members (0.50 $\pm$ 0.351) did not have a significantly different mean P50 ratio when compared to individuals with schizophrenia and controls. Within each diagnostic group, being a smoker was not associated with having a lower P50 sensory gating ratio.
Conclusion: Consistent with prior studies, individuals with schizophrenia have impaired sensory gating. While nicotine acutely may transiently improve P50 sensory gating, chronic nicotine dosing (i.e. tobacco smoking) is not associated with improved sensory gating. A longitudinal study of change in smoking status among the smoking groups is needed to explore whether there is a cohort effect.

GUZMAN, A

DEVELOPING A PROBE TO ASSESS BILINGUALISM. A. GUZMAN, (B.A) and J.G. HARRIS, (Ph.D) Department of Psychiatry, University of Colorado Denver, Denver, CO.

Can language proficiency of a bilingual patient be validly assessed and what is the best method for doing so? In this study we will examine the performance of two groups of highly fluent bilingual (English/Spanish) subjects, those who learned English as a second language early (before the age of 7) versus late learners (after the age of 7), on two distinct language proficiency measures. It is known that early learners develop early and automatic links between languages at the semantic level as has been demonstrated using semantic priming paradigms. The main purpose of this study is to measure the specificity of a traditional "on-line" versus an "off-line" approach to assess language proficiency in bilinguals. These results will help to guide clinical decision-making regarding patients who might require a neuropsychological assessment in both of their languages to comprehensively evaluate cognitive function and those who have a "dominant" language and would be accurately evaluated in solely one language.

HA, D

CHARACTERIZATION OF AN ANTI-ANKRD26 ANTIBODY AND DETECTION OF A 38kDA PROTEIN IN MICE TISSUES. Duc Ha[1*], Tapan Bera[1], Ira Pastan[1]; [1]Laboratory of Molecular Biology, Center for Cancer Research, NCI, NIH; *University of Colorado - Denver School of Medicine

Introduction: The primate-specific gene family of POTE, gene expressed in normal prostate, ovary, testis, and placenta tissues, has been shown to also be expressed in many cancers, including prostate, breast, and lung cancers. The POTE gene family, consisting of 13 paralogs dispersed among eight different chromosomes, evolved through duplication from an ancestral gene, Ankrd26. To understand the role of the Ankrd26 gene, mutant Ankrd26 mice have been created. In mice with mutation of the Ankrd26 gene by insertion of β-galactosidase cDNA into the Ankrd26 gene, phenotypes in obesity, gigantism, and insulin resistance were observed. Ankrd26 is a 34-exon gene spanning a 60-kb region of chromosome band 6qF. Its 7.5kb transcript predicts a 192kDa protein if translated in its entirety. To generate antibodies to study Ankrd26 protein, rabbits have been immunized with a peptide containing the first 250 amino acids of the predicted Ankrd26 protein in fusion with maltose binding protein. Immunoglobulin G was isolated through size-exclusion chromatography. Its reactivity was characterized by Western blotting.

Methods and Results: Western analysis was used to characterize anti-Ankrd26 antibody reactivity against pure Ankrd26 protein and protein lysates from cells transfected with a construct expressing Ankrd26. Results indicate that anti-Ankrd26 antibody can recongize pure Ankrd26 protein and Ankrd26 protein in cell lystates. Reactivity of Anti-Ankrd26 antibody in mice tissue lysates. Protein lysates were isolated from mouse hypothalamus, brain, kidney, testis, and liver tissues and 50ug of protein were used for Western analysis. Results did not reveal a protein

corresponding to the predicted 192kDa size. Instead, a strong 37kDa band was observed in mouse hypothalamus and brain tissues and weaker bands of the same size were detected in testis and liver tissues.

To investigate the possibility that the detected protein is encoded from the Ankrd26 gene, a search for mouse Ankrd26 cDNA sequences in the Expressed Sequence Tags (EST) database revealed an EST (accession AK084890) from 13-day mouse embryonic lung containing a 5'UTR and a 3'UTR with a coding sequence of 1044bp, translating to a possible protein with a predicted size of 38kDa. We then designed a probe corresponding to this sequence of cDNA and performed Northern analysis to detect for a possible smaller Ankrd26 transcript. Northern analysis was performed on RNA from mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis. A smaller transcript of approximately 1.5kb was observed in brain, liver, and kidney RNA, predicting for a 38kDa Ankrd26 protein.

Discussion and Conclusion: Anti-Ankrd26 antibody can detect transfected Ankrd26 protein in HEK 293T cells. In mouse tissues, the antibody detects a 38kDa protein in hypothalamus, brain, testis, and liver. There are two Ankrd26 mRNA transcripts in mouse tissues, one at 7.5kb that would encode for a predicted protein size of 192kDa, and a smaller 1.5kb transcript that would code for a predicted 38kDa protein. The 38kDa protein is present in both Ankrd26 mutant and wild type mice.

Mutant Ankrd26 mice with β-galactosidase cDNA insertion into intron 24 display obesity, gigantism, and insulin resistance phenotypes. A 38kDa protein encoded from Ankrd26 exons 1-10 would be intact in both wild type and mutant Ankrd26 mice. If such protein exists, its function is unknown.

HADDAD, NE

WEB-BASED GUIDED IMAGERY AND PROBLEM SOLVING FOR HEALTH SCIENCES STUDENT BURNOUT. **NE Haddad, (MD, SOM), JN Lillemon, and RE Feinstein, Department of Medicine, University of Colorado, Denver, CO.**

Burnout is prevalent in health sciences students and has implications for health, professional development, attrition from school, and the quality of patient care they will provide later as health professionals. Difficulties in intervening to reduce burnout relate to the intensive workload and limited free-time that school imposes. Thus, this study was developed as a web-based self-administered stress reduction intervention offered to students in all years of medical, dental, pharmacy, physician assistant and nursing school at University of Colorado at Denver. A web-based intervention offers flexibility, ease in access, confidentiality, and cost effectiveness. The purpose of this study is to assess whether relaxation training or cognitive behavioral therapy (CBT), administered via a website, can reduce burnout amongst health sciences students.

This study is a web-based randomized placebo control trial of 150 health sciences students, who will be randomized into one of three groups: 1) Relaxation intervention 2) CBT intervention 3) Control. The relaxation group will utilize two guided imagery mp3s and the CBT Group a CBT worksheet. Both intervention groups will be asked to utilize their intervention for 20 minutes, 3x per week for three months. We will use the standardized and validated Maslach Burnout Inventory (MBI) to assess primary outcome, burnout. Secondary outcomes will be assessed using a locus of control (LOC) scale and distress thermometer. The data in all groups will undergo an intent-to-treat analysis and be analyzed using repeat measures of ANOVA.

The hypothesis is that students in both intervention groups will experience at least a 30% reduction in scores on the MBI at three months as compared to control, with dose-response effect. The CBT intervention will be more effective than the relaxation intervention. Secondary hypotheses are 1) That students who have an internal LOC will utilize these interventions more frequently and show greater stress reduction than students who have an external LOC and 2) The distress thermometer may be an easier way to assess stress/burnout and will have a significant correlation with scores on the MBI.

A web-based stress reduction intervention is more effective than placebo, practical and cost-effective to administer, and helpful in reducing health sciences student burnout. Locus of control may predict which students will benefit most from this type of intervention.

HOW DOES AUDITORY SENSORY GATING IN NEWBORNS WITH PRENATAL NICOTINE AND/OR DRUG EXPOSURE DIFFER FROM NEWBORNS WITH NORMAL PRENATAL EXPOSURES? AC Herndon (MSPH), SK Hunter, and RG Ross, Psychiatry Scholars Program, Department of Psychiatry, University of Colorado, Denver, CO

Purpose: This study looked at the effect of prenatal multi-drug and nicotine exposure on auditory sensory gating in infants. Auditory sensory gating, using a paired-click paradigm, provides a physiological measure of the brain's ability to disregard irrelevant information (Adler, 1982) and is related to attentional control. Evoked responses (ERPs) to the paired clicks are recorded and compared using a ratio (T/C Ratio) of peak amplitudes following the auditory stimuli (Stimulus 2;Test/Stimulus 1; Conditioning). Individuals with intact auditory gating tend to exhibit ratios of less than 0.4 (Seigel, 1984). We hypothesized that infants prenatally exposed would exhibit a deficit in auditory sensory gating as compared to infants with no prenatal exposure.

Methods: Auditory ERPs were collected on 23 case (i.e., infants born to poly-drug abusing mothers) and 27 control infants. Continuous EEG was recorded from site Cz and used in ERP analysis. Bipolar electrooculagram (EOG) and electromyogram (EMG) were used to help determine when the infant entered active sleep. To investigate whether auditory sensory gating differed based on prenatal exposures to drugs, the mean T/C ratios for cases and controls were compared using a student's t-test with a two-sided alpha of .05. All procedures were approved and monitored by the Colorado Multiple Institute Review Board.

Results: Cases and controls did not differ in gestational age, size, gender, or race and ethnicity. Latencies and amplitudes of ERPs for conditioning and test clicks were similar. Controls were found to have lower T/C ratios (mean T/C ratio = 0.46) than cases (mean T/C ratio = 0.65).

Conclusions: This is the first study to evaluate auditory sensory gating in infants prenatally exposed to illicit drugs. Infants with prenatal drug exposures were found to have deficits in auditory sensory gating when compared to infants with no prenatal drug exposures. Auditory sensory gating deficits indicate attentional difficulties, and while we cannot say whether that these infants will continue to exhibit problems with attention in the future, it appears that they may have trouble filtering out irrelevant information presently.

Nuclear Translocation of PKCδ in Salivary Acinar Cells

Matthew D Hockin, Tariq Adwan, Mary Reyland. Department of Craniofacial Biology, University of Colorado School of Dental Medicine.

According to the National Institute for Dental and Craniofacial Research, it is estimated that there are approximately 40,000 new cases of xerostomia each year as a result of irradiation induced damage of the salivary gland in head and neck cancer patients. Apoptosis contributes to this irradiation induced salivary gland damage. Our lab has identified PKCδ, a serine/threonine kinase, as a critical regulator of salivary epithelial cell apoptosis and mice in which the PKC□ gene has been disrupted are protected against irradiation induced damage to their salivary gland. Previously we have shown that nuclear accumulation of PKCδ is required for apoptosis, and we have identified a nuclear localization signal (NLS) that regulates nuclear import and in turn the pro-apoptotic function of PKCδ. Since under basal conditions PKC□ is primarily located in the cytoplasm, we predict that a second, apoptosis specific signal, is also required for nuclear import. Our studies show that PKC□ is rapidly phosphorylated on tyrosine residues in response to apoptotic signals and that phosphorylation on two specific tyrosine residues, Y64 and Y155, is required for nuclear localization of PKCδ. **Thus the overall goal of the current studies is to understand how tyrosine phosphorylation on these residues regulates PKC□ nuclear import. Here we have specifically addressed the question of how tyrosine kinases interact with PKC□ in response to apoptotic signals.** Tyrosine kinases interact with other proteins via src-homology (SH2 and SH3) domains. Sequence analysis has revealed a single putative SH3 domain ligand (PxxP) in PKCδ, which overlaps the NLS. **We hypothesize that in response to apoptotic stimuli, a SH3 domain containing tyrosine kinase (TK) binds to the PxxP site and phosphorylates Y64 and Y155 in the δRD, allowing nuclear import of PKCδ.** Using site directed mutagenesis, and Western blotting analysis we were able to show that mutation in the PxxP motif does not effect tyrosine phosphorylation of Y64, but instead abolished phosphorylation at other critical sites in PKC□ that are important for kinase activity. Further studies will address whether the PxxP motif plays a role in nuclear import of PKCδ and whether it constitutes a binding site for SH3 containing tyrosine kinase.

SENSITVITY OF THE AUTISM DIAGNOSTIC OBSERVATION SCHEDULE FOR CHILDREN WITH AUTISM SPECTRUM DISORDERS AND CO-OCCURRING ANXIETY SYMPTOMS K. Homburg, (MD, SOM), A. Blakeley-Smith, K. Ridge, S. Hepburn, and J. Reaven. UCD. Denver, CO.

PURPOSE: Individuals with autism spectrum disorders (ASD) are at increased risk for developing anxiety symptoms compared to the general population. The purpose of this study is to examine the relationship between clinical anxiety symptoms and the presentation of an autism spectrum disorder. The current study investigates the sensitivity of the Reciprocal Social Interaction score of the Autism Diagnostic Observation Schedule (ADOS) to co-occurring anxiety symptoms. Because the ADOS is often used for diagnostic evaluation for autism, anxiety-associated differences in performance might explain individual differences in presentation, thus indicating the need for a more in-depth assessment of anxiety symptoms. METHODS: The dataset was assembled from data obtained in two other studies (CPEA Longitudinal Network; Reaven et al., in preparation). The subjects were children ages 6-14 with an autism spectrum diagnosis based on the diagnostic algorithm of Module 3 ADOS. Subjects were classified as "anxious" if they scored above the 90th percentile on the anxiety subscale of the Developmental Behavior Checklist – Primary Carer version (DBC-P). A Reciprocal Social Interaction composite score for each subject was calculated by summing the score for each individual item in the Reciprocal Social Interaction portion of the ADOS, with higher scores indicating greater impairment. The group means were compared using a one-way ANOVA. RESULTS: The study included 71 subjects, 45 in the Non-Anxious group and 26 in the Anxious group. The groups were matched for age, Full Scale IQ, Verbal IQ, and Nonverbal IQ. The means for the Reciprocal Social Interaction composite score were not significantly different between groups. The Non-Anxious group mean score was 11.56, and the Anxious group mean score was 12.65, $F(1,69) = 1.183$, $p = 0.280$. CONCLUSIONS: Based on the current study, anxiety symptoms do not appear to be associated with degree of social impairment in children with autism spectrum disorders. Limitations of the study will be discussed. Further research is needed to investigate how co-occurring anxiety affects the presentation of ASD.
ACKNOWLEDGEMENTS: Cure Autism Now (CAN).

THE UNIQUE ROLES OF DOST AND DOSS IN DOSR REGULON INDUCTION AND MYCOBACTERIUM TUBERCULOSIS DORMANCY. RW Honaker (Ph.D., GS) , RL Leistikow, IL Bartek, and MI Voskuil

University of Colorado Denver, School of Medicine, Department of Microbiology

In *Mycobacterium tuberculosis*, the sensor kinases DosT and DosS activate the transcriptional regulator DosR, resulting in the induction of the DosR regulon, important for anaerobic survival and perhaps latent infection. The individual and collective roles of theses sensors has been postulated biochemically, but their roles have remained unclear in vivo. This work demonstrates distinct and additive roles for each sensor during anaerobic dormancy. Both sensors are necessary for wild type levels of DosR regulon induction, and concomitantly, full induction of the regulon is required for wild type anaerobic survival. In the anaerobic model, DosT plays an early role, responding to hypoxia. DosT then induces the regulon and with it DosS, which sustains and further induces the genes. DosT then loses its functionality when oxygen becomes limited, and DosS alone maintains induction of the genes from that point onward. Thus the bacterium has evolved a system whereby it responds to hypoxic conditions in a stepwise fashion as it enters an anaerobic state.

VGL-2A IS REQUIRED FOR ENDODERMAL POUCH MORPHOGENESIS DURING ZEBRAFISH CRANIOFACIAL DEVELOPMENT. CW Johnson (Ph.D., GS), W Feng, T Williams, and KB Artinger, Department of Craniofacial Biology, Universtiy of Colorado, Denver, CO.

The development of the vertebrate cranial skeleton results from the specification, growth, patterning, and morphogenesis of tissues derived from all three germ layers in response to a complex network of reciprocal signaling. While many genes involved in these processes have been identified, additional genes involved in craniofacial development remain uncharacterized. We have identified a gene, *vgl-2a,* which is expressed in the pharyngeal endoderm and ectoderm surrounding the neural crest derived mesenchyme of the pharyngeal arches in zebrafish. We have found that reducing expression of *vgl-2a* in zebrafish embryos using morpholino injection

results in a loss of cranial cartilages, demonstrating a previously undescribed requirement for *vgl-2a* in craniofacial development. Inhibiting fibroblast growth factor (FGF) and retinoic acid (RA) signaling results in altered expression of *vgl-2a* within the region of the pharyngeal arches, suggesting that these signaling pathways regulate *vgl-2a* expression in these cell populations. We have also demonstrated that reducing expression of *vgl-2a* results in a defect in endodermal pouch morphogenesis, a process that requires FGF and RA signaling and that is essential for later development of the pharyngeal skeleton.

KLEIN, RL

STRUCTURAL LUNG INJURY IN SCHOOL AGE CHILDREN WITH CYSTIC FIBROSIS. RL Klein, (MD, SOM), SL Heltshe, SD Sagel, Department of Pediatrics, The Children's Hospital, University of Colorado Denver School of Medicine.

Cystic fibrosis (CF) is a genetic disorder characterized by chronic airway infection and inflammation leading to bronchiectasis, progressive obstructive lung disease, and marked shortening of life expectancy. There is a paucity of data regarding bronchiectasis and structural lung injury in school age children with mild lung disease. Structural changes often precede functional changes in children with CF. Since the greatest rate of decline in pulmonary function occurs in late childhood and adolescent years, it stands to reason that structural changes may play a significant role in this decline. High resolution computed tomography (HRCT) of the chest is currently the best tool to assess evolving bronchiectasis and structural lung injury in CF. The objectives of this study were to describe abnormalities in lung structure in school age children with CF and to evaluate the relationships between HRCT scores and pulmonary function measurements and sputum biomarkers of inflammation. HRCT scans were performed on 34 children with CF (mean age 11 yrs, range 6-15; FEV_1 %predicted: mean 94, range 60-122). HRCT scans were scored using a validated Brody scoring system (Brody, J Pediatr, 2004). In brief, each lobe of the lung was evaluated for bronchiectasis, peribronchial thickening, mucus plugging, parenchymal opacities, and air trapping. Subscores for these abnormalities and total Brody HRCT scores were calculated. The subjects underwent spirometry, lung volumes, and induced sputum collection for biomarkers of airway inflammation on the same day as the HRCT scan. The mean total Brody score was 8.7 (range 2-27). Despite the fact that many of these subjects had normal pulmonary function, approximately 90% of subjects had some evidence of bronchiectasis and almost 50% had evidence of bronchiectasis in all lung lobes. Mucus plugging was present in 56% of subjects and air trapping in 65%. There were no significant correlations between HRCT scores and pulmonary function except for a weak association with RV/TLC, an index of air trapping ($r=0.34$, $p=0.07$). The total Brody score was weakly related to sputum neutrophil elastase ($r=0.34$, $p=0.08$), an important protease, and inversely related to another protease, sputum matrix metalloproteinase-9 ($r=-0.54$, $p=0.001$). The total Brody score was inversely related to two important antiproteases, tissue inhibitor of matrix metalloproteinase-1 ($r=-0.42$, $p=0.02$) and secretory leukoprotease inhibitor ($r=-0.61$, $p<0.001$). In conclusion, the majority of these school age children with CF already have established bronchiectasis despite the fact that many of them have normal pulmonary function. HRCT and pulmonary function measurements appear to provide discordant information in that there were not significant relationships between structural injury and pulmonary functions. HRCT scores correlated with a few sputum biomarkers of airway inflammation such that increasing structural injury was associated with decreasing antiprotease levels.

KOVACS, JM

USE OF NMR TO DEFINE CR2:C3D INTERACTIONS IN SOLUTION REVEALS DUAL SCR1-2 INTERFACE WITH C3D: CONFIRMATION USING A NOVEL LIGAND-SELECTIVE INHIBITORY PEPTIDE. JM Kovacs, (Ph.D., GS), JP Hannan, EZ Eisenmesser, VM Holers, Department of Medicine, University of Colorado Denver, Aurora, CO

Complement receptor 2 (CR2, CD21) is a cell membrane protein, with 15 or 16 extracellular short consensus repeats (SCRs), that promotes B cell responses and bridges innate and acquired immunity. The most distally located SCRs (SCR1-2) mediate the interaction of CR2 with its four known ligands (C3d, EBV gp350, IFN-alpha, CD23). Inhibitory mAbs against SCR1-2 block binding of all ligands, and all ligands cross-compete for binding SCR1-2. To develop ligand-specific inhibitors that would also assist in identifying residues unique to each receptor-ligand interaction, phage were selected from randomly generated constrained and un-constrained libraries by panning with recombinant SCR1-2, followed by specific ligand-driven elution. Derived peptides were tested by competition ELISA. Two peptides, C3dp1:APQHLSSQYSRT and gp350cp1:CSEGSLKGC, exhibited ligand specific inhibition at 100 and 150 micromolar IC50, respectively. C3d was titrated into N-15 labeled SCR1-2, which revealed chemical shift changes indicative of specific inter-molecular interactions. With backbone assignments made, the

chemical shift changes were mapped onto the crystal structure of SCR1-2. With regard to C3d, the binding surface includes regions of SCR1, SCR2 and the inter-SCR linker, specifically residues R13, Y16, R28, Y29, S32, T34, K48, D56, K57, Y68, R83, G84, N101, N105, S109 and R122. The CR2 binding surface incorporating SCR1 is inconsistent with a previous X-ray CR2-C3d co-crystal analysis, but consistent with mutagenesis, x-ray neutron scattering and inhibitory mAb epitope mapping. C3d titration also revealed a two site binding model characterized by unique modes of residue specific chemical shift changes in each SCR and different binding affinities for each binding surface. This entirely solution based structural kinetics analysis has led to a new hypothesis for the differential ligand binding capabilities of CR2. Titration with C3dp1 yielded chemical shift changes (R13, Y16, T34, K48, D56, K57, Y68, R83, G84, N105 and S109) overlapping with C3d, indicating that C3dp1 interacts at the same CR2 site as C3d. These peptides represent a novel method of selectively inhibiting single ligand binding to CR2 and have the potential to modulate CR2 function in vivo. R01 CA053615 (VMH); American Heart Association Fellowship (JMK)

CLEMONS, JE

LOW DOSE DIETHYLSTILBESTROL FOR THE TREATMENT OF ADVANCED PROSTATE CANCER. JE Clemons, (M.D., SOM), LM Glodé, and TW Flaig, Department of Medicine, University of Colorado, Denver, CO.

The purpose of this study was to assess the efficacy and safety of low dose (1mg) diethylstilbestrol (DES) for the treatment of advanced prostate cancer. DES is a synthetic estrogen previously used extensively to treat prostate cancer until its significant cardiovascular toxicity was described in numerous studies examining the use of higher doses (2-5mg) of DES. A retrospective chart review was performed to extract efficacy and safety data from patients treated with low dose (1mg) DES who had progressive, castrate-resistant prostate cancer (CRPC), despite anti-androgen withdrawal. The study population was generated from those who filled a DES prescription between 1/1/04 and 3/1/08, creating a baseline pool of 63 subjects. The PSA responses were used to determine efficacy according to the formal PSA Working Group definitions for PSA progression and PSA response. Specific IRB approval was obtained for this analysis. Of the 63 patients, 58 had sufficient data to analyze for efficacy; all were included in the safety analysis. A PSA response of 50% was seen in 22 of 58 patients (37.9%) and a stable PSA or a PSA decrease that did not reach 50% was seen in an additional 18 patients, thus, 40 out of 58 patients (69.0%) experienced a stable or reduced PSA response. The average time to progression for these patients with either category of PSA response was 29.0 weeks (median 23.1 weeks); among patients that did not responde, the average time to progression was 6.8 weeks (median 6.4). Significant cardiovascular events observed during the treatment period included 2 patients with DVTs, both of whom recovered fully after treatment. Other significant adverse events included 1 patient with primary fibrinolysis syndrome that was associated with the initiation of taxotere and DES and 1 patient with grade III mastitis. Additional adverse events, well documented with the use of DES, included breast tenderness and gynecomastia, which was experienced by 37/58 patients (63.8%); 27.6% of the patients underwent breast irradiation due to DES-induced gynecomastia. A secondary observation involves the use of DES after chemotherapy in 10 patients with PSA responses seen in 5. Additionally, 8 patients were re-treated with DES after a drug holiday or intervening medical therapy and 6 of those patients had a PSA response in this setting.

Conclusions: Low dose DES is an active therapy in patients with CRPC Considering the toxicity of competing therapeutic options including cytotoxic chemotherapy, 1 mg of DES has an acceptable level of cardiovascular toxicity. These findings, along with the very low financial cost of DES, serve as the basis for the continuing utilization of DES as a treatment for advanced prostate cancer. While only a small amount of data is available, the activity of DES in the post chemotherapy-setting and after a drug holiday merits consideration of additional investigation.

MARTIN, J

EFFECTS OF NICOTINE ON DEFAULT BRAIN MODE FUNCTIONAL CONNECTIVITY. J Martin, (MD, SOM), L Martin, J Tanabe, and J Tregellas, Department of Psychiatry, University of Colorado Denver, Denver, CO.

Default brain mode (DBM) functional connectivity in the brain has been identified as a network of regions demonstrating concurrent functional activity when an individual is resting in a conscious, but inactive, state. The exact functions of the default mode network are not fully understood, but it is believed to be involved in a variety of tasks, including reflective activity (Greicius et al. 2003). Recent evidence has demonstrated that nicotine deactivates portions of the DBM network and enhances visuospatial attention when individuals are engaged in

tasks requiring visual activity (Hahn et al. 2007). The purpose of this study is to identify effects that nicotine may have on the DBM network of individuals in a resting, inactive, state.

Fifteen individuals who reported themselves as non-smokers without a diagnosis of a psychiatric disorder participated in this study. Each participant was studied using functional magnetic resonance imaging (fMRI) and was scanned pre- and post-administration of a placebo patch and on another date pre- and post-nicotine patch administration. fMRI data was acquired during a period where the individual was presented with no stimuli and asked to remain awake but lay still and quiet. Data was analyzed using spatial independent component analysis and the DBM signal was identified via spatial correlation with a mask. Spatial differences between default mode components in pre- and post-placebo and pre-and post-nicotine groups were examined.

Nicotine administration resulted in decreased activity of the DBM. Functional activity was most markedly diminished bilaterally in the posterior cingulate cortex, specifically the retrosplenium (BA29 and BA30).

The decrease in DBM activity as a result of nicotine administration suggests that nicotine partially deactivates the resting state of the brain even without the presence of an attention demanding task or salient stimulus. The retrosplenium is involved in episodic memory (Maguire 2001), thus nicotine may interfere with this process. Default mode connectivity has been demonstrated to be aberrant in schizophrenic patients (Garrity et al. 2007). As a large percentage of schizophrenic individuals smoke, our study to observe the effects of nicotine on the default mode in this population is currently ongoing.

MCKEAN, DM

THE ROLE OF THE GLYCEROPHOSPHATIDYL INOSITOL-ANCHOR IN TRANSFORMING GROWTH FACTOR BETA SIGNALING AND FOREBRAIN DEVELOPMENT

DM McKean (Ph.D., GS) and L Niswander

In humans, holoprosencephaly (HPE) occurs in 1 out of 250 pregnancies, usually resulting in spontaneous abortion. HPE is a defect of the developing forebrain, which is critical for higher thinking, as well as for sensory and motor functions. From an ENU mutagenesis screen in the mouse, I have identified a recessive mutation in the Pig-N gene that causes HPE in homozygous mutant embryos. I will verify that the mutation in Pig-N is responsible for the HPE phenotype, demonstrate that GPI-anchored proteins are mis-localized, and will test my hypothesis that the activity of the GPI-anchored Cripto protein is disrupted in Pig-N mutants, which is causative for the HPE phenotype.

Antisense morpholino injections targeting PigN will be used in zebrafish embryos to show that knockdown of PigN protein recapitulates the HPE phenotype in a different animal model. GPI anchored protein localization will be analyzed by crossing the Pig-N mutant mouse line into a GPI-GFP transgenic mouse line. Cripto activity will be analyzed by immunocytochemical localization of Smad2 proteins in Pig-N mutant vs. wildtype mouse embryo fibroblasts.

Knockdown of Pig-N in zebrafish recapitulates the holoprosencephaly phenotype, as demonstrated by bone/cartilage staining of morphants and uninjected controls. GPI anchored proteins are mislocalized in the Golgi when Pig-N is disrupted, whereas in wildtype cells, GPI anchored proteins are found in lipid raft domains of the plasma membrane.

I have determined that a mutation in Pig-N is responsible for the HPE phenotype. Moreover, the mutation in Pig-N results in a non-functional Pig-N protein as evidenced by the mislocalization of GPI-anchored proteins. I have not yet shown that Cripto activity is disrupted and causative for the forebrain defect.

MICALIZZI, DS

THE ROLE OF TRANSFORMING GROWTH FACTOR-BETA SIGNALING IN SIX1-INDUCED EPITHELIAL-TO-MESENCHYMAL TRANSITION AND METASTASIS. DS Micalizzi, (M.D./Ph.D., GS), KL Christensen, P Jedlicka, JC Harrell, KB Horwitz, AL Welm, WP Schiemann and HL Ford, Department of Molecular Biology, University of Colorado, Denver, CO.

Activation of developmental pathways through misexpression of embryonic genes is an important tumor-promoting mechanism. The Six1 homeodomain protein, a key developmental transcription factor, is overexpressed in 50% of primary and 90% of metastatic human breast tumors, suggesting that it plays a role in tumor progression. The objective of this work is to provide evidence that Six1 induces breast cancer metastasis, and to identify the mechanism by which it induces metastatic disease. To study the role of Six1 in tumor progression, we

screened the expression profile of MCF7-Six1 and -control cells and identified multiple TGF-□ target genes regulated by Six1. Six1-mediated activation of TGF-□ signaling was confirmed by increased phospho-Smad3 levels and nuclear localization, and increased activity of a Smad-responsive reporter, 3TP. Since TGF-□ is a classic inducer of the epithelial to mesenchymal transition (EMT), we tested if Six1 could induce an EMT. Indeed, Six1 overexpression results in an EMT, marked by increased cytoplasmic localization of E-cadherin and □-catenin, downregulation of the epithelial marker cytokeratin-18, and upregulation of the mesenchymal marker fibronectin. Furthermore, the increase in soluble □-catenin corresponded to increased □-catenin-dependent transcription. Expression of a dominant negative T□-SR□ in MCF7-Six1 cells resulted in a partial reversal of the EMT, restoring E-cadherin and □-catenin localization, as well as nuclear □-catenin transcriptional activity to levels observed in control cells. Based on the recognized role for TGF-□ and EMT in metastasis, we tested the metastatic potential of MCF7-Six1 cells and show that Six1 induces metastasis to the bone and lymph nodes, sites relevant in human breast cancer. Finally, we discovered that Six1 correlates with decreased time to relapse, metastasis and poor survival in publicly available microarray datasets of human breast cancer. In conclusion, Six1 expression in mammary carcinoma cells activates TGF-□ signaling, induces properties of EMT dependent on increased TGF-□ signaling and initiates metastasis. Importantly, we show that Six1 correlates with poor prognosis in human breast cancer, consistent with data obtained in our animal models. Together, our data strongly suggests that Six1 mediates tumor aggressiveness in human breast tumors. Based on the Six1's developmentally restricted expression and its contribution to tumor metastasis, we propose that Six1 is a novel therapeutic target whose inhibition should decrease metastasis, with limited side-effects.

MILLER, MR

VARIANT NEAR THE NFKBIA GENE ASSOCIATED WITH INSULIN RESISTANCE IN HISPANIC AMERICANS: THE IRAS FAMILY STUDY. Miller MR[1](PhD, GS), Zhang W[1], Sibbel SP[1], Langefeld CD[2], Bowden DW[2], Haffner SM[3], Bergman RN[4], Norris JM[1], Fingerlin TE[1]. Colorado School of Public Health, Department of Epidemiology. (1: Denver, CO, 2: Winston-Salem, NC, 3: San Antonio, TX, 4: Los Angeles, CA)

Objective: The Inhibitor kappa B kinase beta/Nuclear Factor kappa B (IKKβ/NF-κB) pathway is known to play in important role in inflammatory response and has also recently been implicated in the mediation of insulin resistance. Nuclear Factor kappa B inhibitor alpha (IκBα) is an inhibitor of NF-κB, and a decrease of IκBα in human skeletal muscle is associated with fatty acid-induced insulin resistance. To date, no studies have tested for a relationship between the gene that codes for IκBα (NFKBIA) and insulin resistance. We hypothesized that one or more variants in the NFKBIA gene, or its 5' or 3' untranslated region, would be associated with insulin sensitivity (S_I), an important risk factor for type 2 diabetes (T2D), in Hispanic-American families.

Methods: The Insulin Resistance and Atherosclerosis Family Study (IRAS FS) is a multi-center study designed to investigate the genetic determinants of glucose homeostasis and adiposity phenotypes. Large families (12 to 13 members per family) were recruited without regard to diabetes status. We typed 11 SNPs spanning 20 kb in the NFKBIA gene in two Hispanic American (HA) samples: 520 individuals from 30 families in the San Luis Valley in Colorado (SLV) and 520 individuals in 60 families in San Antonio, Texas (SA). At least one typed SNP had an $r^2 \geq 0.65$ with all known (at the time of typing) common variants in the gene. The S_I measure was based on Minimal Model analysis of a frequently sampled intravenous glucose tolerance test. Individuals with type 2 diabetes were excluded. We tested for association between each SNP and S_I using a variance components measured genotype approach as implemented in Sequential Oligogenic Linkage Analysis Routines (SOLAR).

Results: We identified one SNP in the 3' region of the NFKBIA gene that was significantly associated with S_I, adjusting for age and sex. SNP rs1951276 (G→A) was associated with S_I in the SA sample under a dominant model (uncorrected $p = 1.84 \times 10^{-5}$, conservative Bonferroni correction $p = 3.64 \times 10^{-4}$). In the SA sample, subjects with at least one A allele for NFKBIA rs1951276 had ~30% lower S_I compared to individuals homozygous for the G allele. The association was in the same direction but not statistically significant ($p = 0.320$, ~10% decrease in S_I) in the SLV sample but remained highly significant in the combined sample ($p = 4.06 \times 10^{-4}$, ~20% decrease in S_I).
Conclusions: These results corroborate other evidence that the IKKβ/NF-κB pathway may mediate insulin resistance in humans.

MILLER, R

DISSECTING THE SIGNAL INVOLVED IN LIPID CHEMOTAXIS BY *PSEUDOMONAS AERUGINOSA*. RM Miller, (Ph.D., GS), AP Tomaras, AP Barker, and ML Vasil, Department of Microbiology, University of Colorado Denver, Aurora, CO 80045

Some bacteria use type IV pili to move across solid surfaces in a type of motility called twitching. Twitching motility is important for the opportunistic pathogen *Pseudomonas aeruginosa* in completing many diverse processes including the formation of biofilms. *P. aeruginosa* twitches directionally up a gradient of the phospholipids phosphatidyl-ethanolamine (PE) and phosphatidylcholine, and a specific phospholipase C, PlcB, has been shown to be required for this complex behavior. Additional requirements for phospholipid degradation during this process were investigated through a global transcriptional analysis during twitching-mediated movement up a PE gradient and by genetic analysis of mutants in metabolism and transport of PE or its moieties. Based on the outcomes of these experiments, it was determined that *P. aeruginosa* metabolizes PE, through PlcB and other lipid modifying enzymes (e.g. lipases), to enable it to ultimately move up a gradient of the long chain fatty acid (LCFA) moieties of PE. However, *P. aeruginosa* is only able to recognize unsaturated, but not saturated, LCFAs for this purpose. Moreover, mutants deficient in the enzymes of the glyoxylate shunt, which are unable to utilize LCFAs as a substrate for growth, can still directionally twitch to unsaturated LCFAs. Therefore, this directed motility can be referred to as "metabolically-independent chemotaxis", rather than strictly as "energy taxis". Chemotaxis requires a specific signal, and modified unsaturated fatty acids commonly act as signals in numerous organisms. For example, leukotrienes, a form of oxidized fatty acids, can act as neutrophil chemoattractants within the human body. In our assays, the unsaturated LCFA chemoattractant is exposed to oxygen for long periods of time. Thus, we hypothesize that *Pseudomonas aeruginosa* specifically recognizes oxidized unsaturated LCFAs. Currently, the addition of alpha-tocopherol, an anti-oxidant, to the unsaturated LCFA decreases the distance that *P. aeruginosa* travels towards the chemoattractant. We are continuing to confirm that the presence of alpha-tocoperol is indeed limiting oxidation and is not itself decreasing the chemotactic response. If these unsaturated LCFAs are indeed oxidized, this form of signaling would be novel as no other prokaryote has been shown to use the same system to date.

MILROY, LL

INFERIOR ALVEOLAR NERVE INJURY MAY BE INFLUENCED BY GENDER DIFFERENCES. LL Milroy, (D.D.S. SoDM) DJ Kleier, RE Averbach, & BJ Potter, Department of Surgical Dentistry, School of Dental Medicine, University of Colorado, Aurora, CO

A number of case reports have documented injury to the inferior alveolar nerve during endodontic treatment of mandibular posterior teeth. The majority of these cases involve the second molars of female patients. **Objective:** Our goal was to determine if gender differences in second molar root proximity to the inferior alveolar canal may contribute to this finding. **Hypothesis:** We hypothesized the distance between the root apices of mandibular second molars and the inferior alveolar canal is shorter in females than it is in males. To pilot test this hypothesis we reviewed Cone Beam Computed Tomography (CBCT) images from 16 patients: 8 males and 8 females. Patients were between the ages of 28 and 69 and had received these scans for a variety of diagnostic purposes. Images of the mandibular second molar region were viewed in a buccal-lingual cross-sectional plane with the scanner's Xoran Software. Using the measuring tool provided by the software, the distance from the most apical part of the mesial and distal roots to the superior border of the mandibular canal was recorded. Both roots were measured on two separate occasions and the results averaged. **Results:** We found that the average distance in the male population was 5.46mm (mesial root) and 5.40mm (distal root), compared to 4.31mm (mesial root) and 3.32mm (distal root) in the female population. Using two-sample equal-variance t-test with a significance set at $p \leq .05$ we found that the means of the two populations were significantly different for both the mesial ($p \leq .04$) and distal ($p \leq .002$) roots. **Conclusions:** For the limited population in this pilot study we concluded that there was a statistically significant difference in the proximity of the mandibular second molar root apices to the mandibular canal between genders. This difference could provide a possible explanation regarding the increased frequency of inferior alveolar nerve injuries reported in female patients.

NELSON, AM

EXTRACELLULAR DOPAMINE CONCENTRATIONS PARALLEL COCAINE-INDUCED DIFFERENCES IN LOCOMOTOR ACTIVATION OF INDIVIDUAL RATS. AM Nelson (MD/PhD, MS/GS) and N.R. Zahniser, Dept of Pharmacology and Med Scientist Training Program, University of Colorado Denver, Aurora, CO

Individuals differ in their responsiveness to abused stimulant drugs, such as cocaine and amphetamine, which produce their activating and rewarding effects primarily by binding to the neuronal dopamine (DA) transporter (DAT). Outbred male Sprague-Dawley rats can be classified as either low or high cocaine responders (LCRs or HCRs, respectively) based on the median split of open-field locomotor activity induced by an acute dose of cocaine (10 mg/kg, i.p.). LCR/HCR classification is predictive of differences in repeated cocaine-induced locomotor sensitization, conditioned place preference, and motivation to self-administer cocaine, demonstrating the utility of this unique model of individual differences in cocaine responsiveness. The observed behavioral differences reflect, in part, that the initial exposure to cocaine inhibits the DAT more effectively in HCRs than LCRs, a difference that is more pronounced in nucleus accumbens (NAc) than dorsal striatum (dSTR). To investigate if the differential inhibition of DAT in LCRs and HCRs is translated into differences in extracellular DA in dSTR and NAc, before and/or after acute cocaine, we used in vivo microdialysis concomitantly with open-field locomotor activity measurements. None of the rats subsequently defined as LCRs/HCRs or saline-treated controls differed in their locomotor activity during the first 60 min in an inescapable novel environment (open-field chamber). The following day locomotor activity and microdialysis samples were collected for 2 hrs before (baseline) and after injection of saline or cocaine (10 mg/kg, i.p.). LCRs/HCRs did not differ in baseline extracellular DA, as predicted by their lack of baseline locomotor activity differences during the same time period (dSTR: HCRs = 1.54 ± 0.10 nM, LCRs = 1.57 ± 0.06 nM; NAc: HCRs = 0.78 ± 0.14 nM, LCRs = 0.74 ± 0.18 nM). However, following cocaine, extracellular DA levels in dSTR and NAc of HCRs were significantly higher than those of both LCRs and saline-treated rats. In agreement with our previous in vivo DAT function results, these differences were more pronounced in NAc, where HCRs showed 3.5-fold greater extracellular DA during the 40 min after cocaine, compared to LCRs. Irrespective of LCR/HCR classification, there were significant positive correlations between individual rats' cocaine-induced locomotor activity and extracellular DA in dSTR (Pearson r = 0.49) and NAc (r = 0.89). These findings confirm the greater role of NAc DA, compared to dSTR DA, in cocaine-induced locomotor activity. More importantly, our results show that the same low dose of cocaine results in significantly higher striatal levels of extracellular DA in HCRs than LCRs, and that these changes parallel their unique cocaine activation profiles. Taken together, our findings suggest that DA plays an important role in low initial cocaine responsiveness and support further investigation of the LCR/HCR phenotypes as they predict cocaine addictive behaviors.

NEUWELT, A

ACETAMINOPHEN ENHANCES THE CYTOTOXICITY OF CISPLATIN WHICH IS REVERSED BY N-ACETYLCYSTEINE IN HEPATOBLASTOMA CELLS. Alexander Neuwelt, Sean McNatt, Jeffrey Wu, Mike Pagel, Steven Warmann, Narcyz Knap, Marcin Losin, Edward Neuwelt, Piotr Czauderna, Michal Wozniak. Medical University of Gdansk, Poland, Department of pediatric neurosurgery, UCHSC.

Malignancies with low levels of intracellular glutathione have increased sensitivity to cisplatin (CDDP)-based chemotherapeutic regimens. Acetaminophen (AAP) is a commonly prescribed analgesic and antipyretic that when taken in overdose causes liver damage via a mechanism involving glutathione depletion and production of a toxic metabolite primarily via cytochrome P450 2E1 (CYP2E1). Kobrinsky et al. (2005) published a case study of the successful treatment of a patient with recurrent hepatoblastoma that had failed front-line therapies using a novel chemotherapeutic regimen combining high-dose AAP with CDDP, then rescued 8 h later with N-acetylcysteine (NAC). In this work, in vitro studies were performed using HUH6 and HepT1 human hepatoblastoma cell lines cultured in AAP and the combination of AAP and CDDP with or without delayed NAC rescue. In vivo pharmacology and toxicology tests were also performed in control rats. In vitro, AAP decreased glutathione (GSH) levels in hepatoblastoma cell lines in a dose- and time-dependent manner. We also demonstrated that AAP combined with CDDP has enhanced cytotoxicity over CDDP alone by cell viability assay and Western blotting analysis. Cytotoxicity was decreased when NAC was administered simultaneously with CDDP and AAP. However, the protective effect decreased when NAC was administered at delayed time points. High dose AAP (1000 mg/kg) is hepatotoxic as evidenced by elevated serum ALT and AST levels in AAP-treated rats. In addition, AAP treatment substantially lowered GSH levels in the liver but not in the blood or brain 6 h after treatment. The expression of CYP2E1protein depends upon species and cell type. We concluded that a chemotherapeutic regimen containing both AAP and

CDDP with delayed NAC rescue would be a promising approach that merits further pre-clinical examination, particularly in hepatoblastoma but also in other malignancies where the cytochrome P450 system can be modulated. In current studies, underway at the University of Colorado, we are expanding these studies to include atypical rhabdoid (ATRT) and germ cell tumor cell lines.

OTTEN, DR

ASSOCIATION OF EGO DEFENSE MECHANISMS, DEPRESSION, AND CATECHOLAMINE-O-METHYLTRANSFERASE GENOTYPING AMONG PARENTS OF CHILDREN WITH PEDIATRIC CANCER.
DR Otten, (MD, SOM), TP Beresford, and AS Hoffenberg, Department of Psychiatry, University of Colorado, Denver, CO.

Purpose: This study looks to investigate the association of ego defense mechanisms and depression scores of parents of children with cancer with their genotype for the COMT allele and their cortisol levels. A secondary goal for this study will be the investigation of the association of parents' ego defense mechanism profiles and their children's cancer survival three and five years later.

Background: The enzyme catecholamine-O-methyltransferase (COMT) degrades the catecholamine neurotransmitters dopamine, epinephrine, and norepinephrine. A polymorphism of the gene that codes for this enzyme results in differential enzymatic activity, and those with methionine (Met158) alleles have decreased COMT activity. Individuals with this low-activity allele are prone to anxiety, depression, and neuroticism, thereby increasing their cortisol levels. Furthermore, studies have indicated that the maturity of ego-defense adaptation among adult cancer patients not only affected their psychological adjustment but also was associated with increased cancer survival. Therefore, we hypothesize that among parents of children with cancer, the individuals who are less depressed or anxious are more likely to have lower cortisol levels, more likely to have the high-activity COMT allele, and are more likely to have "mature" ego defense mechanisms. We also believe that these parents' children with cancer will have a longer survival probability than children with cancer whose parents have "immature" adaptation styles.

Methods: The parents will be administered the 21-item Beck Depression Inventory and the 40-item Defense Style Questionnaire while their child undergoes chemotherapy infusions. A salivary cortisol and DNA sample (via saliva) will also be gathered during this session. In addition they will fill out a 15-item survey regarding compounding variables, their child's cancer diagnosis, and demographic information. Follow up will follow at 3- and 5-years to determine child survival via the Cancer Registry.

Results: The study protocol was submitted to COMIRB, full board. It was reviewed, and approval was deferred to a future date, as a few questions needed clarification. Meanwhile, a new research member was identified in the Department of Pediatric Oncology, and new details are under discussion to be incorporated in the protocol application.

Conclusions: The results of this study would help the child's treatment team reach out to at-risk parents, maximizing positive outcomes and minimizing distress in both the parent and the child.

PANDIPATI, S

NOREPINEPHRINE-INDUCED LONG-TERM MODIFICATIONS IN RAT OLFACTORY BULB CIRCUIT DYNAMICS. S Pandipati (MD, PhD, MSTP) and NE Schoppa. Department of Physiology and Biophysics, UCD-AMC, Aurora, CO.

Norepinephrine (NE), a catecholamine neuromodulator released from the locus coeruleus (LC), may be essential for many forms of olfactory learning. Many changes in brain circuitry leading to this olfactory learning appear localized to the olfactory bulb (OB), which receives a large fraction of inputs from the LC. The cellular mechanisms responsible for NE-mediated olfactory learning remain unknown. Here, we tested the effects of NE on the OB circuitry both acutely and in the long-term using patch-clamp and local field potential (LFP) recordings in rat OB slices. At the level of the granule-to-mitral cell inhibitory synapse, NE appears to acutely reduce GABAergic transmission by 51.4 $\pm$ 7.4 percent. During responses to patterned olfactory nerve stimulation mimicking the breathing cycle, NE induced a long-term 235 $\pm$ 84.1 percent increase in gamma frequency (30–70 Hz) synchronized oscillations. Additionally, we tested to see if clonidine, a α_2-adrenergic receptor (AR) agonist, could mimic the effects of NE. We found that clonidine caused a decrease in GABAergic transmission at the granule-to-mitral

inhibitory synapse by 31.4 $\pm$ 12.5 percent. Clonidine also resulted in a long-term enhancement of gamma oscillations in the LFP by 83 $\pm$ 20.6 percent. This leads us to the conclusion that NE acutely disinhibits mitral cells that in turn may be permissive to the long-term enhancement of gamma oscillations. NE appears to have these effects at least partially through the activation of α_2-ARs.

PYRGAKI, C

THE MAMMALIAN HOMOLOG OF THE DROSOPHILA GRAINYHEAD LIKE 2 TRANSCRIPTION FACTOR PLAYS A ROLE IN FUSION AND TUBULE FORMATION DURING DEVELOPMENT. C.Pyrgaki (phD, GS) and L.A. Niswader. Department of Pediatrics, University of Colorado, Denver, CO.

Development is a very complicated process and it requires the very well orchestrated action of a number of genes. Disruption of the function of any of these genes leads to conditions known as birth defects. We have a clear understanding of the function of only a very small percent of the genes involved in mammalian development. Therefore, the challenge that we are facing in our effort to better understand development is not only to identify the genes involved in the process of development, but also elucidate their function during that process. In the current study and with the use of the mouse as a model organism and with a forward genetic approached we were able to assign a novel function to a transcription factor that is a mammalian homologue to the Drosophila Grainyhead

Methods We used ENU to generate mouse lines with autosomal recessive mutation that cause birth defects. The line of interest (Line2) had three severe phenotypes: exencephaly, cleft palate and omphalocele. Backcrossing of the carrier animals into a strain other than the original mutagenized male helped us map the gene responsible for the phonotypes to a 2Mb region of mouse chromosome 15.

Using Sequence capture, a novel method that allows for a more efficient, high throughput sequencing of DNA regions up to 5Mb long we identify the mutation that lead to the embryonic phenotype.

Results: A point mutation (T to A transversion) on position 301 of the coding sequence of the gene Grainyhead like 2 (Grhl2), or BOM, leads to a stop codon and that causes a truncation of the last 450 aminoacids of the protein. In addition to the closure defects, it appears the mutant embryos also have a severe lung phenotype. The lungs are much smaller compared to wild type littermates, and their structure seems to be also disturbed. Similarly are affected the kidneys, displaying significantly smaller size and altered structure. The loss of function of that gene does not disrupt the A-P or D-V patterning of the embryo as it was shown by in situ hybridization experiments and therefore the severe phenotype of these embryos is not due to patterning defects.

Conclusions and future directions; Grhl2 seems to affect two different types of developmental processes in the mouse embryo. First processes that involve active closure of tissues, such as neural tube closure, face closure and body wall closure. Also, it affects the morphogenesis of organs that require formation of tubules in their structure. We aim to identify genes that Grhl2 control in order to understand how closure and formation of tubular structure occurs in the mammalian embryo.

REDZIC, JS

IDENTIFICATION AND CHARACTERIZATION OF A SOLUBLE FORM OF CD147 IN HUMAN BLOOD

Jasmina S. Redzic (Ph.D, BMG), Jennifer Schlegel, Adrie vanBokhoven, M. Scott Lucia, Steven K. Nordeen, Kirk C. Hansen, Elan Z. Eisenmesser, Department of Biochemistry and Molecular Genetics, University of Colorado Denver, School of Medicine, Denver, CO.

CD147 (also known as Extracellular Matrix Metalloproteinase Inducer or EMMPRIN) plays a central role in numerous cancers that include breast and lung and is a highly expressed receptor in disseminated cancer cells such as those in prostate cancer. The CD147 receptor regulates multiple protein families integrally involved in tumorigenesis where it is also released in several forms as a soluble protein. While CD147 is already a tissue "biomarker" for many cancers, CD147 has not been identified within human blood despite the potential to use CD147 as a diagnostic for cancer. Our goal is to determine if CD147 does exist in blood, what form(s) of the receptor are present and in what quantities. Using a combination of multiple techniques that include immunoprecipitation, Western analysis, and mass spectrometry, we have discovered a highly glycosylated form of CD147 present in human serum that comprises the extracellular region of the full-length CD147 receptor present in ng/mL quantities. Our identification of a soluble CD147 present within human blood opens the door to exploring another potential mechanism whereby this receptor contributes to metastasis. Our identification of soluble CD147

in the blood is a crucial first step in determining whether CD147 can be used as diagnostic and prognostic indicator for multiple cancers.

ROEDE, JR

IMPACT OF PEROXIREDOXIN 6 GENE DELETION AND OVER-EXPRESSION ON ETHANOL-MEDIATED LIVER DAMAGE IN MICE. JR Roede[1] (Ph.D., GS) , AB Fisher[2] and DR Petersen[1], [1]Department of Pharmaceutical Sciences, University of Colorado Denver, Denver, Colorado. [2]Institute of Environmental Medicine, University of Pennsylvania.

Oxidative stress is implicated in the etiology of many diseases including alcoholic liver disease (ALD). Peroxiredoxin 6 is a cytosolic peroxidase that has been demonstrated to protect various tissues, such as skin, lung and cardiac muscle, against acute oxidative insults. Taken together, peroxiredoxin 6 was hypothesized to also protect the liver from oxidative stress generated during the process of chronic ethanol metabolism. To test this, both peroxiredoxin 6 knockout mice (KO) and transgenic, peroxiredoxin 6 over-expressing mice (TG) were fed an ethanol containing diet and various biomarkers of ALD were assessed along with the effects of chronic ethanol consumption on the antioxidant defenses. After 9 weeks of ethanol consumption all backgrounds exhibited increased plasma ALT activity, steatosis, CYP2E1 induction and lipid peroxidation. Differences in antioxidant protein expression and activity were also observed. Significantly induced catalase and glutathione S-transferase activity in ethanol-fed KO and TG mice along with elevated levels of glutathione peroxidase activity were noted. These results could be attributed to either compensatory responses due to the genetic manipulations or ethanol-mediated responses. It can then be concluded that both ethanol-fed KO and ethanol-fed TG mice developed early stage ALD and that oxidative stress and overall pathology were not exacerbated by the absence of peroxiredoxin 6 and not prevented or attenuated by its over-expression.

RUMER, KK

BIPHASIC EFFECT OF LEPTIN ON HUMAN CYTOTROPHOBLAST INVASION

KK Rumer, (M.D./Ph.D. SOM/GS), VD. Winn. Dept. of OB/GYN, University of Colorado Denver, Aurora, CO.

Purpose: Preeclampsia (PE) complicates ~4-8% of pregnancies and accounts for significant maternal and neonatal morbidity and mortality. Impaired invasion of placental cytotrophoblasts (CTBs) is an established defect in PE. It has been shown that leptin is elevated in the serum and placentas of women with PE. In fact, elevated serum leptin in women who develop PE has been noted as early as 13 weeks. Our recent microarray studies of the placental basal plate, the maternal/fetal interface and location of CTB invasion, also showed increased expression of leptin in PE. Despite the common association of leptin and PE, the role of excess leptin in PE pathogenesis has not been determined. We hypothesized that high levels of leptin would inhibit human CTB invasion.

Methods: An immortalized human trophoblast (HTR8SVneo) cell line and primary human CTBs (pCTBs) isolated from second trimester placentas (n=4, gestational age range 13-24 weeks) under Institutional Review Board approved protocols were used for these studies. HTR8SVneo experiments were repeated three times. Cells were plated on Matrigel-coated invasion chambers and incubated in serum free medium containing leptin at 0, 10, 80, or 160 ng/ml. Medium was replaced daily and chambers harvested at 48 (HTR8SVneo) or 72 (pCTBs) hours. Invasion was quantified and counts were averaged from 2-5 membranes per leptin concentration for each experiment. Invasion was normalized to the average invasion at 0 ng/ml and reported as fold change (fc). Statistical analysis was performed using one-way ANOVA with Tukey post-test analysis.

Results: Leptin had a biphasic effect on human CTB invasion. No difference in invasion was seen at 10 ng/ml leptin compared to 0 ng/ml for both cell types although pCTBs showed a trend toward increased invasion (fc=3.0 $p>0.05$). Upon 80 ng/ml leptin treatment, invasion increased in both cell types, for HTR8SVneo fc=1.8 ($p<0.05$) and for pCTBs fc=3.7 ($p<0.05$). At 160 ng/ml, invasion returned to baseline with a trend toward inhibition of invasion for the pCTBs (fc=0.4 $p>0.05$).

Conclusions: The biphasic effect of leptin on human CTBs suggests there is an optimal leptin concentration for promoting CTB invasion. Thus, the hyperleptinemia seen in women with PE may be causal in insufficient CTB invasion during the first stage of PE pathogenesis.

RUYGROK, ML

VALIDATION OF THREE TERMINATION OF RESUSCITATION CRITERIA FOR GOOD NEUROLOGIC SURVIVAL AFTER OUT-OF-HOSPITAL CARDIAC ARREST. ML Ruygrok, (MD, GS), RL Bynyny MD, MSc, JS Haukoos MD, MSc, Department of Emergency Medicine, Denver Health Medical Center, Denver, CO.

Background: Several termination of resuscitation (TOR) criteria have been proposed to identify patients who will not survive to hospital discharge (SHD) after out-of-hospital cardiac arrest. However, only one set has been derived to specifically predict SHD with good neurologic function. The objectives of this study were to externally validate the Basic Life Support (BLS) TOR, Advanced Life Support (ALS) TOR, and Neurologic (NEURO) TOR criteria and compare their abilities to predict SHD with good neurologic function after out-of-hospital cardiac arrest.

Methods: This was a secondary analysis of the Denver Cardiac Arrest Registry. Consecutive adult non-traumatic cardiac arrest patients in Denver County from January 1, 2003, through December 31, 2004, were included in the study. The BLS TOR, ALS TOR, and NEURO TOR criteria were applied to the cohort, and their predictive proportions and 95% confidence intervals (CIs) were calculated for each set of criteria.

Results: Of the 715 patients included in this study, the median age was 65 (IQR: 52 – 78) years, and 69% were male. In addition, 223 (31%) had return of spontaneous circulation, 175 (24%) survived to hospital admission, 58 (8%) SHD, and 42 (6%) SHD with good neurologic function. The proportion of patients with good neurologic SHD correctly identified for continued resuscitation was 100% (95% CI: 92%-100%) for all three TOR criteria. The proportion of patients with poor neurologic SHD, or no SHD, correctly identified as eligible for TOR was 36% (95% CI: 32%-40%) using the BLS TOR criteria, 25% (95% CI: 22%-29%) using the ALS TOR criteria, and 6% (95% CI: 4%-8%) using the NEURO TOR criteria. Use of the BLS TOR criteria would have reduced transport of the largest number of patients.

Conclusions: All three TOR criteria had equally high abilities to identify patients requiring continued resuscitation. The BLS TOR criteria, however, had the best combined ability to predict good neurologic survival and poor neurologic survival or death. These findings and its relative simplicity support the use of the BLS TOR criteria.

STROM, T

RARE NERVE LESIONS OF NON-NERVE SHEATH ORIGIN: A 17 YEAR RETROSPECTIVE SERIES. T Strom (M.D., SOM)[1], BK Kleinschmidt-DeMasters[1,2,3], A Donson[4], NK Foreman[2,4], KO Lillehei[2] Departments of [1]Pathology, [2]Neurosurgery, and [3]Neurology, The University of Colorado Health Sciences Center, and [4]Neuro-Oncology, The Children's Hospital, Aurora, Colorado.

Purpose: Peripheral nerve masses are frequently encountered in surgical pathology practice. However, once a peripheral nerve mass is determined *not* to be a nerve sheath neoplasm, differential diagnostic considerations drop off sharply. The goal of this study is to review our experience with nerve and nerve root masses.

Methods: Computer-based, 17-year retrospective search of our pathology database from 1991-2008, including only cases where symptoms/signs were referable to a nerve lesion, necessitating surgical resection. Rare neoplasms were further studied by cytogenetic analysis or gene microarray to compare findings with similar tumors arising in more common anatomical sites.

Results: 458 cases of nerve lesions/masses were identified. After elimination of common lesions (nerve sheath tumors, ganglion cysts, traumatic or Morton neuromas, malignant peripheral nerve sheath tumors and paragangliomas), 37 cases (8%) remained, almost all of which were of non-nerve sheath origin. Notable rare nerve lesions included cavernous angioma, massive capillary hemangioma, proximal traumatic neuroma devoid of collagen, metastatic neuroendocrine pancreatic carcinoma, meningiomas invading nerve fascicles, and primary Ewing sarcoma, extrarenal rhabdoid tumor (RT), and undifferentiated sarcoma. The latter presented in a child with diffuse, rope-like nerve enlargement several years before evolving into a more typical MPNST. Ewing sarcoma showed a rearrangement of the EWSR1 locus at 22q12 and the RT manifested loss of INI-1 protein expression. The

undifferentiated sarcoma was found to have a gene expression pattern that clustered with other malignant peripheral nerve sheath neoplasms, but not Ewing sarcomas or rhabdomyosarcomas.

Conclusions: Pathologists and clinicians should be aware of the wide diversity of benign, metastatic, and primary diseases that can rarely affect peripheral nerve, often mimicking nerve sheath tumors.

TARULLO, DB

STUDENT HEALTH REFUGEE EDUCATION COLLABORATION: A NEEDS ASSESSMENT. DB Tarullo, (MD, SOM), LT Gonzales, MP Miller, HJ Miller, and E Aagaard, Department of Medicine, University of Colorado at Denver and Health Sciences Center.

Background: 35,000 political refugees live in Denver, and 1,100 more arrive annually. They represent a population vulnerable to health disparities and associated disease risk.

Purpose: To determine the health-related needs of refugees in the Denver area with the long-term goal of designing a medical student service elective to be integrated into the University of Colorado Denver Medical School curriculum. The elective will strive to help overcome some of the healthcare disparities of the refugee population while enhancing the cultural competence of students.

Methods: We will use surveys and structured interviews to establish the health-related needs of the refugee population. We will collect 400 responses from refugees (roughly 1.2% of the refugee population in the Denver area); 45 one-on-one interviews, 60 focus groups, and 295 self-administered surveys. We will also interview 30 healthcare providers who serve refugees and 20 refugee community leaders (key informants). This process is ongoing with responses to date from 9 key informants, 2 providers, and 1 focus group of 10 individuals. The current data is being used to refine our survey prior to dissemination.

Results: Preliminary results show concerns falling into 4 broad categories: treatment of disease, causes of disease, language difficulties, and cultural expectations. Common diseases refugees have difficulty finding treatment for include preexisting malaria, tuberculosis, hepatitis A, B, & C, HIV infection, PTSD, and malnutrition. In addition, many develop hypertension, heart disease, diabetes, cancer, depression, anxiety disorder, and dental caries after arrival. Perceived causes of disease or its worsening include changes in diet, socioeconomics, and experiences with unhelpful healthcare providers. Language issues include difficulties with interpreters, providers unfamiliar with communication with an interpreter, inadequate ESL classes, and limited native vocabulary to describe medical conditions. Cultural expectations include conflicts between evidence-based medical practices and native folk-medicine, religious beliefs incompatible with western medicine, and unrealistic expectations held by both refugees and providers. These and future findings from the survey will be used to design an elective to address some of the issues described. Our methods describe a feasible mechanism to develop a sustainable medical student service project that meets the needs of the population it intends to serve. Future research must investigate whether the elective we develop truly achieves this goal.

VEO, B

TRANSLATION INITIATION OF THE HUMAN TAU mRNA THROUGH AN INTERNAL RIBOSOMAL ENTRY SITE

Bethany Veo (Ph.D., GS) and Les Krushel
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Tau is a microtubule associated protein that is known to cause the formation of neurofibrillary tangles when hyperphosphorylated. The accumulation of neurofibrillary tangles is a pathological phenotype common in several neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), and supranuclear palsy. It has been previously shown that altering the protein level of tau has beneficial effects on the severity of the symptoms associated with AD. The regulation of protein synthesis can occur through two mechanisms transcription and translation. We focused our efforts on the translational regulation of tau mRNA to see if a regulatory element is present that may influence tau production. The two major mechanisms of translation are cap-dependent and cap-independent translation. While most mRNAs are thought to be translated through a cap an increasing number of cellular mRNAs are found to utilize a

cap-independent method. Initiation of translation independently of the cap requires the use of an internal ribosomal entry site or IRES. To investigate if the tau 5' UTR could initiate translation internally the tau 5' UTR was inserted upstream of the 2nd cistron in a dicistronic construct and transfected into a neuroblastoma cell line. In vivo analysis of the dicistronic RNA constructs suggested that the tau 5' UTR could initiate translation internal of the cap. Likewise, in vivo transfections of ApppG capped monocistronic RNAs did not hinder the ability of the tau 5' UTR to initiate translation internally. Furthermore, the endogenous level of tau protein did not decrease when cap-dependent synthesis is shut down by siRNA treatment against the cap binding protein eIF4E. Thus, also suggesting that tau protein synthesis is facilitated by an additional mechanism that does not rely on the recognition of the m7G cap by eIF4E. Our examination of the tau 5'UTR reveals that tau utilizes an IRES to facilitate translation. These studies may indicate a method for targeting tau production in neurodegenerative diseases, which have a taupopathy phenotype.

WARMACK, JD

PEDIATRIC MENTAL HEALTH AND SUBSTANCE ABUSE SCREENING PRACTICES. JD Warmack, (M.D., SOM), PD Riggs (M.D.), Department of Psychiatry, University of Colorado Denver School of Medicine, Denver, CO.

Pediatricians play an important role in early identification of adolescent substance abuse and mental health problems, but there is little published research about current screening and referral practices. Research is needed to identify deficits and to inform development of more effective screening, referral, and intervention.

Five physicians in Denver participated in a semi-structured interview created by the primary investigator (Warmack) evaluating current screening and referral practices of primary care physicians treating adolescents. Survey questions included: practice setting; estimates of the number of adolescents seen annually and of the percent of adolescents in their practice with substance use or psychiatric disorders; current screening, treatment referral and follow-up methods; knowledge of published screening guidelines; perceived barriers, satisfaction ratings and suggestions for improving this system of care. All procedures were approved and monitored by the Colorado Multiple Institutional Review Board.

Of five physicians interviewed (3 pediatric private practices, 1 university-based family practice, and 1 county-hospital affiliated adolescent medicine clinic), all estimated that >50% of adolescents used or abused substances and 3 estimated >40% had psychiatric disorders. 4/5 used self-administered questionnaires developed by their practice group; 1/5 reported non-standardized screening based on clinical judgment. None of the physicians used published screening practice guidelines. Referral practices were driven by patients' insurance provider lists. All reported low satisfaction ratings with substance and mental health treatment. Low satisfaction ratings were attributed to poor treatment access and lack of clinical progress information or follow-up recommendations from referral agencies.

Although most physicians in this pilot study use some mental health and substance screening measures, low satisfaction ratings may indicate that initiatives to improve standardized screening and referral practices in primary care settings may be ineffective without concomitant improvement in systemic and economic barriers to mental health and substance treatment access and continuing care.

WILLIS, VC

TREATMENT WITH CL-AMIDINE, A PEPTIDYL ARGININE DEIMINASE INHIBITOR, SIGNIFICANTLY REDUCES THE SEVERITY OF COLLAGEN-INDUCED ARTHRITIS: VC Willis[1] (PhD, GS), A Gizinski[1], NK Banda[1], CP Causey[2], K Cordova[1], B Knuckley[2], B Levitt[1], M Glogowska[1], WP Arend[1], PR Thompson[2], VM Holers[1]; [1]University of Colorado, Denver, CO; [2]University of South Carolina, Columbia, SC

Antibodies to citrullinated protein antigens (ACPA) are specific for RA and can be detected several years before the clinical onset of disease. Post-translational citrullination of epitopes to which ACPA are directed is carried out by enzymes known as peptidyl arginine deiminases (PADs). We have previously shown that ACPA develop in collagen-induced arthritis (CIA) and contribute to joint damage. In this study we used Cl-amidine, a PAD inhibitor that specifically inhibits all five PAD enzymes, to determine whether limiting the development of citrullinated epitopes can modify the development of ACPA, and if so at what point in disease evolution.

Male DBA/1j mice were immunized with bovine type II collagen (CII) in Complete Freund's Adjuvant at days 0 and 21. Five groups of immunized mice were studied: no drug treatment, vehicle alone (PBS) and 1, 10 or 50 mg/kg/day Cl-amidine via daily IP injection. Clinical disease activity was scored in a blinded fashion on a standard scale. All mice were sacrificed on day 35. Collagen antibody-induced arthritis (CAIA), a model of the antibody-mediated effector pathway of CIA, was induced by IP injection of 4 mg of Arthrogen monoclonal antibodies, followed after 3 days by 50 µg LPS IP.

Cl-amidine treatment reduced clinical disease activity scores by 42%, 53% and 55% in the respective treatment groups (1,10, 50 mg/kg/day). There were no observed differences in T cell proliferation and IgG1 or IgG2a antibody levels to bovine CII between treatment and control groups. However, lower IgG1 and IgG2a autoantibody levels to mouse CII, and decreased epitope spreading to citrullinated epitopes, was observed. Conversely, Cl-amidine treatment had no effect on the development of CAIA disease activity scores.

Cl-amidine significantly reduced inflammation and tissue injury in CIA but not CAIA. These results suggest that PADs are essential components in the development of autoimmunity to citrullinated epitopes and that modulation of their activities can inhibit the subsequent development of target organ damage.

WILSON, B and KAMAYA, S

STUDENT BEHAVIOR AS A PREDICTOR OF USMLE STEP 1 SCORES. B Wilson (MD, SOM) S Kamaya (MD, SOM)

Objective:Starting early in their medical education, students at the University of Colorado Denver School of Medicine are introduced to a variety of study techniques geared toward improving their USMLE© Step 1 experience/score. However, many of these suggestions are made to students without evidence to support which of these behaviors may be most effective. This study examines the impact of various students' USMLE© Step 1 study behaviors on Step 1 score outcomes.

Methods: Upon completion of the USMLE© Step 1 exam, 127 rising third-year medical students at the University of Colorado Denver School of Medicine were given a 30 question survey assessing a multitude of variables associated with individual self-reported study behaviors for the USMLE© Step 1 exam. Some of the variables included (1) amount of hours per week students' studied during basic science courses, (2) the amount of hours per day students studied during the designated study time, (3) class attendance in the first two years of the curriculum, (4) amount of practice questions completed, (5) whether or not a schedule was created and (6) a high self-identified and/or school-identified risk for poor performance. This survey was assessed along with other individual student data including first year curriculum course grades and MCAT© section scores. Data was analyzed using regression models for key variables.

Results: Initial regression models shows three variables are significant predictors of USMLE© Step 1 scores. These variables are (1) MCAT© Physical Sciences scores ($p < .0001$), (2) number of test questions studied ($p < .036$), and (3) total hours spent studying ($p < .011$). Both number of test questions completed and number of hours spent studying are variables controlled by medical students and are examples of effective studying behaviors. However, when number of hours spent studying and total number of practice questions completed were grouped together in a regression analysis, total number of hours studied became insignificant.

Conclusions: Individual student behavior does impact USMLE© Step 1 exam scores. Specifically, completing more questions yields higher Step 1 scores. This does have implications for the message medical schools give to their students in preparation for this important exam.

WYATT, MF

ANKLE-BRACHIAL INDEX KNOWLEDGE AMONG INTERNAL MEDICINE RESIDENTS – IMPACT OF AN EDUCATIONAL INTERVENTION. MF Wyatt (MD, MS2), C Stickrath MD, A Shah MD, A Smart MD, J Hunt MD, IP Casserly MD. Department of Medicine, University of Colorado, Denver, CO.

Introduction: The ankle-brachial index (ABI) is the standard screening tool used in clinical practice to detect peripheral arterial disease (PAD) and is a powerful predictor of adverse cardiovascular and cerebrovascular events. Based on our experience, the ability of internal medicine residents to perform, calculate, and interpret an ABI is suboptimal. **Objective**: 1. To quantify common errors made by internal medicine residents in the measurement, calculation, and interpretation of ABI; 2. To provide feedback and education to residents on these errors and

measure the impact of this intervention to improve subsequent performance. **Methods**: Internal medicine residents from all three years of the University of Colorado Internal Medicine Residency were invited to participate in this study. Under the supervision of a trained observer, participants were asked to perform the following three tasks: 1. Perform an ABI measurement on a normal subject in a standardized environment (Score 15); 2. Calculate the right and left ABI based on arm and leg pressure data from a hypothetical patient (Score 3); and 3. Provide the correct interpretation for a range of ABI values (Score 6). Based on the critical elements for each task, a score was assigned for each task as shown. The total possible score was 24. Following a standardized educational session, participants were asked to repeat the three tasks under the supervision of the same trained observer. The difference in the mean score between baseline and follow-up was assessed using a paired t test. **Findings**: A total of 27 residents (13 interns, 10 second years, 4 third years) participated in the study. At baseline, the percent of participants who correctly performed, calculated, and interpreted the ABI measurement were 4%, 11%, and 44%, respectively. Following the educational intervention, the percent of participants who correctly performed, calculated, and interpreted the ABI measurement were 90%, 74%, and 84%, respectively. The composite mean score for all tasks at baseline was 10.3, and following the educational intervention was 22.3 ($p < 0.001$). **Conclusions**: The level of knowledge regarding the correct performance, calculation, and interpretation of the ABI is poor, regardless of year. However, these deficiencies can be successfully addressed through a brief focussed educational intervention.